1992

Central serotonergic stimulation of renin secretion

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CENTRAL SEROTONERGIC STIMULATION OF RENIN SECRETION

by

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A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

May

1992

ACKNOWLEDGMENTS

It is not often in life that one receives a second chance. Fortunately for me, the Department of Pharmacology at Loyola allowed me the opportunity to do just that. Special thanks has to go to our chairman Dr. Israel Hanin for taking a calculated risk by accepting me. Thanks also goes to the other members of my committee for their help and support, Dr. George Battaglia, Dr. Mark Brownfield and Dr. William Simmons. A retrospective thank-you to Dr. Michael Mangiapane, formerly of the University of Rochester, for not letting me give up. I would like to extend my appreciation to the Arthur J. Schmitt Foundation for their generosity and support.

The experiments conducted for this dissertation involved teamwork by many people. Thank-you for all your help, Joe Yracheta, Kayoko Kunimoto, Erica Bakkum, Qian Li and Andrew Levy. And, to Dr. Louis Van de Kar, who takes compliments about as well as I give them, you have become not only my mentor, but my friend. I seriously doubt that any advisor can train a student about the business of being a scientist better than Dr. Van de Kar, yet still believe that if science isn't fun, you're in the wrong line of work. Thanks Loek. Finally, thanks to my parents, Louise and George Rittenhouse, and my sisters Beth, Ann and Amy, for fostering such a stimulating and vigorous environment growing up. Always try for the best!

DEDICATION

To Denise, thanks for waiting. I'll be home soon.

VITA

The author was born on March 8, 1957 in Bryn Mawr, Pennsylvania. He received his secondary education at The Haverford School, Haverford, Pennsylvania, graduating in 1975. His college education was at Middlebury College, Middlebury, Vermont where he received a Bachelor of Arts degree in biopsychology in 1979.

A basic science fellowship was awarded upon matriculation to the Department of Pharmacology, Loyola University Chicago in 1987. In May 1988 he entered the laboratory of Dr. Louis Van de Kar, and an Arthur J. Schmitt Fellowship was awarded in 1991. The author is a student member of the Society for Neuroscience and the American Society of Pharmacology and Experimental Therapeutics. He has presented papers at five national meetings while a graduate student at Loyola, and is author or coauthor on seventeen publications. A post-doctoral position has been arranged with Dr. Susan Leeman at Boston University.

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LIST OF ABBREVIATIONS

1.	ANG I	angiotensin I
2.	ANG II	angiotensin II
3.	ANOVA	analysis of variance
4.	ANP	atrial natriuretic peptide
5.	BP	blood pressure
6.	cAMP	cyclic adenosine monophosphate
7.	cGMP	cyclic guanine monophosphate
8.	CNS	central nervous system
9.	5,7-DHT	5,7-dihydroxytryptamine, a 5-HT selective neurotoxin
10.	DMN	dorsomedial nucleus of the hypothalamus
11.	DOI	(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl
12.	EEDQ	1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
13.	EDTA	ethylene diamine tetra acidic acid
14.	HR	heart rate
15.	5-HT	5-hydroxytryptamine, serotonin
16.	ICV	intracerebroventricular
17.	ia	intra-arterial
18.	ip	intraperitoneal
19.	iv	intravenous
20.	LY53857	6-methyl-1-[1-methylethyl]ergoline-8-carboxylic acid
21.	NMDA	N-methyl-d-aspartate
22.	8-OH-DPAT	8-hydroxy-2-(di-N-propylamino)tetralin
23.	OVLT	organum vasculosum of the lamina terminalis
24.	PCA	*p*-chloroamphetamine

25.	PCPA	*p*-chlorophenylalanine
26.	PRA	plasma renin activity
27.	PRC	plasma renin concentration
28.	PVN	paraventricular nucleus of the hypothalamus
29.	RIA	radioimmunoassay
30.	RU 24969	5-methoxy-3-(1,2,3,4-tetrahydro-4-pyridinyl)-1H-indole
31.	sc	subcutaneously
32.	SHR	spontaneously hypertensive rat
33.	VMN	ventromedial nucleus of the hypothalamus

DESCRIPTION OF DRUGS

1. DOI ..... a 5-HT_{1C}/5-HT_2 agonist
2. Fluoxetine ..... a 5-HT uptake inhibitor
3. Ibotenic Acid ..... a cell-selective neurotoxin
4. LY53857 ..... a 5-HT_{1C}/5-HT_2 antagonist
5. *P*-chloroamphetamine (PCA) ..... a 5-HT releaser
6. Prazosin ..... an α_1 antagonist
7. Ritanserin ..... a 5-HT_{1C}/5-HT_2 antagonist
8. RU 24969 ..... a 5-HT_{1A}/5-HT_{1B} agonist
9. Spiperone ..... a 5-HT_{1A}/5-HT_2/D_2 antagonist
10. Xylamidine ..... a peripheral 5-HT_2 antagonist

CHAPTER I

INTRODUCTION

The objective of this dissertation was to investigate the location of serotonergic receptors mediating renin release. Serotonin agonists have been shown to increase plasma renin activity (PRA) and plasma renin concentration (PRC) when injected peripherally (Van de Kar, 1991). The pathway and location of the receptors involved in this endocrine control remain unclear.

The overall function of the kidney is to maintain body fluid homeostasis. This encompasses maintaining blood pressure, blood volume and electrolyte balance. In this way, the kidney is the ultimate controller of the body's blood pressure set-point (Guyton et al., 1972). The renin-angiotensin system is a vital component of the kidney's regulatory mechanisms. The active product of this cascade is the peptide angiotensin II. Angiotensin II is a potent vasoconstrictor, stimulates aldosterone release from the adrenal gland, and activates receptors in the brain to induce thirst. The control of renin secretion from the kidney is a complex array of interconnected mechanisms including: renal stretch receptors which sense blood pressure changes, the macula densa which senses sodium changes, the sympathetic nervous system, and many hormones (Hackenthal et al., 1990). The primary regulatory hormone is angiotensin

II, which feeds back to the kidney to inhibit renin release (Vallotton, 1987).

Serotonin (5-HT) is found predominately in three locations, the gut, circulating platelets, and the brain (Grahame-Smith, 1988). Established as a neurotransmitter since the mid 1950's, 5-HT is now known to influence the secretion of many hormones including: ACTH and corticosterone (Fuller, 1990), oxytocin (Saydoff et al., 1991), prolactin (Van de Kar and Bethea, 1982) and vasopressin (Brownfield et al., 1988). That renin may also be regulated by 5-HT was determined in the early 1980's (Zimmerman and Ganong, 1980; Van de Kar et al., 1981).

Serotonin pharmacology has seen an outburst of drug development since Peroutka and Snyder (1979) described multiple families of 5-HT receptors in the brain. Currently, conclusive evidence exists for 5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_{1C}, 5-HT_2 and 5-HT_3 receptor subtypes (Frazer et al., 1990). Several studies suggest that renin is regulated through 5-HT_2 receptors (Alper, 1990a; Van de Kar et al., 1989; Bagdy et al., 1992). In attempting to answer the questions posed by this dissertation, many pharmacological "tools" were utilized. The important serotonergic drugs employed were the 5-HT_{1A}/5-HT_{1B} agonist RU 24969, the 5-HT_{1C}/5-HT_2 agonist DOI, and the 5-HT releaser *p*-chloroamphetamine (PCA). A listing of all the drugs used in these studies can be found on page xi.

A debate exists concerning two issues. One, whether 5-HT agonists act at peripheral (Alper and Snider, 1987) or central sites (Karteszi et al., 1982; Van de Kar et al., 1982) to stimulate renin release. Two, if centrally controlled, whether the 5-HT receptors are located in either the paraventricular (PVN) or ventromedial nucleus (VMN) of the hypothalamus

(Gotoh et al., 1987; 1988). The specific aims of this dissertation attempted to resolve these two questions.

First, to establish if the renin releasing effect of 5-HT agonists has a central site of action, the 5-HT agonists DOI, RU 24969 and the 5-HT releaser PCA were injected through cannulae into the lateral cerebral ventricles of conscious rats. A dose-response experiment was performed with each drug. The receptor mediating renin release appears to be of the 5-HT_2 subtype. Therefore, to test whether a 5-HT_2 antagonist could block the effect of the 5-HT agonist, a 5-HT_2 antagonist was pre-injected through the same cannulae.

An experiment to determine if peripheral receptors contribute to the renin releasing effects of 5-HT agonists was also performed. A 5-HT_2 antagonist was injected through cannulae into the lateral ventricles of the brain, followed by peripheral administration of a 5-HT agonist. In addition, the effects of each 5-HT agonist on blood pressure and heart rate were determined in separate control experiments.

Second, once a central site was established, experiments were conducted to determine where in the hypothalamus 5-HT receptors were located that mediate the release of renin. Chemical lesions were made by injecting the neurotoxin ibotenic acid into the PVN or VMN. Ibotenic acid lesions were also made in the dorsomedial nucleus which served as a hypothalamic control site. Ibotenic acid destroys cell bodies while sparing axons which pass through the injection site. PRA and PRC were measured following intraperitoneal (ip) administration of RU 24969 or PCA.

The ramifications of these studies could influence how we view renin regulation, and the important contribution the brain has in this process.

Furthermore, these studies could lead to development of anti-hypertensive agents designed for specific sites and receptor targets.

The hypothesis of this dissertation is that cell bodies in the hypothalamic paraventricular nucleus contain 5-HT receptors that stimulate renin secretin. The results obtained from these studies suggest that neurons located in the PVN are indeed necessary to mediate renin release induced by a 5-HT agonist, and a 5-HT releaser. The receptors involved in this function are likely serotonergic. The mechanism whereby the PVN communicates with the kidney remains unresolved.

The body of the dissertation is organized as three papers, either published (chapter 3) or submitted for publication (chapters 4 and 5). The text of these chapters has remained unaltered from the form they were in when submitted for publication. A general discussion follows these chapters.

CHAPTER II

REVIEW OF RELATED LITERATURE

This dissertation was designed to investigate the influence of brain serotonergic neurons on the renin-angiotensin-system. The long-term goal of this project is to provide additional information on the role of 5-HT and renin in the etiology of hypertension. Background information is divided into two sections: literature related to the renin-angiotensin system, and literature related to serotonin.

The Renin-Angiotensin System

The Renin-Angiotensin-Aldosterone System

History. The discovery of renin occurred in 1897 when Tigerstedt and Bergman injected renal extracts into rabbits and produced a pressor response. This idea was not extended until 1934 when Goldblatt generated "renal" hypertension by occluding the renal arteries, thus demonstrating the connection between renin and high blood pressure (Keeton and Campbell, 1980). By 1940, Page recognized that renin was an enzyme which produced an even more potent pressor substance, termed "angiotonin". Nearly simultaneously, Braun-Menéndez discovered the peptide "hypertensin" generated from renin incubated with plasma. The name angiotensin was

agreed upon in 1957 by consensus (Keeton and Campbell, 1980). Tobian (1959) developed the hypothesis of a renal "stretch", or baroreceptor, that was sensitive to changes in blood pressure and blood volume. Thus began the pursuit of how sodium and blood volume were related to the development of hypertension. The macula densa hypothesis that changes in electrolytes could be "sensed" by the kidney was advanced by Vander (1965).

The renin-angiotensin system (RAS) is a unique enzymatic-hormonal cascade that can be stimulated by hypotension (10 to 20 mm Hg below normal), fluid loss caused by hemorrhage, diarrhea or vomiting, and low sodium diet. Thus, there appear to be four major mechanisms controlling renin secretion: 1. hemodynamic or renal perfusion pressure, mediated by stretch receptors, 2. neurogenic, mediated by sympathetic adrenergic (β and/or α) receptors, 3. sodium load, mediated by the macula densa, and 4. hormonal, mediated by a multitude of substances including ANG II, prostaglandins, adenosine, vasopressin, atrial natriuretic peptide and other peptides and ions. Renin is thus highly regulated by numerous integrated factors discussed more fully below (Vallotton, 1987; Dzau and Pratt, 1986; Keeton and Campbell, 1980).

Renin is the rate limiting enzyme in the sequence producing the active octapeptide angiotensin II (ANG II). Renin is a 40,000 dalton aspartyl protease secreted by the juxtaglomerular cells located along the afferent arterioles of the kidney. Renin has high substrate specificity, with only one known substrate (Hackenthal et al., 1990).

Renin substrate, angiotensinogen (60-65 kDa), is secreted in relatively constant amounts by the liver. Angiotensinogen concentration

in plasma is normally below the Km of the renin-angiotensinogen reaction (Dzau and Pratt, 1986). ANG II feeds back to increase the synthesis of angiotensinogen mRNA (Nakamura et al., 1990). Some evidence exists suggesting that the hypothalamus may be involved in angiotensinogen regulation (Kjos et al., 1991). A ten amino acid peptide with little intrinsic activity of its own, angiotensin I, is cleaved by renin from this substrate. The production of angiotensin I is measured in plasma renin activity (PRA) and plasma renin concentration (PRC) assays.

The ubiquitous endothelium-bound converting enzyme then transforms angiotensin I to ANG II, by cleaving off an additional two amino acids. Converting enzyme, a peptidyl dipeptidase, is found throughout the vasculature, including the kidney and liver, with highest concentrations lining capillary endothelium of the lungs (Vallotton, 1987). Unlike renin, converting enzyme lacks substrate specificity. Converting enzyme plays a dual role as kininase II, which is the enzyme responsible for the inactivation of bradykinin, a potent vasodilator (Vallotton, 1987).

Because renin and its substrate are blood borne, the formation of ANG II can occur anywhere along the vasculature where converting enzyme is located. Also, since ANG II is blood borne, its targets are widely located. ANG II receptors have been identified in targets reflecting its diverse actions: the adrenal (zona glomerulosa), vascular smooth muscle, uterus, bladder, glomeruli, platelets, pituitary, and brain (Dzau and Pratt, 1986). Two ANG II receptor subtypes have recently been characterized, defined as AT_1 and AT_2 (Whitebread et al., 1989; Chiu et al., 1989). AT_1 receptors are coupled to G regulatory proteins, stimulate phosphoinositol turnover, and have been localized in the brain (Tsutsumi

and Saavedra, 1991). AT_2 receptors have not been thoroughly characterized. Binding to ANG II receptors is stereoselective and saturable (Dzau and Pratt, 1986).

The subsequent actions of ANG II include stimulation of the secretion of the Na^+-conserving mineralocorticoid hormone aldosterone from the adrenal cortex. Aldosterone acts at the distal tubule as the primary sodium conserving hormone in the body. ANG II is the principal regulator of aldosterone secretion. ANG II stimulates pressor pathways located in the brain (Hartle and Brody, 1984). Receptors for blood borne ANG II have been identified with autoradiography in the subfornical organ, median eminence, organum vasculosum of the lamina terminalis (OVLT) and the area postrema (van Houten et al., 1980). These sites are circumventricular organs which lie outside the blood-brain barrier. Immunohistochemistry has stained for ANG II cell bodies in the magnocellular neurons of the hypothalamic PVN, as well as for fibers in the brainstem and limbic sites (Lind et al., 1985). ANG II also stimulates thirst through CNS receptors located in the subfornical organ. The drinking behavior induced by ANG II is prevented by the ANG II antagonist saralasin (Mangiapane and Simpson, 1980). Finally, ANG II is a powerful vasoconstrictor of arteriolar smooth muscle. Thus, these combined actions are vital for the maintenance of blood volume and fluid homeostasis.

The importance of the RAS in maintaining blood pressure is evidenced by the ability of both β-blockers such as propranolol, which inhibit renin secretion, and converting enzyme inhibitors such as captopril to effectively lower BP in hypertensive patients (Dzau and Pratt, 1986; Keeton and Campbell, 1980; Vallotton, 1992). The role of the RAS in

hypertension is discussed more fully below. The RAS does not appear to play as prominent a role in maintenance of normal blood pressure as does the sympathetic nervous system. Ganglionic blockade lowers normal pressure to a greater extent than RAS blockade. Thus, the sympathetic nervous system plays the primary role, while the RAS acts in more of a support capacity, becoming fully activated at pressures of approximately 80 to 90 mm Hg (Zimmerman et al., 1984). Some evidence suggests that during normal fluctuations in BP, the RAS may contribute substantially to normalizing the pressure, for example as occurs during orthostatic hypotension (Hackenthal et al., 1990).

A recent area of research has focused on localized tissue renin-angiotensin systems (Dzau and Pratt, 1986). The postulated locations include blood vessels, brain, adrenal, testis, heart and kidney. The actions of ANG II in the brain, as outlined above include stimulation of the sympathetic nervous system and blood pressure, increased release of vasopressin and ACTH, inducement of drinking behavior and elevated Na^+ appetite. While these responses can be elicited by either iv or intracerebroventricular (ICV) administered ANG II, the variety of responses suggests the presence of a local synthetic pathway (Reid, 1984; Mendelsohn et al., 1990). Initial evidence for a brain RAS developed when binding sites for ANG II (Castren and Saavedra, 1988) and cells containing ANG II (Healy and Printz, 1984) were observed in the PVN, clearly a structure within the blood brain barrier. However, whole brain angiotensinogen mRNA levels are substantially higher than renin mRNA levels (Dzau et al., 1986). While the idea of a brain RAS is provocative, uneven and nonoverlapping distributions of the brain RAS components have

caused skepticism (Brownfield et al., 1982; Moffett et al., 1987). A final possibility which circumvents the low brain levels of renin mRNA is that proposed by Ferrario et al. (1990), who suggested that angiotensin (1-7) is produced in the brain by an alternate enzymatic pathway. Angiotensin (1-7) produces an increase in vasopressin release, but not dipsogenic or vasoconstrictor activity.

The RAS can respond within minutes to a sudden change in arterial pressure, while the aldosterone response to pressure changes may take hours to occur. The RAS is a relatively short-term pressure regulator. In normal kidneys, the renin-angiotensin system prevents wide fluctuations in arterial pressure, despite tremendous changes in salt intake (Guyton, 1990).

Long-term arterial pressure control is maintained by a renal-body fluid feedback mechanism. This means that as mean arterial pressure rises, the kidney will continue to excrete water and Na^+ until the pressure is returned back to the original level or "set-point". It is thus considered to have "infinite gain" since the kidney will eventually return any pressure back to normal (Guyton, 1972). A large volume increase is necessary to elevate blood pressure acutely, while a small volume is needed to chronically raise pressure (Guyton, 1990).

A pathological elevation in angiotensin results in Na^+ and water retention. The increased Na^+ concentration and fluid volume lead to increased arterial pressure. The resulting pressure diuresis maintains Na^+ and body fluid balance (Hall et al., 1986). Thus if an animal is experimentally salt loaded, and angiotensin levels are maintained, hypertension will develop. How could renin stay elevated chronically?

First, by low renal blood flow (such as produced by a stenosis), and second by factors affecting water and electrolytes beyond the macula densa, presumably in the caudal distal tubule (Guyton, 1990). In essential hypertension, the output of the kidneys is normal, in the face of arterial pressures that are abnormally high. The reason for this abnormal renal function is completely unknown.

Renin-Angiotensin and Hypertension

Hypertension is defined as any pressure above a systolic of 140 mm Hg, or a diastolic of 90 mm Hg. 60 million people suffer from high blood pressure (Ferrario, 1990). The etiology of pathogenic hypertension remains unknown for the majority of cases, termed essential hypertension. Due to the complex regulatory mechanisms involved in maintenance of arterial pressure (RAS, sympathetic nervous system, baroreceptors, chemoreceptors, renal fluid volume) it is very likely that many factors will have to be considered as causative. Thus, hypertension may be considered a family of diseases.

It is not surprising then, to discover that the sodium-renin profile of patients suffering from essential hypertension covers a wide spectrum. A certain percent have low renin values, which would be expected with elevated blood pressure. But 30% have high renin values, which is unexpected. In addition, 30% have "normal" renin values, which in the face of abnormally high blood pressure, may not be normal after all (Laragh, 1978). Experimentally, this corresponds to a rat model termed 2-kidney, 1-clip, where a silver clip is placed on one renal artery, inducing an unnaturally low pressure in the clipped kidney. This kidney

produces excessive amounts of renin, although sodium and water excretion remain normal, since the remaining kidney is perfectly functional. In this model, converting enzyme inhibitors are highly effective at lowering the induced hypertension caused by the elevated plasma renin levels (Laragh, 1978). Clinically, this model could represent a stenotically injured renal artery, or other vascular injury to the kidney. A recent report has now shown that high renin-sodium profiles correlate significantly with future risk of heart attack (Alderman et al., 1991).

If more than one factor is necessary for the development of hypertension, it is likely that more than one gene is involved. In Dahl salt-sensitive rats and spontaneously hypertensive rats (SHR) at least four independently segregating loci have been estimated to contribute to their hypertension (Morris, 1991). It has also been shown that restriction fragment length polymorphism marking the renin gene of the SHR was associated with their elevated blood pressure, suggesting that a structural defect in the renin gene, or a closely linked gene may be responsible for producing hypertension in SHR (Kurtz et al., 1990).

The contribution of the renin-angiotensin system to the pathogenesis of some forms of hypertension is now well documented (Hall et al., 1986; Laragh, 1978; Shibutani et al., 1988; Waeber et al., 1986). In rats, there is evidence that chronic angiotensin infusion and other manipulations which increase plasma renin activity cause hypertension (Reid, 1984). The onset of several forms of renin-dependent hypertension is dependent on structures in the brain, primarily the hypothalamus (Brody, 1988). PVN ablation in young SHR attenuated the normal development of their hypertension (Ciriello et al., 1984).

That the RAS contributes to maintaining hypertension is also evidenced by the effectiveness of intervening at different points of the system: renin, converting enzyme or ANG II. Direct inhibition of renin has two important advantages. Renin is the rate limiting enzyme and is substrate specific. Unfortunately, drug developers have yet to design an orally effective renin inhibitor (Corvol et al., 1990; Weber et al., 1990). A peptide analog of ANG II, saralasin, has been developed that directly antagonizes ANG II receptors. However, it suffers from the same disadvantage as renin inhibitors: lack of oral activity. Both saralasin and renin inhibitors are effective depressor agents when administered iv. By far the most efficacious RAS antagonists are the converting enzyme inhibitors, such as captopril and enalapril, which are extensively used as treatments for essential hypertension (Waeber et al., 1986; Laragh, 1978).

Considerable attention has been given to the possible contributions tissue RAS may have in producing hypertension. Thus, paracrine or autocrine actions of ANG II may be important in cardiovascular regulation. For example, adrenal RAS has been found to augment aldosterone release in stroke prone SHR (Kim et al., 1991). Local generation of cardiac ANG II aggravated arrhythmias (Becker et al., 1989; Hirsch et al., 1990). The existence of a putative brain RAS, has spurred intensive research on the role it may play in the pathogenesis of hypertension (Ferrario, 1990). The ventrolateral medulla may be a central site of action for ANG II regulation of blood pressure. ANG II was found to have a depressor action if injected into the caudal ventrolateral medulla, while injection of ANG II into the rostral ventrolateral medulla produced a pressor response (Sasaki and Dampney, 1990).

However, as discussed above, ANG II receptors have been identified in the circumventricular organs which lie outside the blood brain barrier, yet maintain synaptic connections with cardiovascular regulatory centers. Thus the subfornical organ, OVLT, and the area postrema may be mediating the effects of blood borne ANG II produced systemically (Brody, 1988). Electrolytic lesions in each of these structures produces a decrease of blood pressure in experimental hypertension (Moffett et al., 1987; Hartle and Brody, 1984).

The pathogenesis of essential hypertension remains unknown, and a continued source of intensive investigations. From the brief review presented here, it is clear that the RAS is an important component of normal arterial pressure regulation. It would not be surprising if an abnormality of the RAS proved to be a consequential factor either for the development or maintenance of some forms of essential hypertension.

Renin Regulation

Granulated renin secreting cells are found throughout the kidney vasculature, with highest concentrations in the cells proximal to the glomerulus (Hackenthal et al., 1990). These juxtaglomerular cells are actually metaplastically transformed arteriolar smooth muscle cells, having reduced myofilaments (Churchill, 1988; Kurtz, 1990). If rats are chronically treated with converting enzyme inhibitors or salt deprived, it was found that additional smooth muscle cells are recruited to become renin secreting cells, as evidenced by elevated renin mRNA levels (Gomez et al., 1990). PCR (polymerase chain reaction) techniques have enabled a rough comparison of renin mRNA in various tissues. The conclusion reached

at this point is that there is relatively little renin mRNA in any tissue other than the kidney. For example, the adrenal had 0.3 to 1%, the heart and aorta 0.2%, the hypothalamus 0.1%, the liver 0.1% and the pituitary 0.01% of that found in the kidney (Lou et al., 1991; Okura et al., 1991). However, these minute amounts could still be the minimum necessary for a functional autocrine or paracrine role in local RAS's (Hackenthal et al., 1990).

Synthesis of renin follows a standard pathway for secretory substances. Renin mRNA is translated into preprorenin, then glycosylated on the Golgi apparatus to form prorenin and packaged into granules where it undergoes final activation to renin (Vallotton, 1987). Renin gene expression is regulated, rather than being tonically turned on or off in a constitutive manner. There are excess renin stores in the kidney for several days. As discussed briefly in the RAS section, renin release is controlled by four primary mechanisms. These are: 1. stretch, 2. Na^+ load, 3. hormones, and 4. the sympathetic nervous system. Each of these will be examined individually.

Stretch receptors. Renin secretion can be stimulated by a fall in blood volume or blood pressure. Hodge et al. (1966) discovered that a decrease in blood volume due to hemorrhage could cause ANG II elevations, independent of BP. Decreased renal perfusion pressure produces a decrease in internal Ca^{++} via prostacyclin (PGI_2) and cAMP (Jackson, 1991). When renal blood pressure or blood volume are elevated, it is presumed that renal "stretch" receptors are activated, which cause an increase in Ca^{++} influx, resulting in renin inhibition (Kurtz et al., 1990). Although still under investigation, the putative renal "stretch" receptor may

actually be the juxtaglomerular cell itself. No other site has been proposed, and since the juxtaglomerular cells are transformed smooth muscle cells, it is easy to envision a transduction mechanism involving myofilaments responsive to distension.

Macula densa mechanism. Renin secretion can be stimulated by reduced levels of Na^+ sensed by the macula densa cells located on the distal tubule, adjacent to the afferent arteriole and glomerulus. This close proximity originally led investigators to propose that these cells could act as chemical sensors (Vander, 1965). Only a 10% drop in plasma Na^+ can result in a 400% increase of renin secretion. In contrast, a 10% increase in plasma Na^+ causes just a 60% reduction in renin (Kurtz et al., 1990).

The actual signal transmitted from the macula densa to the renin secreting juxtaglomerular cells remains subject to debate. Two distinct theories exist to explain the phenomenon of tubuloglomerular feedback. The first suggests that ionic changes in the interstitial medium between the macula densa and juxtaglomerular cells are "sensed" by cell surface receptors on the juxtaglomerular cells. In this theory, either Na^+, Cl^- or both are the chemical signal (Navar et al., 1991; Persson et al., 1991). Most evidence favors Cl^- as the actual signal to inhibit renin secretion (Hackenthal et al., 1990). The second theory proposes that energy dependent uptake of Na^+ releases adenosine as a metabolic product from the macula densa. This excess of adenosine activates high affinity A_1 receptors on the juxtaglomerular cells to increase the internal Ca^{++} concentration, and thereby inhibit renin secretion (Jackson, 1991).

Hormones. There are a number of hormones capable of either increasing or decreasing renin release. The primary hormone influencing renin is the

final product of the synthetic pathway, ANG II. ANG II tonically inhibits renin secretion via three negative feedback loops: a) directly to the juxtaglomerular cells, b) through vasoconstriction, ANG II elevates blood pressure, which is an inhibitory signal, and c) ANG II stimulates aldosterone to conserve Na^+, which is also an inhibitory signal (Kurtz, 1990). Chronic ANG II infusion results in a decrease in renin mRNA levels demonstrating regulation of renin gene expression (Johns et al., 1990). As with the other hormonal messengers, ANG II produces an influx of Ca^{++}, which functions as the inhibitory second messenger.

Other hormonal substances can also alter renin secretion. Adenosine is known to inhibit renin secretion and may mediate signals from the macula densa, as described above, in an autocrine fashion (Jackson, 1991). Atrial natriuretic peptide (ANP) produces vasodilation, increases Na^+ excretion, increases glomerular filtration rate and inhibits renin secretion (Atlas et al., 1986). However, it is probably not as important an influence as originally expected, since physiologic doses of ANP did not alter arterial pressure or PRA (Kivlighn et al., 1990; Hackenthal et al., 1990). Vasopressin has an inhibitory influence on renin secretion, but is not a primary regulatory hormone (Hackenthal et al., 1990). Each of these hormones decreases renin release by raising intracellular levels of Ca^{++} concentration.

Sympathetic nervous system. While the signals discussed thus far are predominantly negative, increased sympathetic nerve activity will raise plasma renin levels. This is done predominantly through norepinephrine release and activation of renal β_1 receptors (Hackenthal et al., 1990). However, some evidence also suggests a positive involvement of renal α

receptors and extrarenal β receptors (Blair et al., 1991).

Besides the sympathetic nervous system, other forms of central regulation of renin release have been investigated. Ueda et al. (1967) were the first to stimulate the brain and see an increase in renin release. In their studies, electrodes were placed in the midbrain, just dorsal to the central gray, and PRA increased even in renal denervated dogs. Thus, factors in the brain other than the sympathetic nerves are capable of elevating renin release. Lesions in the PVN with a selective catecholamine neurotoxin inhibited stress-induced elevations of PRA and PRC (Richardson Morton et al., 1990). Histamine antagonists injected into the ventricles of the brain inhibited restraint-induced increases in PRA, while injection of histamine ICV increased PRA (Matzen et al., 1990). Electrical stimulation of different brain sites can cause renin release. Stimulation of the PVN (Porter, 1988) and ventrolateral medulla, as well as lesions in the anteroventral third ventricular (so called AV3V) region result in elevated PRA (Brosnihan and Ferrario, 1984). Electrolytic lesions in nuclei within the hypothalamus result in blunting of the renin response to a variety of stimuli, including low Na^+ diet, head-up tilt, immobilization and PCA (Gotoh et al., 1987; 1988). In addition, there is evidence for a blood borne renin releasing factor produced by the hypothalamus (Urban et al., 1985). Hypothalamic extracts significantly elevated renin secretion from *in vitro* kidney slices (Van de Kar et al., 1987). These studies suggest that structures within the brain may have a prominent influence on renin regulation. As previously mentioned, the circumventricular organs which lie outside the blood brain barrier, are heavily labeled autoradiographically with [^{125}I]ANG II or its antagonist

[^{125}I]saralasin (van Houten et al., 1980; Mendelsohn et al., 1990). If the brain does have an important role in renin regulation, a necessary form of communication from the kidney back to the brain should exist. Since the circumventricular organs contain ANG II receptors, they are in an ideal position to monitor this feedback control. Furthermore, the area postrema, subfornical organ and OVLT maintain direct connections with the PVN, a likely integrator of renin regulation within the hypothalamus (Lind et al, 1985; Van de Kar et al., 1990).

The exocytotic mechanism of renin secretion is still unclear. In normal smooth muscle cells, an increase in intracellular calcium causes an increase in contraction. In other endocrine or transmitter secreting cells, release is also dependent on calcium entry. However, in juxtaglomerular cells a decrease in internal Ca^{++} concentration stimulates renin release. This inverse relationship with Ca^{++} has become known as the Ca^{++} paradox. It has been postulated that all signals regulating renin secretion do so by either increasing or decreasing intracellular Ca^{++} levels (Fray, 1990). For example, a decrease in extracellular Ca^{++} in isolated perfused kidneys reduces renin secretion. Conversely, elevated renal perfusion pressure causes an increase in stretch and an increase in Ca^{++} influx, causing a decrease in renin secretion (Fray, 1990). Decreases in intracellular Ca^{++} have been shown to cause renin secretion, independent of cAMP changes (Pardy et al., 1989). Cell surface receptors are linked through stimulatory (G_s) or inhibitory (G_i) G-proteins to adenylate cyclase or to phospholipase C. In juxtaglomerular cells, cAMP acts as a stimulatory second messenger, while cGMP acts as an inhibitory second messenger (Della Bruna et al., 1991). How these second messengers relay

their stimulatory or inhibitory signals to renin containing granules remains a mystery.

In summary, stimuli that augment renin secretion appear to do so by increasing intracellular levels of cAMP. These are norepinephrine from sympathetic terminals or epinephrine from the adrenal which activate β_1 receptors and cAMP; a decrease in perfusion pressure stimulating prostacyclin and elevating cAMP; and a decrease in Na^+ at the macula densa, which also activates prostacyclin and cAMP (Jackson, 1991). Factors which inhibit renin secretion may all act to increase internal Ca^{++} levels in the juxtaglomerular cells. These signals include the hormones ANG II, vasopressin, atrial natriuretic peptide, adenosine, and increases in plasma volume, arterial blood pressure and distal tubule sodium load (Fray, 1990).

Serotonin

Serotonin Pharmacology

History. 5-Hydroxytryptamine (5-HT) was discovered by Page as a vasoconstrictive substance in serum, derived from platelets, and thus the name serotonin (Rapport et al., 1947). In Italy, Erspamer had identified a vasoconstrictive substance derived from enterochromaffin cells of the GI tract he termed "enteramine", which was soon recognized to be identical to serotonin. By 1953, Page had detected 5-HT in the brain, so that by the early 1950's serotonin was isolated from the three major depots in the body: the gut, platelets, and the brain (Sjoerdsma and Palfreyman, 1990). Early pharmacological investigations concentrated on possible antihypertensive agents, but soon moved towards emotional disorders such as depression and anxiety when the psychoactive ergot LSD was recognized to have a structure containing a serotonin backbone. The efficacy of tricyclic antidepressants and 5-HT uptake inhibitors developed during the early 1970's, instigated the formulation of the serotonergic hypothesis of depression (Van de Kar, 1989).

Biochemistry. The precursor for 5-HT is the essential amino acid L-tryptophan, the availability of which is usually rate limiting. The biosynthesis of 5-HT involves only two enzymes, the first of which is rate limiting. Tryptophan hydroxylase generates 5-hydroxytryptophan, which is decarboxylated by L-aromatic amino acid decarboxylase, to produce the final product 5-hydroxytryptamine. Storage of 5-HT occurs in granules of all cells which secrete 5-HT. Release of 5-HT is a Ca^{++} dependent process. Once released, the primary mode of inactivation is by a presynaptic high

affinity reuptake transporter specific for 5-HT. 5-HT is degraded by monoamine oxidase and aldehyde dehydrogenase to produce the metabolite 5-hydroxyindole acetic acid (Grahame-Smith, 1988). Enterochromaffin cells of the gut contain greater than 90% of the body's 5-HT. Platelets do not synthesize 5-HT, but rather utilize the active uptake mechanism. Substance P often coexists with 5-HT in brain and gut 5-HT granules, although the function of this is not yet known (Tyce, 1990). Other peptides and their receptors have been identified in the raphé nuclei, including CCK (cholecystokinin), TRH (thyrotropin releasing hormone), neurokinin A, and enkaphalin, although their functions are currently unknown (Paris and Lorens, 1989; Boden et al., 1991; Cooper et al., 1991).

Receptor types. Two peripheral receptors were initially characterized based on differential agonist potencies, and termed "M" and "D" receptors. D receptors contracted smooth muscles, while M receptors depolarized cholinergic nerves (Grahame-Smith, 1988). A seminal report in 1979 by Peroutka and Snyder categorized brain 5-HT receptors as belonging to two major "families", the 5-HT_1 or 5-HT_2 subtypes. This classification was based, by definition, on high affinity (nanomolar) binding of 5-HT to 5-HT_1 receptors, and low affinity (micromolar) of 5-HT binding to 5-HT_2 receptors (Hamon et al., 1990). High affinity agonists were quickly developed for 5-HT_1 receptors and high affinity antagonists developed for 5-HT_2 receptors. Other receptor subtypes were rapidly characterized so that currently there are six well recognized receptors: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2 and 5-HT_3 (Frazer et al., 1990). Binding studies have revealed potentially a 5-HT_{1E}, and a 5-HT_4 receptor subtype (Hamon et al., 1990; Bockaert et al., 1990). The previously identified "D" receptor

appears to correspond to the 5-HT_2 receptor, while the "M" receptor was renamed the 5-HT_3 receptor (Frazer et al., 1990).

Amino acid sequences, G-protein linkage, and second messengers are being characterized for each of the 5-HT receptors using molecular biological techniques (Hartig et al., 1990). Clones have been produced for 5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_{1C} and 5-HT_2 subtypes, revealing each to contain 7 membrane spanning domains routinely seen with G-protein coupled receptors. Interestingly, the sequence homology between the 5-HT_{1C} and 5-HT_2 subtypes is greater than 70%, suggesting they are actually members of the same family. These two receptors also share the same G-protein stimulated second messenger system, namely phosphoinositol turnover (Conn and Sanders-Bush, 1987). The remaining 5-HT_1 receptors are all coupled via G_i, to adenylate cyclase. Evidence suggests that 5-HT_3 receptors are part of an ion channel (Aghajanian et al., 1990).

Anatomy. 5-HT neuronal cell bodies were first localized to the midbrain using histofluorescence (Dahlström and Fuxe, 1965). These were grouped into nine clusters of cells, labeled B1 through B9. These can easily be divided into two major groups, caudal (comprising B1-B3) and rostral (comprising B4-B9). Immunohistochemical use of 5-HT antibodies allowed for detailed mapping (Tork, 1990). The caudal cell groups send most of their projections to the spinal cord, while the middle cell groups (B4-B6) send diffuse projections throughout the brainstem and cerebellum. The more rostral nuclei include the median (B8) and dorsal raphé (B7), which send projections to cortical areas containing limbic structures (amygdala and hippocampus), as well as the hypothalamus (Azmitia and Segal, 1978).

Recent advances in autoradiography have enabled the precise localization of the different receptor subtypes in various brain regions (Palacios et al., 1990; Radja et al., 1991). 5-HT_{1A} receptors are found in the neocortex and the limbic structures, hippocampus, septum, amygdala and hypothalamus (Pazos and Palacios, 1985). 5-HT_{1A} autoreceptors are located on dorsal raphé cell bodies (somato-dendritic receptors, Göthert and Schlicker, 1990). The 5-HT_{1B} and 5-HT_{1D} receptors can be localized to basal ganglia structures such as the striatum, substantia nigra, and pallidum. These receptors are ostensibly the same receptor, although there is a species differentiation, with the 5-HT_{1B} subtype appearing in mice and rats, and the 5-HT_{1D} subtype appearing in humans and all other species studied thus far (Waeber et al., 1989). Some evidence suggests that this subtype is a presynaptic autoreceptor (Göthert and Schlicker, 1990), although the evidence is not conclusive. The 5-HT_{1C} receptor has been found in high concentrations in the choroid plexus, where it is believed to regulate cerebral spinal fluid volume and composition (Pazos and Palacios, 1985). The 5-HT_2 receptor has been visualized in the frontal cortex, nucleus accumbens, and the hypothalamus (Pazos et al., 1985). Peripherally, 5-HT_2 receptors have been localized to smooth muscle and platelets (Leysen and Pauwels, 1990). 5-HT_3 receptors have only just been found in the brainstem in such places as the area postrema, where they may link the periphery with the brain (Cooper and Abbott, 1988).

Functions. An early description of behavioral effects attributed to 5-HT is known as the "serotonin syndrome". In rats, it is characterized by hindlimb abduction and rigidity, forepaw treading, lateral headweaving and tremor (Frazer et al., 1990). Humans have experienced restlessness,

shivering and mental confusion (Sternbach, 1991). This hyperstimulation of the 5-HT system has been attributed to activation of 5-HT_{1A} receptors in the spinal cord.

5-HT is considered to play a role in a wide variety of behavioral and homeostatic functions: anxiety (Taylor, 1990; Chopin and Briley, 1987), depression (Meltzer, 1990), panic disorder (Den Boer and Westenberg, 1990), sex, sleep and thermoregulation (Glennon, 1987), obsessive-compulsive disorder (Murphy, 1990), appetite (Leibowitz, 1990), schizophrenia and hallucinations (Leysen and Pauwels, 1990; Glennon et al., 1982), migraine (Humphrey et al., 1990), pain (Sternbach, 1991), emesis (Cooper and Abbott, 1988), memory (McEntee and Crook, 1991) and as a growth factor (Seuwen and Pouyssegur, 1990). These functions are often associated with different receptor subtypes, which correlate with their anatomical location.

Many hormones have been found to be stimulated by serotonergic agents. The current status of neuroendocrine function and receptor subtype regulation consists of: ACTH and corticosterone with 5-HT_{1A} or 5-HT_{1C}/5-HT_2 (Fuller, 1990; Cowen et al., 1990); prolactin with 5-HT_{1B} or 5-HT_{1C}/5-HT_2 (Cowen et al., 1990; Van de Kar, 1991); oxytocin and vasopressin with 5-HT_{1C}/5-HT_2 (Saydoff et al., 1991; Brownfield et al., 1988). 5-HT receptor types regulating renin secretion are discussed fully below.

Drugs. This plethora of receptor subtypes has been utilized by drug developers to design an expanding array of agents, aimed at relieving different ailments attributed to a specific 5-HT receptor related dysfunction (Göthert and Schlicker, 1990; Sjoerdsma and Palfreyman, 1990). The 5-HT uptake inhibitor fluoxetine is widely marketed as an

antidepressant. The 5-HT_{1A} agonists buspirone and ipsapirone are used as anxyiolytics. The 5-HT_{1D} agonist sumatriptan is used in migraine therapy. 5-HT_2 antagonists may prove therapeutic for anxiety, schizophrenia and sleep disorders. Ondansetron and zacopride, 5-HT_3 antagonists, are established therapeutic antiemetics for cancer chemotherapy-induced vomiting. The list of potentially useful serotonergic drugs is as long as ailments attributed to 5-HT dysfunction. Clearly, investigation of the multiple roles that brain 5-HT plays is necessary for integrative understanding, and development of further drugs. For a complete list of the agents used in this dissertation study, please refer to page xi.

Importance of Serotonin in Cardiovascular Diseases

Serotonin, derived from platelets, was originally isolated because of its vasoconstrictor actions. However, central actions of 5-HT may prove to be more relevant physiologically in the pathogenesis and maintenance of hypertension.

Studies have shown that 5-HT may be an important component of CNS cardiovascular regulation (Mikulic et al., 1988; Ramage, 1988; Robinson et al., 1985; Smits et al., 1978). Direct injection of 5-HT into the anterior hypothalamus and electrical stimulation of the dorsal raphé produce acute pressor effects in both SHR and their normotensive controls, Wistar Kyoto rats (Wolf et al., 1981). Recent evidence suggests that 5-HT_2 receptors may be involved in BP regulation (Kushiro et al., 1988). 5-HT_2 receptors are located on vascular smooth muscle and platelets. Thus, 5-HT can augment or reduce blood flow depending on multiple variables including vascular bed, sympathetic tone, dose and route of administration (Van

Neuten et al., 1985).

Injection of 5-HT elicits complex changes in the cardiovascular system. A triphasic response pattern occurs, attributable to activation of different receptor subtypes in different locations. Initially, there is a depressor response thought to be due to immediate bradycardia and lowered cardiac output mediated by 5-HT_3 receptors. Subsequently, 5-HT_2 receptors are considered to produce an increase in total peripheral resistance, an increase in cardiac output and an increase in blood pressure. Finally, a long lasting depressor response occurs mediated by 5-HT_1 receptors (Saxena and Villalón, 1990; Dabiré et al., 1990). The 5-HT_1 depressor response is considered to be due to a central decrease in sympathetic nerve activity, an increase in vagal activity, a decrease in transmitter release from sympathetic terminals, and a decrease in peripheral resistance (Dabiré and Richer, 1991). 5-HT_4 receptors may have a direct inotropic effect on the heart (Saxena and Villalón, 1991). The overall response pattern of 5-HT agonists appears to be that 5-HT_{1A} agonists produce vasodilation and hypotension, while 5-HT_2 agonists are vasoconstrictors causing hypertension, but many mixed response patterns result from dose, route and site of administration.

There is some evidence that 5-HT may contribute to the development of hypertension in SHR. An increased availability of tryptophan produced SHRs with higher blood pressure (Kubo et al., 1990). Intra-arterially injected 5-HT augmented cerebral arterial vasoconstriction in stroke-prone SHR (Mayhan and Faraci, 1990). Presumably, this 5-HT would derive endogenously from platelets, and has implications for stroke and cerebral vascular diseases.

Stimulation of serotonergic neurons in the dorsal raphé nucleus and 5-HT receptive cells in the anterior hypothalamus increases blood pressure both in normotensive and hypertensive rats (Kuhn et al., 1980; Smits et al., 1978; Wolf et al., 1981). Direct injection of 5-HT_{1A} agonists 8-OH-DPAT and flesinoxan into the dorsal raphé produced hypotension and bradycardia (Connor and Higgins, 1990). Intracisternal administration of the serotonin selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) produced a 10 mm Hg drop in BP 10 days after treatment in rats (Mikulic et al., 1988). 5-HT turnover in the hypothalamic paraventricular and supraoptic nuclei and in the median eminence is elevated in SHR (Koulu et al., 1986a). In addition, 5-HT turnover is elevated in the raphé nuclei during the development of hypertension in young SHR (Koulu et al., 1986b). These studies suggest that 5-HT from the dorsal raphé may be involved in the pathogenesis of some forms of hypertension.

There appear to be opposing actions of 5-HT_{1A} and 5-HT_2 agonists on the ventral surface of the medulla. When applied to the ventral surface of the medulla, the 5-HT_{1A} agonists 8-OH-DPAT or urapidil dose-dependently cause hypotension and inhibit sympathetic nerve discharge in anesthetized cats (McCall et al., 1987; Gillis et al., 1989; McCall et al., 1989; Mandal et al., 1990b). However, the 5-HT_2 agonist DOI (±-1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane HCl) causes an increase in blood pressure, with no accompanying bradycardia, and an increase in sympathetic nerve discharge (McCall and Harris, 1988; Mandal et al., 1990a; King and Holtman, 1990). Finally, stimulation of cardiac vagal afferents specifically decrease 5-HT turnover in hypothalamic and brain stem nuclei during acute myocardial ischemia and after coronary artery occlusion (Sole

et al., 1978; 1983).

There are two possible mechanisms whereby 5-HT_{1A} agonists could lower blood pressure: either by 5-HT_{1A} autoreceptor inhibition of dorsal raphé neurons, or by 5-HT_{1A} postsynaptic inhibition of medullary sympathetic neurons. It may be that central 5-HT_{1A} receptors play a more important role than peripheral 5-HT_2 receptors in cardiovascular regulation. Increased vascular reactivity to 5-HT in hypertensives is considered more of a general phenomenon than specific for 5-HT. Although other 5-HT_2 antagonists such as ritanserin and LY53857 are ineffective as antihypertensive agents (Van Zwieten et al., 1990), the selective 5-HT_2 antagonist ketanserin is being used clinically as an anti-hypertensive drug (Van Heuven-Nolsen, 1988). Evidence now suggests that ketanserin is also a relatively potent α_1 antagonist (Koss, 1991), but additional antihypertensive action could be due to blocking 5-HT_2 receptors on vascular smooth muscle. Thus the interaction between platelet derived 5-HT and vascular 5-HT_2 receptors would be interrupted, reducing the vasoconstrictive action of 5-HT in the blood (De Clerck, 1991).

Therefore, the results of the experiments from this dissertation project may lead to further understanding of hypertension, development of more specific anti-hypertensive drugs, and an increased ability to manipulate the systems implicated in these disease states.

Receptor Subtype Mediating Renin Release

Our previous data suggest that activation of 5-HT_2 receptors increases renin secretion. The 5-HT_{1C}/5-HT_2 antagonist LY53857 blocked the increase in renin levels caused by injection (ip) of the nonselective 5-HT agonist

MK-212 and 5-HT releaser fenfluramine, while other selective 5-HT_{1A} agonists, ipsapirone and 8-OH-DPAT, elevated plasma corticosterone but not renin levels (Lorens and Van de Kar, 1987). The mixed 5-$HT_{1A/1B}$ agonist RU 24969 (ip) elevated PRA, PRC, ACTH, and plasma corticosterone and prolactin. Only the RU 24969-induced increases in PRA and PRC were inhibited with the 5-HT_2 antagonists LY53857 and ritanserin (Van de Kar et al., 1989), suggesting that RU 24969 may also interact with 5-HT_2 receptors. This differential control of hormonal release suggests that the serotonergic stimulation of renin release is mediated by 5-HT_2 receptors.

Some dispute exists concerning the separate identity of 5-HT_{1C} and 5-HT_2 receptors, since both are coupled through G proteins to phosphoinositide hydrolysis, and they display similar affinities for a variety of drugs (Hoyer, 1988). 5-HT_{1C} receptors are primarily located in choroid plexus (Conn and Sanders-Bush, 1987), although low levels of mRNA for the 5-HT_{1C} receptor have been identified in the hypothalamus (Mengod et al., 1990). 5-HT_2 receptors are found in the cerebral cortex and hypothalamus (Appel et al., 1990). Since the antagonist spiperone has a 100-fold greater affinity for 5-HT_2 receptors than 5-HT_{1C} receptors (Hoyer, 1988), it provides the best drug currently available to differentiate between 5-HT_{1C} and 5-HT_2 receptor mediated renin secretion.

Other studies have also suggested that renin secretion is mediated via 5-HT_2 receptors. The nonselective 5-HT agonist quipazine injected iv increased PRA in a dose-dependent manner, which was blocked by 5-HT_2 antagonists (Alper and Snider, 1987). However, it was determined that this increase in PRA may have been secondary to a decrease in renal blood

flow (Zink et al., 1990). The 5-HT_{1C}/5-HT_2 agonist DOI increased PRA when injected iv, although this also was attributed to a decrease in renal blood flow (Alper, 1990a). Finally, the nonselective 5-HT agonist *m*-chlorophenylpiperazine (m-CPP) and DOI significantly elevated PRA, although no 5-HT_2 antagonists were used to inhibit the response (Bagdy et al., 1992).

As previously described, 5-HT can influence a host of hormones through differential receptor subtype regulation. Although other receptor types may be involved, studies reported thus far support the 5-HT_2 receptor subtype as the primary serotonergic regulator of renin release from the kidney. Where those receptors are located remains a debate.

Location of 5-HT Receptors Regulating Renin Secretion

Peripheral. Intrarenal infusion of 5-HT into denervated dog kidneys resulted in elevated renin secretion (Takahashi et al., 1991). There was no accompanying change in blood pressure, but an initial decrease in renal blood flow could account for the renin release. This 5-HT-induced renin secretion was attenuated by the 5-HT_2 antagonist ketanserin, suggesting that 5-HT_2 receptors within the kidney could be responsible for serotonergic stimulation of renin release.

Several 5-HT agonists increase PRA in a dose-dependent manner upon ip administration to conscious rats. The 5-HT_1 agonist m-CPP (Saydoff et al., 1989), the 5-HT_{1A}/5-HT_{1B} agonist RU 24969 (Van de Kar et al., 1989), the 5-HT_1/5-HT_2 agonist MK 212 (Lorens and Van de Kar, 1987) all elevated PRA in a dose-dependent manner. When the 5-HT releasing drug PCA was administered ip, renin secretion was also stimulated in a dose-dependent

manner (Van de Kar et al., 1981).

Systemic injection of the 5-HT releaser PCA and the 5-HT_2 agonist quipazine elevated arterial pressure and plasma renin activity (PRA), while reducing heart rate and renal blood flow (Alper and Snider, 1987; Alper et al., 1987). Administration of quipazine iv produced cardiovascular changes (hypotension followed by hypertension) mediated by peripheral and central 5-HT_2 receptors (Vayssettes-Courchay et al., 1990; Vayssettes-Courchay et al., 1991). The 5-HT_2 agonist DOI also increased blood pressure and decreased renal blood flow when administered iv. These responses were abolished by the 5-HT_2 LY53857 as well as by the 5-HT antagonist xylamidine which does not readily cross the blood brain barrier (Alper, 1990a). However, direct infusion of DOI into the kidney resulted in renal vasodilation and an increase in renal blood flow (Shoji et al., 1990), suggesting DOI may act directly at the kidney to stimulate renin secretion.

Central. Central administration of 5-HT into the ventricles (ICV) produces a biphasic blood pressure response. An initial rise within 5 minutes is followed by a longer lasting hypotensive response (Stein et al., 1987; Montes and Johnson, 1990). Additionally, 5-HT injected ICV stimulates Na^+ excretion from the kidney, suggesting that 5-HT may produce its effects on renin secondarily to elevated Na^+ excretion (Stein et al., 1987).

The elevation of PRA following RU 24969 is potentiated when serotonergic neurons are destroyed by ICV administration of the 5-HT selective neurotoxin 5,7-DHT (Van de Kar et al., 1989). These results suggest that stimulation of 5-HT receptors in the brain increase PRA and

PRC and that these receptors can be made supersensitive when serotonergic neurons are destroyed. Thus, the postsynaptic 5-HT receptors, unaffected by the toxic effects of presynaptic 5,7-DHT uptake, become functionally up-regulated in response to the diminished input from the damaged presynaptic 5-HT terminals. Intra-PVN injection of RU 24969 significantly elevated PRA, suggesting that 5-HT receptors in the PVN are involved in the control of renin secretion. The dose of RU 24969 (10 μg/kg) was 50-fold lower than the minimally effective ip dose of 0.5 mg/kg (Van de Kar et al., 1990).

ICV administration of PCA (400-600 μg/rat) caused dose-dependent increases in sodium excretion and blood pressure (Stein et al., 1987). This suggests that CNS mechanisms may be involved in regulating renal function via 5-HT neurons. Previous pharmacological studies showed that pretreatment of rats with the synthesis inhibitor *p*-chlorophenylalanine (PCPA, ip) prevented PRA elevations following PCA injection (ip). 5,7-DHT administered ICV 14 days prior to sacrifice also significantly reduced PRA from basal levels. This demonstrates that serotonergic neurons in the brain can stimulate renin release from the kidneys (Van de Kar et al., 1981). Destruction of 5-HT neurons in the dorsal raphé nucleus with 5,7-DHT also blocked the renin response to PCA (Van de Kar et al., 1982). Electrolytic lesions in the dorsal raphé blocked renin release due to the stressor of conditioned emotional fear (Van de Kar et al., 1984) and after injection of PCA (Gotoh et al., 1988). These experiments indicate that 5-HT may be an important component of the neural control of renin release. The PCA induced elevation in PRA was also blocked by destroying the mediobasal hypothalamus (Karteszi et al., 1982). These studies suggest

that a serotonergic pathway originating in the dorsal raphé nucleus and terminating in the hypothalamus plays a role in increasing renin secretion.

The search for the central site regulating renin release has subsequently focused on the hypothalamic paraventricular and ventromedial nuclei. The PVN has known neuroendocrine functions: vasopressin, CRF, TRH, and oxytocin secretory neurons are located there (Swanson and Sawchenko, 1983). Neuropeptide Y appears to play a prominent role in regulating feeding and energy balance through actions in the PVN (Leibowitz, 1991). The VMN may contain a "satiety center" (Tokunaga et al., 1986), and control thermoregulation (Amir, 1990) and sexual behavior (Schulze and Gorzalka, 1991). The PVN, VMN or both may be necessary regulatory components of the pituitary-adrenal axis vital to the stress response (Filaretov and Filaretov, 1985; Feldman et al., 1987). Electrical stimulation of the PVN increases PRA in a frequency dependent manner (Porter, 1988). Ablation of the PVN both electrolytically and with the cell-selective neurotoxin ibotenic acid, blocked the effects of stress on PRA, PRC, and plasma corticosterone concentration (Richardson Morton et al., 1989). Other studies have shown that the effect of PCA on PRA and PRC was blocked by electrolytic lesions in the VMN (Gotoh et al., 1987). VMN ablation also attenuated the PRA and PRC response to low sodium diet, immobilization, and head-up tilt (Gotoh et al., 1988). Hypothalamic extracts contain a renin-releasing factor which stimulated renin secretion from kidney slices in a dose dependent manner (Urban et al., 1985; Van de Kar et al., 1987).

Taken together, these studies suggest that the hypothalamus, and

specifically the PVN or VMN, may contain 5-HT receptors that exert an important influence on renin secretion. Therefore, the experiments proposed for this dissertation were designed to characterize these sites.

CHAPTER III

EVIDENCE THAT DOI INCREASES RENIN SECRETION AND BLOOD PRESSURE THROUGH CENTRAL AND PERIPHERAL 5-HT RECEPTORS

INTRODUCTION

The present studies were undertaken to obtain clearer information concerning the identity and site of the 5-HT receptor subtype(s) that stimulate renin secretion (Alper and Snider, 1987; Zink et al., 1990; Van de Kar, 1991). Most of the evidence suggests that the 5-HT receptors stimulating renin secretion are located centrally (Van de Kar et al., 1981; Van de Kar and Richardson-Morton, 1986; Van de Kar et al., 1989), and are 5-HT_2 receptors (Alper and Snider, 1987; Van de Kar et al., 1989). However, there is a great similarity between 5-HT_2 and 5-HT_{1C} receptors and most drugs used so far have not distinguished between them. Furthermore, recent evidence suggests that peripheral 5-HT receptors can also stimulate renin secretion (Zink et al., 1990). Therefore, a closer examination of the 5-HT receptor subtype that regulates renin secretion was needed.

DOI is a recent addition to the rapidly expanding arsenal of 5-HT agonists (Shannon et al., 1984; Glennon et al., 1982). DOI is a 5-HT_{1C} and

5-HT_2 agonist, which produces a number of cardiovascular and hormonal consequences. DOI elevates blood pressure and sympathetic nerve discharge when administered iv (McCall et al., 1987). However no concomitant alterations in heart rate were observed (McCall and Harris, 1988; Dabiré et al., 1989). DOI also elevates plasma renin activity (Alper, 1990a) and plasma ACTH/corticosterone (Alper, 1990b; Calogero et al., 1989). A peripheral site of action may exist for DOI's effects on plasma renin activity and blood pressure (Alper, 1990a). However, Clement and McCall (1990) demonstrated that the ventrolateral medulla is the site of action of iv administered DOI-induced elevations of mean arterial pressure and sympathetic nerve discharge in anesthetized cats. Therefore, it remains unclear whether DOI's effects on renin secretion via 5-HT_{1C}/5-HT_2 receptors involve only peripheral, or both peripheral and central sites of action.

The present experiments were designed to investigate whether 5-HT_{1C}, 5-HT_2 or some other receptor subtypes are involved in mediating the renin response to DOI. Furthermore, experiments were designed to determine whether the receptors are located predominantly in the brain, or in peripheral sites. For this purpose, selective 5-HT antagonists were tested on their ability to attenuate the renin and blood pressure response to DOI. The antagonists used were: ritanserin, a 5-HT_{1C}/5-HT_2 antagonist (Schotte et al., 1989; Van Nueten et al., 1986); spiperone, a 5-HT_{1A}/5-HT_2/dopamine (D_2) antagonist, which has about a 100-fold higher affinity for 5-HT_2 receptors than 5-HT_{1C} receptors (Hoyer, 1988; Goffinet et al., 1990); and xylamidine, a 5-HT_2 antagonist postulated to have primarily a peripheral site of action (Copp et al., 1967; Leysen, 1990). To distinguish different sites of action mediating the effects of DOI, we

injected DOI *via* two routes of administration: either peripherally into the femoral artery (ia) or centrally into the lateral cerebral ventricles (ICV). A decrease in blood pressure stimulates renin secretin through activation of a renal "stretch receptor" (Tobian et al., 1959). Because DOI has potent cardiovascular effects, blood pressure was measured to test whether the renin response to DOI was related to these changes.

METHODS

Animals

Male Sprague-Dawley rats (275-300g) were purchased from Sasco-King Animal Laboratories. The rats were housed 2 per cage in a climate and illumination-controlled room. Water and rat chow were available *ad libitum*. All experiments were conducted between 11:00 A.M. and 3:00 P.M. All protocols were approved by the Loyola University Institutional Animal Care and Use Committee.

Drugs

DOI was purchased from Research Biochemical Inc. (Natick, MA). Spiperone was purchased from Sigma (St. Louis, MO). Xylamidine was donated by Wellcome Research Laboratories (Beckenham, UK). Ritanserin was donated by Janssen Pharmaceutica (Belgium).

Surgery

Implantation of ICV cannulas and intrafemoral catheters was performed under pentobarbital anesthesia. Animals were pretreated with 50 mg/kg ampicillin to prevent infection and 0.2 mg/kg atropine methyl bromide to reduce excessive secretions. The ICV cannulae (Plastics One, Roanoke, VA) were implanted stereotaxically (0.5 mm caudal, 4.5 mm ventral, and 1.4 mm lateral from bregma) and then anchored onto 4 jewelers screws with dental cement and secured with stylets. Animals were allowed 2 weeks' recovery from surgery before the experiments.

Catheters (PE 50 tubing) were inserted under pentobarbital anesthesia

into the femoral artery and led subcutaneously to exit between the scapulae. Catheters were filled with a 50% sucrose solution containing 1000 U/ml heparin. Arterial blood pressure recordings were made 24 hours after catheter implantation.

Experimental Protocols

Initially, we wanted to determine the effective dose range for DOI to elevate renin secretion. Therefore, we injected increasing doses of DOI, from 0.05 mg/kg to 2.0 mg/kg, ip, 30 minutes before decapitation. This dose range was chosen due to the high selectivity reported for DOI (Shannon et al., 1984).

Trunk blood of the decapitated rats was collected into centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. The blood was centrifuged at 1000 x g for 25 minutes at 4°C and stored at -40°C until hormone determinations were completed.

To determine *receptor subtypes* mediating the effects of DOI, two experiments were performed. In the first experiment, the 5-HT_{1C}/5-HT_2 antagonist ritanserin was administered to conscious rats in doses of either 0 (vehicle), 0.01 or 0.1 mg/kg sc. Ritanserin was dissolved in 95% ethanol, then diluted with saline resulting in a final 10% ethanol-saline solution. The vehicle used was the same 10% ethanol-saline solution. Ritanserin has an extensive half life of greater than 2 hours (Van Nueten et al., 1986). The greater the time interval between drug injections, the less likely stress effects will occur. Therefore, the ritanserin injection was performed 1 hour prior to DOI administration. Rats were killed by decapitation 30 minutes following injection of DOI (ip). The

doses of DOI were based on the results from our initial dose-response experiment (fig. 3.1). These doses were: 0 (saline), 0.5, 1.0 or 10.0 mg/kg. The highest dose of DOI was increased to 10.0 mg/kg with the expectation of overcoming the effect of the antagonist.

For the second experiment, spiperone was dissolved in 95% ethanol, then diluted with saline to a 10% ethanol-saline solution. The vehicle was also a 10% ethanol-saline solution for this experiment. Spiperone was injected in doses of 0 (vehicle), 0.01 or 0.1 mg/kg sc, 30 minutes before DOI administration (0, 1.0, 10.0 mg/kg, ip). The reported ED_{50} for spiperone blockade of quipazine-induced corticosterone secretion is 1.3 mg/kg (Fuller, 1990). In addition, the doses we used were well below those reported to inhibit ACTH, corticosterone and β-endorphin (Koenig et al., 1987; King et al., 1989). A 30 minute interval between spiperone and DOI injection was chosen because, unlike ritanserin, no information was available as to the duration of occupancy of 5-HT_2 receptors in vivo. Rats were sacrificed 30 minutes after DOI injection.

To investigate the *location* of the receptors mediating the effects of DOI, three experiments were performed. Initially, we administered the peripherally acting 5-HT_2 antagonist xylamidine, 0.1 mg/kg ip, 30 minutes before DOI (0, 0.5, 1.0, 10.0 mg/kg ip). Rats were decapitated and trunk blood collected 30 minutes after injection of DOI.

ICV injection of DOI. DOI was injected in doses below those peripherally effective (0, 1, 10, 100, 200 μg/kg) into the lateral cerebral ventricles via chronic, indwelling cannulae, in a volume of 20 μl/kg. To determine the receptor specificity of DOI, rats were pretreated with vehicle (10% ethanol-saline) or 2.0 μg/kg ritanserin, 5 minutes prior

to DOI injection. The volume was also 20 μl/kg. Rats were killed 15 minutes after DOI injection.

For the third experiment, 0, 10, or 200 μg/kg of DOI were injected while recording arterial pressure changes for 30 minutes. Drugs were injected either ia or ICV. Blood samples (0.4 ml) were collected into tubes containing 50 μl EDTA, before and 5, 15, and 30 minutes after injection of DOI. Since 1.0 ml is necessary for the PRA assay, the plasma volume of 0.4 ml was not sufficient to assay both PRA and PRC. Therefore, only PRC was determined in these samples.

Radioimmunoassays (RIAs)

Plasma renin activity. Plasma renin activity was measured by RIA for generated angiotensin I (ANG I), as previously described (Richardson Morton et al., 1989). Briefly, 1.0 ml plasma samples were incubated for 3 hours at 37°C to generate ANG I. The antiserum against ANG I was used at a dilution of 1:16,000 with total binding of 35%. The sensitivity limit of the RIA is 10 pg ANG I per tube. Intra-assay variability is 4.4%, with inter-assay variability 12.6%. (Richardson Morton et al., 1989).

Plasma renin concentration. Plasma renin concentration was determined by addition of a saturating concentration of exogenous renin substrate (0.1 ml of plasma from nephrectomized, dexamethasone injected rats) to 50 μl plasma samples and incubating at 37°C for 1 hour. The RIA of ANG I was the same as described above for plasma renin activity.

Statistics

Eight rats per group were used in all experiments unless otherwise stated in figure legends. RIA data were analyzed using RIA_AID software (Robert Maciel Associates, Arlington, MA). Statistical analysis of the data was performed using computer software (STATPAC, NWA, Portland, OR) by a 1-way or 2-way analysis of variance (ANOVA). Differences between the group means were analyzed by Newman Keuls' multiple range test (Steel and Torrie, 1960).

RESULTS

When injected ip into conscious male rats, DOI significantly [1-way ANOVA $F_{(5,31)}$=15.35, $p<0.001$] elevated both plasma renin activity and concentration (fig. 3.1). Plasma renin activity was increased eight-fold by the 2.0 mg/kg dose of DOI. The values for plasma renin activity and concentration resulting from the 2.0 mg dose of DOI (25 and 45 ng ANG I) are well above our previously reported maximal response to other 5-HT agonists (Van de Kar et al., 1989), demonstrating the high potency of DOI.

Receptor subtype

In order to evaluate the specific 5-HT receptor mediating the effect of DOI on renin secretion, rats were pretreated with different 5-HT antagonists prior to increasing doses of DOI. When injected ip into conscious male rats, DOI again significantly [$F_{(3,84)}$=19.47, $p<0.0001$] elevated both plasma renin activity and concentration (fig. 3.2). The PRC response to the 10 mg/kg dose of DOI was significantly greater than the 1.0 mg/kg dose of DOI. The highest DOI dose (10 mg/kg) caused the same eight-fold increase in plasma renin activity and concentration as was seen with 2 mg/kg in our initial study (fig. 3.1), suggesting that a response plateau was reached by 2.0 mg/kg. Both the high (0.1 mg/kg) and the low dose (0.01 mg/kg) of ritanserin completely blocked the effect of all doses of DOI (fig. 3.2). The two-way ANOVA revealed a significant interaction between DOI and ritanserin [$F_{(6,84)}$=16.56, $p<0.0001$]. The post-hoc Newman Keuls' test showed that saline pretreated rats injected with 10.0 mg/kg DOI had significantly higher plasma renin values whether compared to their own controls or any of the ritanserin treated rats ($p<0.01$).

To differentiate between 5-HT_{1C} and 5-HT_2 receptor subtypes involved, we pretreated rats with spiperone, which has a higher affinity for 5-HT_2 receptors than 5-HT_{1C} receptors (Hoyer, 1988). As shown in figure 3.3, after injection of spiperone, both plasma renin activity and plasma renin concentration exhibited a shift to the right in the dose-response curve for DOI, with a significant suppression of the maximal response. The 2-way ANOVA showed a significant interaction between the effects of DOI and spiperone on renin activity [$F_{(4,63)}$=6.38, $p<0.0001$]. Newman-Keuls' test revealed that the group with the high dose of DOI (10.0 mg/kg) was significantly greater than vehicle control as well as the corresponding 10.0 mg/kg DOI groups treated with spiperone ($p<0.01$). None of the groups treated with spiperone were different from each other. It is important that a low dose of spiperone (0.01 mg/kg) was effective, suggesting that 5-HT_2 rather than 5-HT_{1C} receptors mediate the effect of DOI.

Location of receptors

To distinguish central from peripheral 5-HT receptors mediating the renin response to ip administered DOI, we first injected the peripherally acting 5-HT_{1C}/5-HT_2 antagonist xylamidine. DOI dose-dependently increased plasma renin activity and concentration [$F_{(3,51)}$=15.3, $p<0.0001$]. Figure 3.4 shows that the DOI dose response curve was shifted to the right in the xylamidine pretreated rats. A two-way ANOVA indicated that the interaction between DOI and xylamidine approached, but did not achieve statistical significance [$F_{(3,51)}$=2.3, $p>0.08$]. However, a post-hoc Newman Keuls' test revealed that there was a significant reduction in renin activity and concentration in rats injected with 10 mg/kg DOI and pretreated with xylamidine, compared to those pretreated with saline

($p<0.01$), suggesting a decrease in the maximal response. The PRA response to the 1.0 mg/kg dose of DOI was significantly higher than the corresponding group treated with xylamidine ($p<0.01$). These data suggest a role for peripheral 5-HT_2 receptors in the renin response to DOI.

To investigate the central effects of DOI directly, we injected low doses of DOI into the lateral cerebral ventricles. The plasma renin concentration data demonstrate a dose-response effect due to DOI more clearly than the plasma renin activity data (fig. 3.5), although both were affected [PRA: $F_{(4,55)}=19.4$, $p<0.0001$; PRC: $F_{(4,55)}=19.24$, $p<0.0001$]. Only the 200 μg/kg dose of DOI significantly elevated plasma renin activity and concentration compared to the saline controls (Newman Keuls' test, $p<0.01$). ICV injected ritanserin (2 μg/kg) did not significantly affect the renin response to ICV administered DOI.

DOI has impressive cardiovascular effects, whether administered ICV or ia. Representative arterial pressure tracings for vehicle (A), 200 μg/kg DOI-ia (B), and 200 μg/kg DOI-ICV (C) are shown in figure 3.6. Note also the larger increase in pulse pressure following ICV DOI injection (fig. 3.6C). Figure 3.7 shows the complete data for all the groups. We found that DOI, injected ICV at 10, and 200 μg/kg produced a maximal elevation in BP of 10 $\pm$ 1.5 and 40 $\pm$ 3.6 mm Hg respectively, within 10 minutes post-injection (fig. 3.7, middle panel). In contrast, ia injection of 200 μg/kg DOI caused a more delayed rise (15 minutes post-injection) in BP as well as a lower maximal response of 28 $\pm$ 3.2 mm Hg (fig. 3.7, middle panel). Both ICV and ia administration of 200 μg/kg DOI caused significant blood pressure elevations at all time points compared to saline controls (Newman Keuls' test $p<0.01$). However, blood pressure

increases after ICV injections were significantly higher at 5 ($p<0.05$) and 10 minutes ($p<0.01$) than the corresponding values for ia injection. The renin data accompanying these changes in blood pressure are also summarized in figure 3.7 (top panel). At 5 minutes, plasma renin concentration was significantly increased in rats receiving 200 μg/kg DOI-ICV, compared to rats receiving vehicle control or DOI-ia injections (Newman Keuls' test, $p<0.01$). At 15 minutes, rats injected ICV still had elevated renin secretion ($p<0.01$) whereas rats injected ia had just attained a significant increase in plasma renin concentration ($p<0.05$), compared to saline controls, at the same time point. DOI-ICV 200 μg/kg caused a reduction in heart rate ($p<0.05$), whereas DOI-ia 200 μg/kg did not cause any change (fig. 3.7, bottom panel). There were no significant changes after ICV injection of 10 μg/kg DOI in any of the parameters measured.

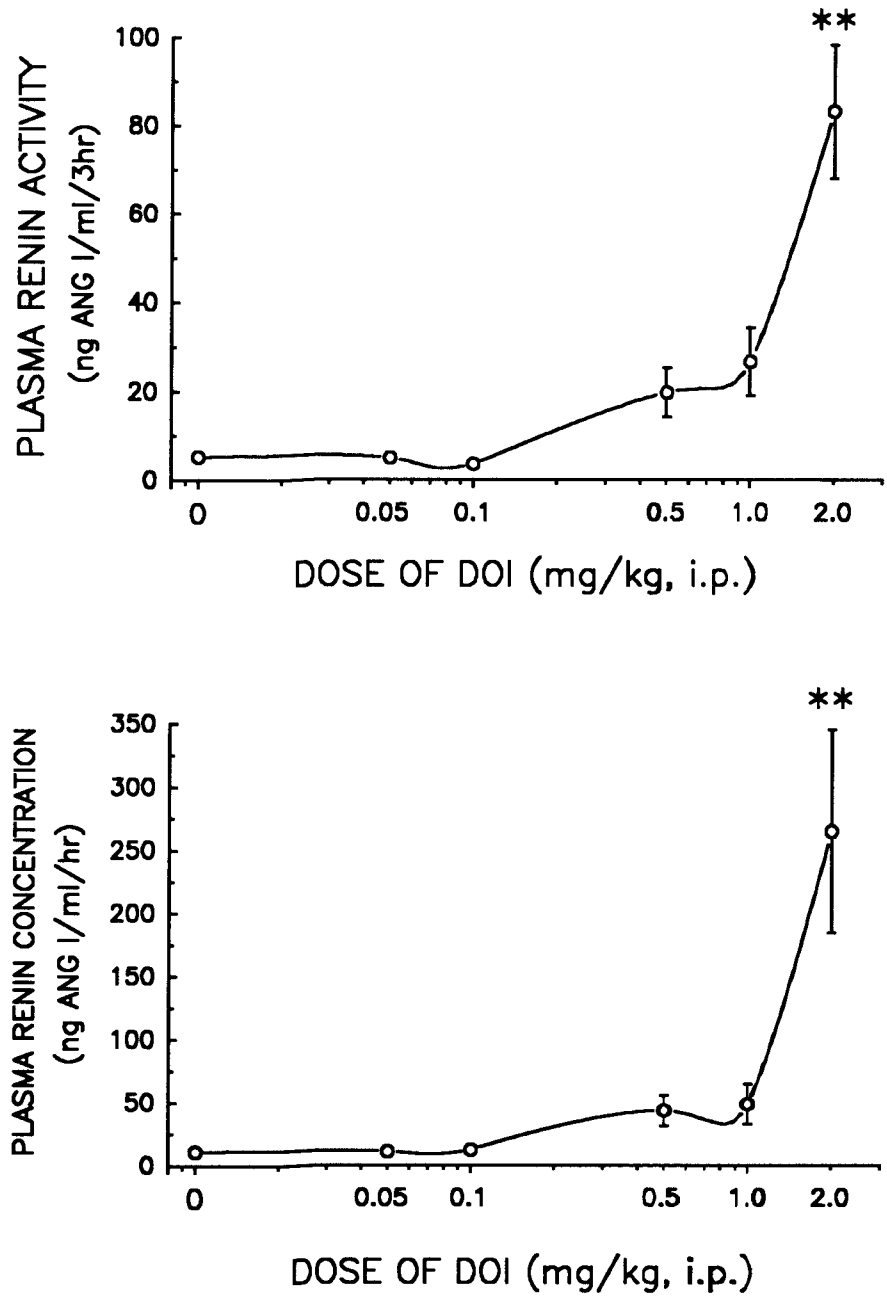

Figure 3.1. The effect of increasing doses of DOI on plasma renin activity and concentration. DOI was administered ip. The data represent the mean ± S.E.M. of eight rats per group. ** Significant difference from the corresponding control (saline) group, $P<0.01$ (one-way ANOVA and Newman Keuls' test).

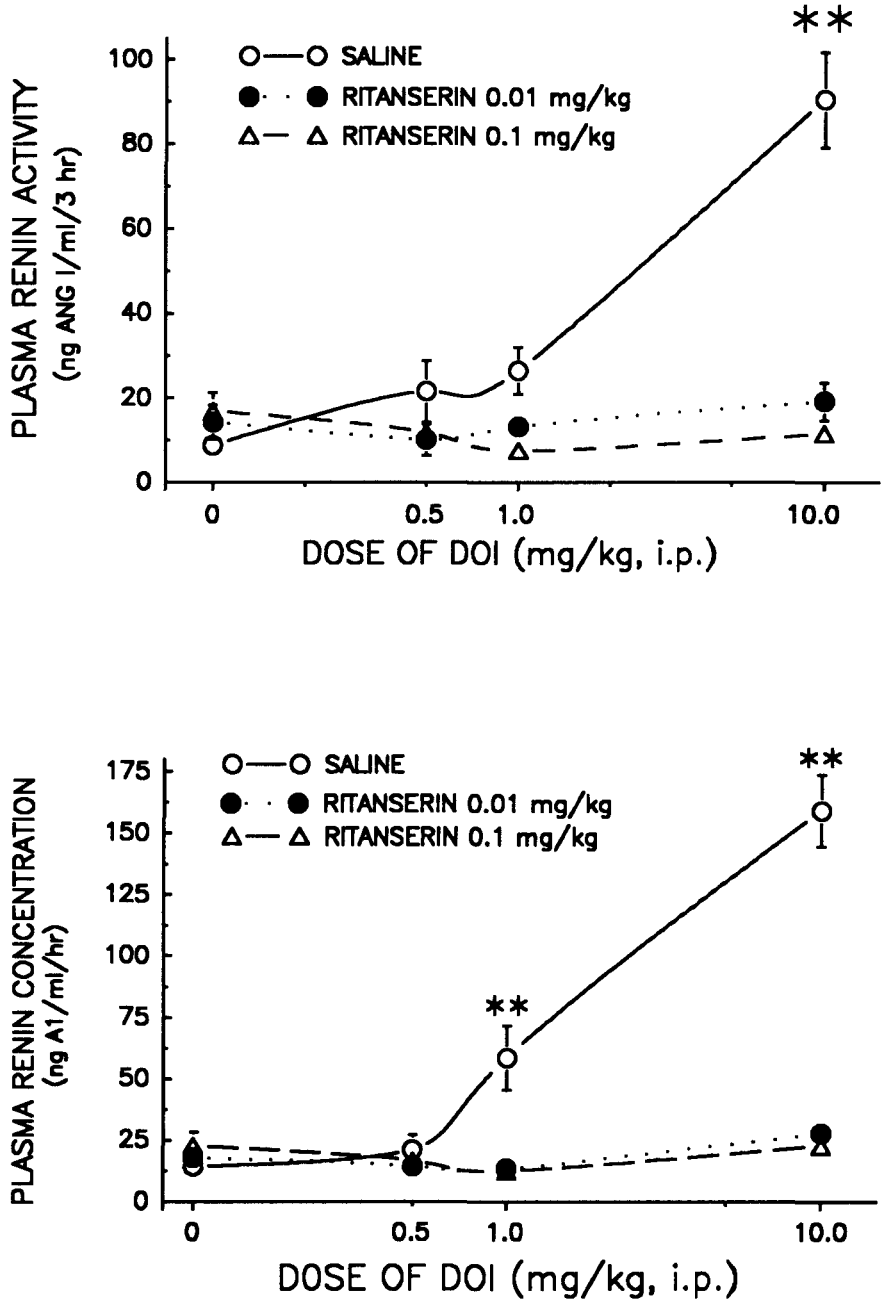

Figure 3.2. The effect of increasing doses of DOI on plasma renin activity and concentration in rats pretreated with either 0.01 or 0.1 mg/kg ritanserin. DOI was administered ip, ritanserin sc. The data represent the mean ± S.E.M. of eight rats per group. ** Significant difference from the corresponding control (saline) group, $P<0.01$ (two-way ANOVA and Newman Keuls' test).

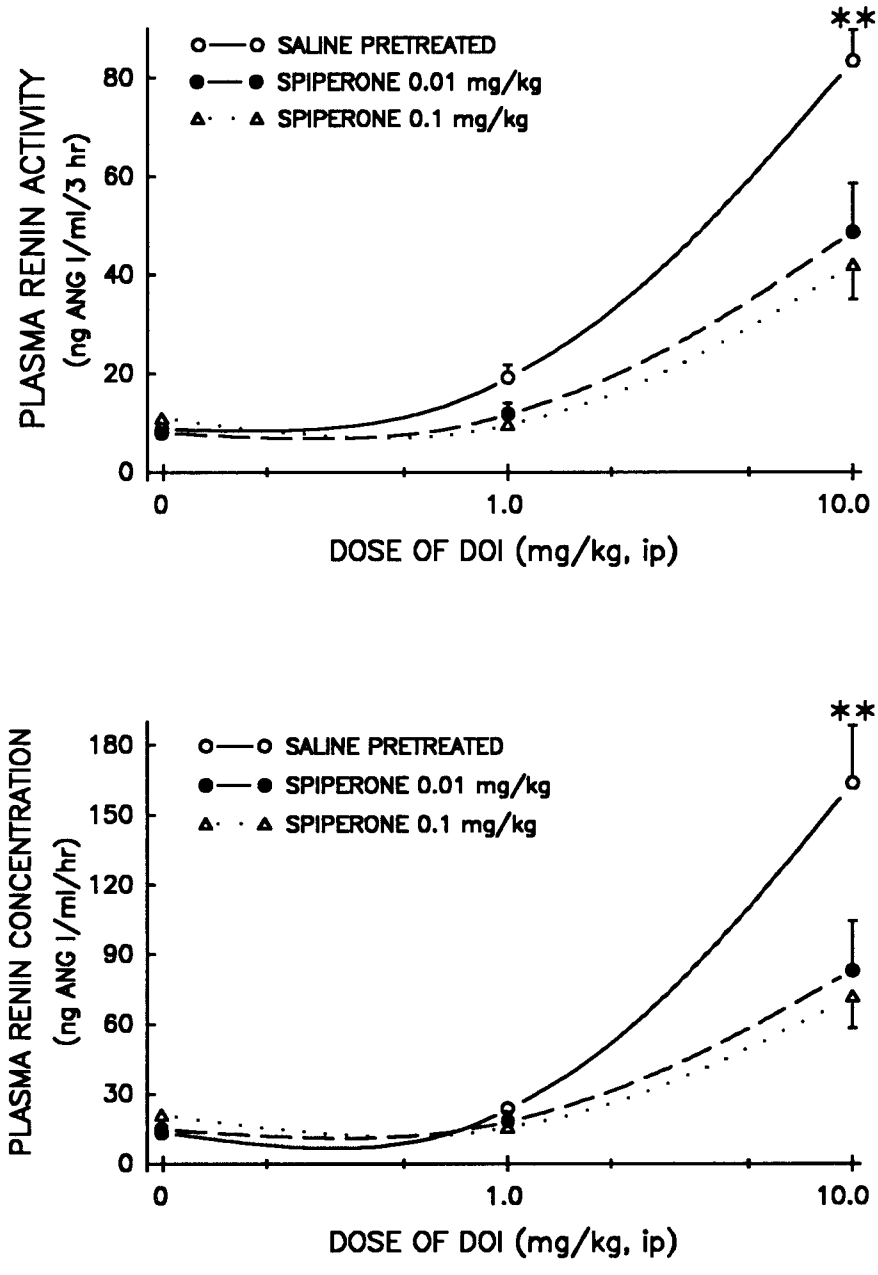

Figure 3.3. The effect of increasing doses of DOI on plasma renin activity and concentration in rats pretreated with either 0.01 or 0.1 mg/kg spiperone. DOI was administered ip, spiperone sc. The data represent the mean ± S.E.M. of eight rats per group. ** Significant difference from the corresponding control (saline) group and all spiperone groups, $P<0.01$ (two-way ANOVA and Newman Keuls' test).

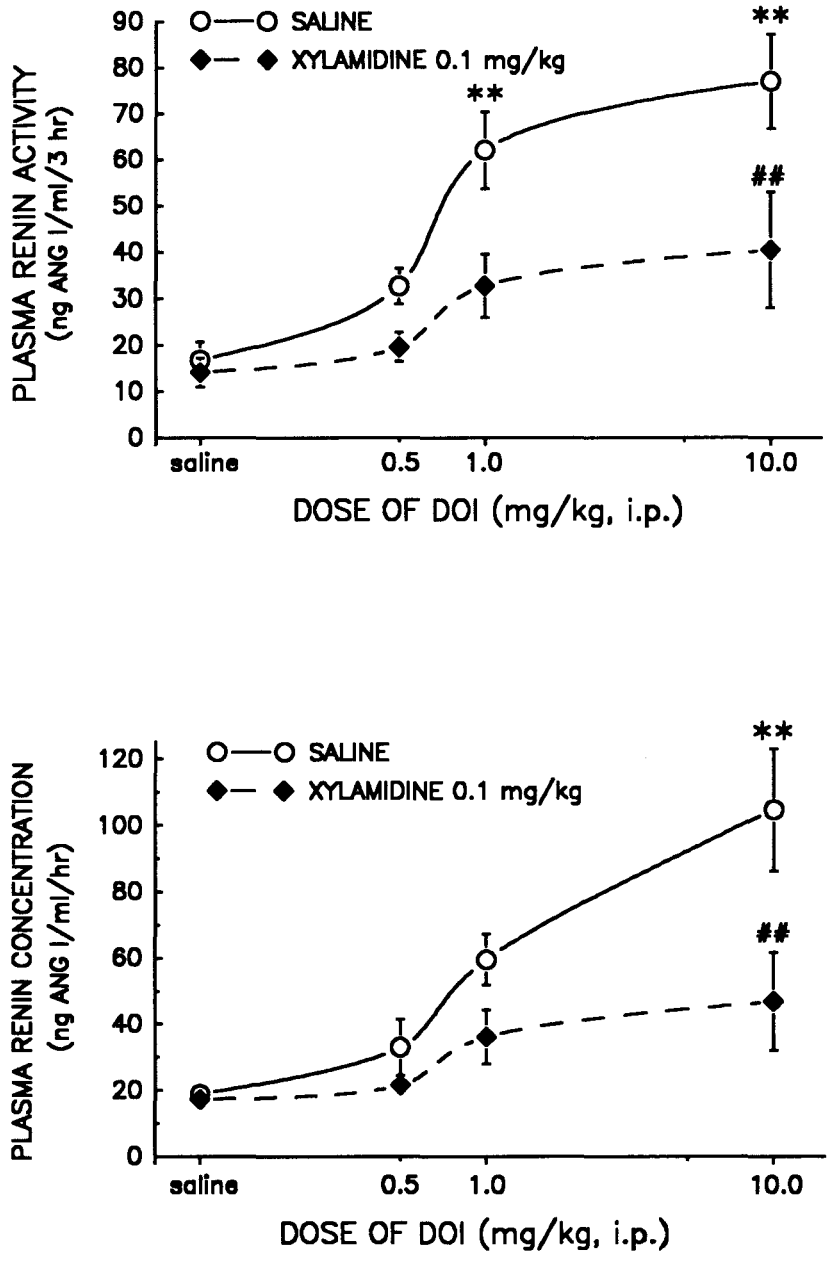

Figure 3.4. The effect of DOI on plasma renin activity and concentration in rats pretreated with 0.1 mg/kg xylamidine. DOI and xylamidine were injected ip. The data represent the mean ± S.E.M. of six to eight rats per group. ** Significant difference from the corresponding control group, $P<0.01$; ## significant difference from 10.0 mg/kg DOI group, $P<0.01$ (two-way ANOVA and Newman Keuls' test).

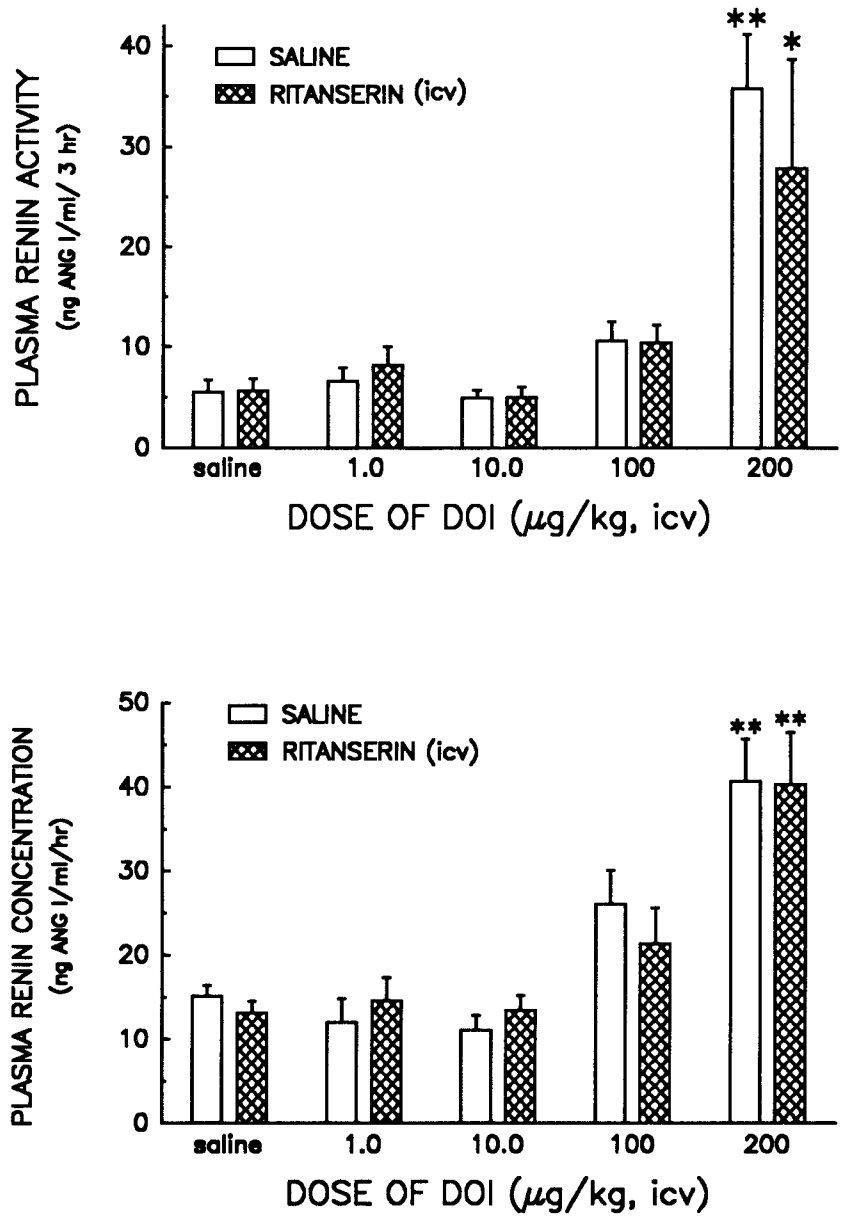

Figure 3.5. The effect of ICV injected DOI on plasma renin activity and concentration in rats pretreated with ICV ritanserin. Ritanserin was dissolved in 2.5% ethanol, and injected at a dose of 2.0 μg/kg in 20μl. The data represent the mean ± S.E.M. of six to eight rats per group. * Significant difference from corresponding control group, $P<0.05$; ** significant difference from corresponding control group, $P<0.01$ (two-way ANOVA and Newman Keuls' test).

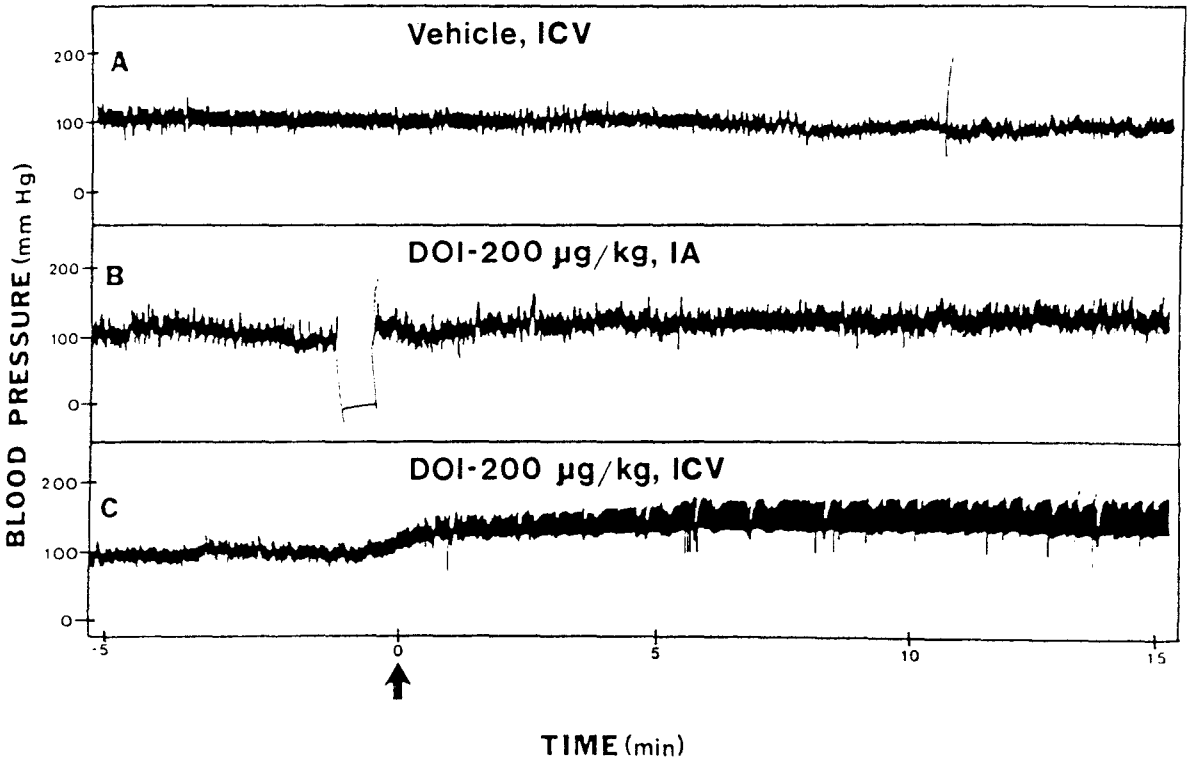

Figure 3.6. Arterial pressure tracings recorded from the femoral artery in freely moving rats. Panel A: Control, ICV injection of vehicle. Panel B: ia injection of 200 µg/kg DOI; injection was complete at time 0. Panel C: ICV injection of 200 µg/kg DOI; injection was complete at time 0; DOI was injected at a rate of 1.0 µl/10 sec; injection volume was 20 µl/kg.

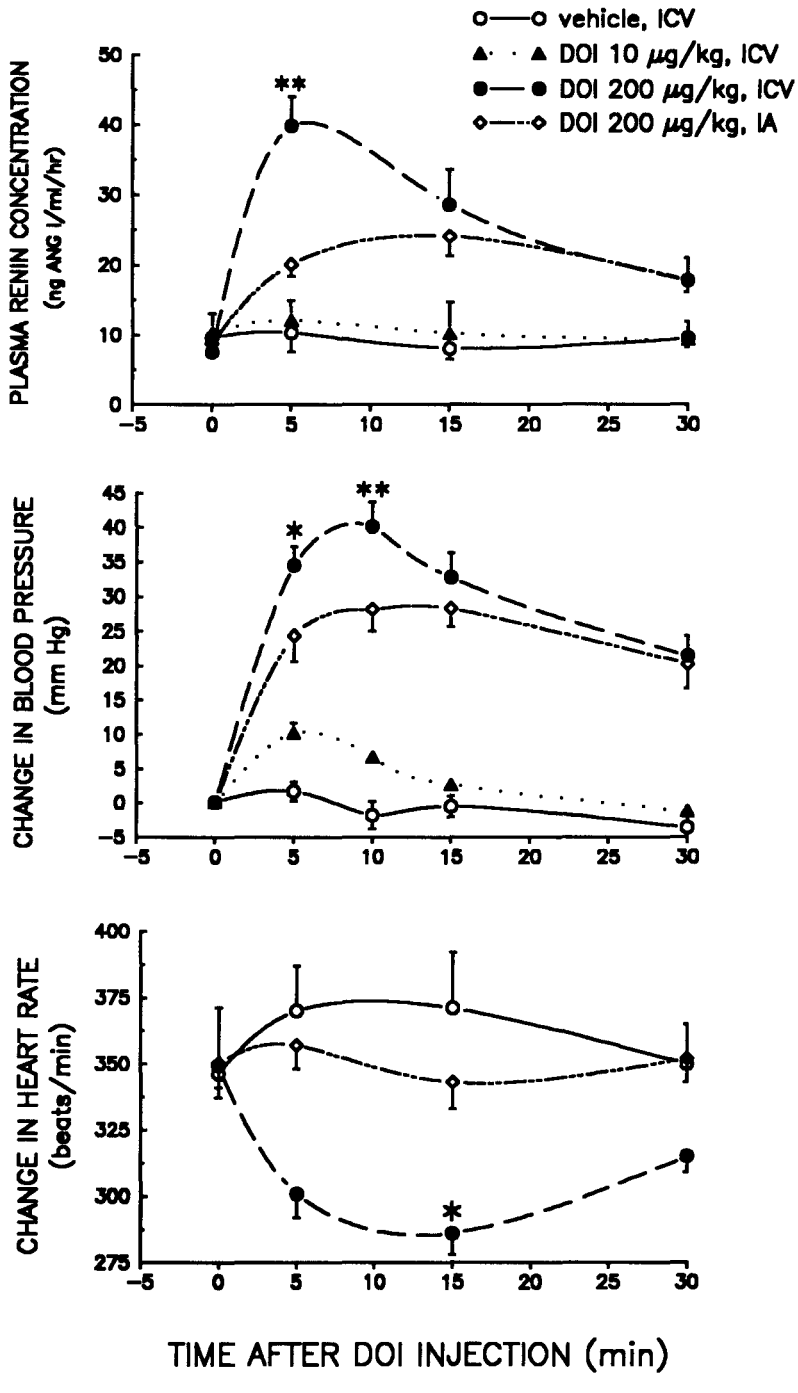

Figure 3.7. Time course after DOI injection of vehicle, 200 μg/kg ICV, 10 μg/kg ICV, or 200 μg/kg ia. Top panel: PRC. Middle panel: change in BP. Bottom panel: change in HR; there was no change in HR after injection of DOI 10 μg/kg ICV. Therefore, it was omitted from the figure in the interest of clarity. For each panel, ** or * is significantly different from the corresponding ia group ($P<0.01$ and $P<0.05$ respectively). The number of rats per group: top = 8; middle = 11; bottom = 8 (repeated measures, 2-way ANOVA).

DISCUSSION

The data in this study suggest that DOI, a 5-HT_{1C}/5-HT_2 agonist, stimulates renin secretion in part through activation of 5-HT_2 receptors. Furthermore, these data suggest that the DOI-induced increase in renin release is mediated by both central and peripheral sites of action. Therefore, DOI's mechanism of action is probably more complex than previously thought.

The ability of both ritanserin and spiperone to antagonize the effect of DOI on renin secretion at low doses (0.01-0.1 mg/kg) suggests that 5-HT_2 receptors, for which they both have a high affinity, were blocked. Evidence suggests that activation of 5-HT_{1C} receptors increases ACTH and corticosterone secretion (King et al., 1989). The antagonists ritanserin and spiperone have a high and approximately equal affinity for 5-HT_2 receptors. Ritanserin also binds with the same affinity to 5-HT_{1C} receptors, while spiperone binds with an approximately 100-fold lower affinity to 5-HT_{1C} receptors (Hoyer, 1988). This difference in affinity is also manifested in vivo by the low potency (a high ED_{50} of 1.5 mg/kg) for spiperone's ability to antagonize the effect of the 5-HT agonist quipazine on corticosterone secretion (Fuller and Snoddy, 1990). The spiperone doses we used were well below those reported to inhibit ACTH, corticosterone and β-endorphin responses to 5-HT agonists (Koenig et al., 1987; King et al., 1989). Doses of spiperone as high as 3 mg/kg did not inhibit the effect of the 5-HT_{1C} agonist MK-212 on ACTH secretion (Koenig et al., 1987; King et al., 1989). Therefore, the ability of spiperone to inhibit the DOI-induced increase in plasma renin at doses of 0.01-0.1

mg/kg suggest that it blocked 5-HT_2 receptors.

A comparison of the increases in plasma renin activity between figures 3.1 and 3.2 indicates that the DOI doses of 2 and 10 mg/kg produced comparable changes in PRA and PRC, suggesting that a maximal plateau had been achieved. The dose of 10 mg/kg was used in an attempt to surmount the antagonist effects of ritanserin, spiperone and xylamidine. The inability of the high dose of DOI to overcome the antagonist effect of ritanserin suggests that it might be noncompetitive or irreversible. Leysen et al. (1985) observed that the inhibition of [^{3}H]ketanserin binding by ritanserin was noncompetitive. Our data also indicate that ritanserin may inhibit the DOI-induced increase in renin secretion in a noncompetitive manner. If the inhibition were competitive in nature, the maximal response achieved at the highest dose of DOI would have been decreased, but not completely suppressed, in the presence of the antagonists. However we observed a significant reduction of the maximal response at the high dose of DOI in the presence of ritanserin. Thus, we conclude that ritanserin may be functioning in a noncompetitive manner, presumably via 5-HT_2 or 5-HT_{1C} receptors.

Binding experiments indicated that a ritanserin dose of 0.1 mg/kg occupies 50% of brain 5-HT_2 receptors (Leysen et al., 1985). Surprisingly, even our low dose of 0.01 mg/kg ritanserin completely blocked the renin response, suggesting the high selectivity of this antagonist for 5-HT_2 receptors. It should be noted that 5-HT_2 receptors exist in high and low affinity states defined by their coupling to the G-protein. ^{3}H-Ketanserin labels low affinity sites. The affinity of 5-HT agonists and antagonists for the 5-HT_2 high affinity sites (^{125}I-DOI binding sites) is higher than

their affinity for ^{3}H-ketanserin sites. Furthermore, the B_{max} of ^{125}I-DOI for 5-HT_2 (agonist) sites is at least 10-fold lower than the B_{max} for ^{3}H-ketanserin sites (Teitler et al., 1990). Thus, it is conceivable that low doses of 5-HT_2 antagonists would be effective in vivo in inhibiting the neuroendocrine effects of DOI.

The combined evidence of the xylamidine and ICV experiments suggests that DOI stimulates 5-HT_2 receptors both centrally and peripherally. We chose to use the 5-HT_{1C}/5-HT_2 antagonist xylamidine because of its apparent inability to cross the blood brain barrier (Copp et al., 1967). Consequently, any inhibition of DOI's stimulation of renin release by xylamidine, could be attributed to peripheral effects. We found that xylamidine caused a shift to the right in the renin dose-response curves, suggesting a role for peripheral 5-HT_2 receptors. In addition, the inability of centrally administered ritanserin to inhibit the ICV DOI-induced increase in renin secretion (fig. 3.5) suggests that DOI may be leaking to the periphery and acting there, or that it acts centrally on a different receptor. However, the dose-response effect of ICV injected DOI on plasma renin concentration suggests that DOI can mediate its effects via interacting with central 5-HT receptors.

Alper (1990b) found that as little as 100 μg/kg DOI was needed to increase renin when injected intravenously. Alper concluded that DOI can stimulate renin secretion through a peripheral mechanism, specifically by reducing renal blood flow. Zink et al. (1990) demonstrated that quipazine also increased renin secretion through this same peripheral mechanism. Shoji et al. (1989) found that 5-HT_1 and 5-HT_2 receptors in the renal vasculature were responsible for the decrease in renal blood flow

following intrarenal serotonin infusion. Hence, DOI could increase renin release by acting in the renal arterioles. Vayssettes-Courchay et al. (1990b) showed that the pressor response after iv injection of quipazine is mediated by peripheral vascular and myocardial 5-HT_2 receptors.

However, our data suggest 5-HT mediated changes in renin responses involve, in part, a central mechanism. The ICV responses to DOI, increased blood pressure and renin concentration with decreased heart rate, were all significantly higher than ia injection of the same dose of DOI (fig. 3.7). Thus, these data suggest a site of action for DOI localized within the brain.

Other evidence exists which would suggest a central site of action for DOI to stimulate renin secretion. Porter (1988) found that electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) caused an increase in plasma renin activity with no change in blood pressure. Richardson-Morton et al. (1989) demonstrated that cell bodies in the PVN mediate stress-induced elevations in renin secretion. Appel et al. (1990) found higher [^{125}I]-DOI binding in the PVN than other hypothalamic nuclei. Gotoh et al. (1987; 1988) reported that lesions in either the PVN or the ventromedial nucleus of the hypothalamus (VMN) inhibit the plasma renin activity response to the 5-HT releaser p-chloroamphetamine (PCA). Another 5-HT agonist, RU 24969, injected into the PVN also increased plasma renin activity and concentration (Van de Kar et al., 1990).

An additional site of action for DOI could be in the circumventricular organs, such as the subfornical organ or the area postrema. These organs, which lie outside the blood brain barrier, are highly vascularized and

have connections to cardiovascular and autonomic control centers in the brain (Miselis, 1981; Shapiro and Miselis, 1985). Thus, they may be a bridge connecting "peripheral" receptor sites with central renin regulatory sites. Immunoreactive 5-HT cell bodies in the area postrema (Lind, 1986) and fibers in the area postrema and subfornical organ (Newton et al., 1985) have been reported. 5-HT_3 receptors have been identified in the area postrema using autoradiography (Radja et al., 1991).

Elevated blood pressure is an effective inhibitor for renin secretion through the renal "stretch" receptor (Tobian et al., 1959). Thus, if DOI were stimulating renin release exclusively through a cardiovascular mechanism, then elevated blood pressure should depress, not raise renin secretion as shown in figure 3.7. Since no change in heart rate was reported by several investigators (Dabiré et al., 1989; McCall and Harris, 1988) following DOI administration it is likely that an increase in sympathetic nerve activity overrides the baroreceptor reflex response to increased blood pressure. Our data show that ICV injection of DOI potently decreases heart rate (fig. 3.7), while ia injection of DOI causes no change. These data also support a central site of action for DOI. Kushiro et al. (1988) have implicated central 5-HT_2 receptors in blood pressure regulation, since ICV ketanserin blocked the pressor response caused by ICV 5-HT injection. Several investigators have suggested that the ventral surface of the medulla may be the site of DOI's pressor response (King and Holtman, 1990; Gillis et al., 1989).

Our experiments showed that it did not matter which route of administration was used for DOI to increase blood pressure. Either ia or ICV, DOI was a vigorous pressor agent. However, ICV injection clearly

resulted in a more robust response of each parameter measured: renin, BP or HR (fig. 3.7). In addition, the large expansion in pulse pressure (fig. 3.6) following ICV injection of DOI suggests that elevated sympathetic outflow is causing a direct increase in cardiac contractility. Therefore, distinct receptor subtypes may be activated on either side of the blood brain barrier. As mentioned above, the circumventricular organs, renal vasculature, or myocardium are possible peripheral sites of action, while sites within the brain include the paraventricular or ventromedial nuclei of the hypothalamus and/or the ventrolateral medulla.

In conclusion, our data indicate that DOI stimulates renin secretion through 5-HT_2 receptors. Our data indicate that both central and peripheral components are involved in DOI's stimulation of renin secretion and blood pressure.

CHAPTER IV

EVIDENCE THAT RU 24969 AND P-CHLOROAMPHETAMINE STIMULATE RENIN SECRETION THROUGH CENTRAL SEROTONERGIC MECHANISMS

INTRODUCTION

The control of renin secretion may have both central and peripheral sites of regulation. Peripherally administered serotonin (5-HT) agonists and releasers reliably elevate plasma levels of renin (Van de Kar, 1991). Other evidence suggests that the 5-HT receptors regulating this endocrine function are located in the brain (Van de Kar et al., 1981; Karteszi et al., 1982; Van de Kar et al., 1989). Previous investigations have shown that the serotonin agonist DOI stimulates renin release through activation of both central and peripheral 5-HT_2 receptors (Rittenhouse et al., 1991; Alper, 1990a). The aim of the present study was to determine if the 5-HT agonist RU 24969 and the 5-HT releaser *p*-chloroamphetamine (PCA) act in the brain to stimulate renin release from the kidney.

RU 24969 is an indole derivative considered to be a mixed 5-HT_{1A}/5-HT_{1B} agonist (Glennon, 1987). However, the effect of RU 24969 on renin secretion has been blocked by the 5-HT_{1C}/5-HT_2 antagonist ritanserin, and therefore RU 24969 is thought to increase renin secretion through activation of 5-HT_{1C}/5-HT_2 receptors (Van de Kar et al., 1989). Increasing

doses of RU 24969 have not been administered directly into the brain. Intravenous administration of RU 24969 causes a dose dependent decrease in blood pressure (Cherqui et al., 1988), suggesting cardiovascular factors may contribute to the renin response.

The mechanism by which PCA stimulates renin secretion remains subject to debate. When injected peripherally (ip), PCA causes a dose-dependent increase in renin secretion (Van de Kar et al., 1981). Intravenous administration of PCA does not increase renin secretion unless preceded by the α_1 antagonist prazosin (Alper et al., 1990). PCA releases both 5-HT and catecholamines from nerve terminals (Sanders-Bush and Steranka, 1978; Sharp et al., 1986). Evidence suggests that the increase in renin caused by PCA is mediated by either 5-HT from the dorsal raphé (Van de Kar et al., 1981) or the sympathetic nervous system (Alper and Ganong, 1984). PCA has also been shown to increase blood pressure when administered peripherally (Alper et al., 1987). However, recent reports suggest that renal toxicity may be responsible for increased renin secretion subsequent to peripherally injected PCA (Ikonomov et al., 1991). Thus, we decided to explore more thoroughly the mechanism underlying PCA's ability to elevate plasma renin levels.

In the present studies, we injected either RU 24969 or PCA into the lateral cerebral ventricle (ICV), using doses below those peripherally effective (Van de Kar et al., 1981; Van de Kar et al., 1989). The 5-HT_{1C}/5-HT_2 antagonist LY53857 was used to verify the 5-HT receptor subtype influencing renin secretion (Hoyer and Schoeffter, 1991). In separate experiments, blood pressure and heart rate were measured, subsequent to ICV drug administration. Additional control experiments were performed

with fluoxetine, a 5-HT uptake inhibitor (Fuller et al., 1991), and prazosin, an α_1 antagonist (Cavero and Roach, 1980) to investigate PCA's mechanism of action. The purpose of these experiments was to determine if 5-HT receptors in the brain mediate the renin response to RU 24969 and PCA in conscious male rats, or whether other mechanisms are involved.

METHODS

Animals

Male Sprague-Dawley rats (275-300g) were purchased from Sasco-King Animal Laboratories. The rats were housed 2 per cage in a climate and illumination (12:12 hr light/dark cycle, lights on at 7:00 AM) controlled room. Water and rat chow (Wayne Lab Blox, Lab Mills Inc., Chicago, IL) were available *ad libitum*. Experiments were conducted between 11:00 A.M. and 3:00 P.M. All protocols were approved by the Loyola University Institutional Animal Care and Use Committee, and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

Implantation of ICV cannulae and intra-femoral catheters were performed under pentobarbital anesthesia (50 mg/kg, ip). Animals were pretreated with ampicillin (50 mg/kg, sc) to prevent infection and atropine (0.2 mg/kg, ip) to reduce excessive secretions.

The ICV guide cannulae (33 gauge, Plastics One, Roanoke, VA) were implanted stereotaxically using the coordinates: 0.5 mm caudal, 4.6 mm ventral, and 1.4 mm lateral from bregma (Paxinos and Watson, 1986). The cannulae were anchored onto 4 jewelers screws with dental cement, and secured with stylets. Animals were allowed 2 weeks recovery from surgery before the experiments. For each experiment, cannulae placement was verified after decapitation by injection of fast green dye through the cannulae into the ventricle. Rats having improperly placed cannulae were not used in the data analysis.

Catheters (PE 50 tubing) were inserted into the femoral artery and led subcutaneously to exit between the scapulae. Catheters were filled with a 50% sucrose solution containing 1000 u/ml heparin. Arterial blood pressure recordings were made 24 hours following catheter implantation on a Grass polygraph.

Experimental Protocols

Experiment 1. To investigate whether a 5-HT antagonist injected into the brain could inhibit a peripherally administered 5-HT agonist, ICV cannulae were implanted stereotaxically into male rats. Following 2 weeks recovery from surgery, the 5-HT_{1C}/5-HT_2 antagonist LY53857 (50 μg/kg) was injected ICV into conscious rats. Fifteen minutes later, RU 24969 was injected ip (0, 1.0, 5.0, 10.0 mg/kg). Rats were decapitated 30 minutes after the RU 24969 injection, and their trunk blood collected into chilled tubes containing 0.5 ml EDTA. Plasma was stored at -40° C until determination of renin.

Experiment 2. This experiment was designed to determine if RU 24969 had a direct action in the brain to elevate plasma renin levels. ICV cannulae were implanted as described above. On the day of the experiment, LY53857 (10 μg/kg) was injected ICV into conscious rats. Fifteen minutes later, RU 24969 was also injected ICV (0, 10, 100, 200 μg/kg). Rats were decapitated 30 minutes later, and their trunk blood collected into chilled tubes containing 0.5 ml EDTA. Plasma was stored at -40° C until determination of renin.

Experiment 3. This control experiment was performed to determine if cardiovascular factors influence RU 24969's ability to stimulate renin

secretion. ICV cannulae were implanted. Two weeks later, catheters were inserted into the femoral artery under pentobarbital anesthesia. Twenty-four hours later blood pressure (BP) and heart rate (HR) were recorded. RU 24969 (200 μg/kg) was administered either centrally through the ICV cannulae or peripherally through the femoral artery. BP and HR were recorded for 30 minutes after drug injection.

Experiment 4. Fluoxetine is a 5-HT uptake inhibitor (Fuller et al., 1991). To verify that PCA is stimulating renin secretion through a serotonergic mechanism and not another transmitter, this control experiment was performed. Fluoxetine was administered 3 hours prior to sacrifice (10 mg/kg, ip). PCA was administered 1 hour prior to sacrifice (8 mg/kg, ip). These time periods were chosen due to the extended duration of action of each drug (Fuller et al., 1975). Plasma was collected and stored at -40° until renin determination.

Experiment 5. This experiment was designed to determine if PCA had a direct action in the brain to elevate plasma renin levels. After the 2 week recovery period following ICV surgery, LY53857 (20 μg/kg) was injected ICV into conscious rats. Fifteen minutes later, PCA was also injected ICV (0, 50, 500, 1000 μg/kg). Rats were decapitated 1 hour later, and their trunk blood collected into chilled tubes containing 0.5 ml EDTA. Plasma was stored at -40° C until determination of renin.

Experiment 6. Results from experiment 5 suggested that cardiovascular parameters could be influencing the renin response to PCA, which prompted the following experiment. ICV cannulae were implanted as described above. Two weeks later, catheters were inserted into the femoral artery under pentobarbital anesthesia. Twenty-four hours later

blood pressure (BP) and heart rate (HR) were recorded. PCA (1000 μg/kg) was administered either centrally through the ICV cannulae or peripherally into the femoral artery. BP and HR were recorded for 1 hour following drug injection. The vehicle (saline) injected controls were measured for only 30 minutes.

Experiment 7. An additional experiment was performed to control for PCA's vasoconstrictive actions. ICV cannulae were implanted stereotaxically. After 2 weeks recovery, the α_1 antagonist prazosin was injected (1.0 mg/kg, sc). Fifteen minutes later, PCA was injected ICV (500 μg/kg) into conscious rats. Rats were decapitated 1 hour later, their trunk blood saved for renin determination, and cannulae placement verified with fast green dye.

Drugs

PCA and prazosin were purchased from Sigma Chemical Co. (St. Louis, MO). RU 24969 was donated by Roussel Uclaf (Romainville, France). LY53857 and fluoxetine were donated by Eli Lilly & Company (Indianapolis, IN). Prazosin was dissolved in ethanol then adjusted with distilled H_2O to a 10% ethanol in distilled H_2O solution. The other drugs were dissolved in a 0.9% saline solution. ICV injected drugs were in a volume of 20 μl/kg, while ip injected drugs were in a volume of 1.0 ml/kg.

Radioimmunoassays (RIA's)

Plasma renin activity. Plasma renin activity (PRA) was measured by RIA for generated ANG I, as previously described (Richardson Morton et al., 1989). Briefly, 1.0 ml plasma samples were incubated for 3 hours at

37°C to generate ANG I. The antiserum against ANG I was used at a dilution of 1:16,000 with total binding of 30%. The sensitivity limit of the RIA is 10 pg ANG I per tube. Intra-assay variability was 4.4%, with inter-assay variability 12.6%. (Richardson Morton et al., 1989). PRA is a reflection of the ability of the native enzyme renin in a given plasma sample to generate ANG I from endogenous substrate (angiotensinogen). It is therefore substrate dependent.

Plasma renin concentration. Plasma renin concentration (PRC) was determined by addition of exogenous renin substrate (0.1 ml of plasma from nephrectomized, dexamethasone injected rats) to 50 μl plasma samples and incubating at 37°C for 1 hour. The RIA of ANG I was the same as described above for plasma renin activity. PRC represents the maximal response possible since a saturating concentration of renin substrate is added to each plasma sample, and thus is substrate independent.

Statistics

Eight rats per group were used in all experiments unless otherwise stated in figure legends. RIA data were analyzed using RIA_AID software (Robert Maciel Associates, Arlington, MA). Statistical analysis of the data was performed using computer software (STATPAC, NWA, Portland, OR) by either a 2-way analysis of variance (ANOVA) or 1-way ANOVA with repeated measures. The group means were compared by Newman Keuls' multiple range test (Steel and Torrie, 1960).

RESULTS

RU 24969

Our first experiment was designed to determine if a centrally administered antagonist could block the effect of a peripherally injected agonist. Figure 4.1 shows a shift to the right and a reduction in the renin maximal response to RU 24969 in rats pretreated with the 5-HT_{1C}/5-HT_2 antagonist LY53857 (ICV). RU 24969 caused a significant elevation of both PRA ($F_{(3,60)}$=8.13, P<0.0001) and PRC ($F_{(3,60)}$=13.23, P<0.0001). In animals pretreated with LY53857, there was a significant decrease in the renin response to RU 24969 (PRA: $F_{(1,60)}$=8.97, P<0.005; PRC: $F_{(1,60)}$=17.97, P<0.0001). Although there was not a significant interaction between LY53857 and RU 24969 on the PRA response, post-hoc analysis revealed that at the highest dose of RU 24969, there was a significant decrease in PRA caused by LY53857 (P<0.05). LY53857 produced a significant reduction in the PRC response to RU 24969 (interaction $F_{(3,60)}$=2.21, P<0.05).

In figure 4.2, RU 24969 administered centrally (ICV, 10-200 μg/kg) elevated plasma renin in a dose-dependent manner (PRA, $F_{(3,64)}$=15.0, P<0.0001; PRC, $F_{(3,64)}$=11.84, P<0.0001). Post-hoc Newman Keuls' analysis showed that the 100 and 200 μg/kg doses of RU 24969 significantly increased PRA compared to saline pretreated rats (P<0.05 and P<0.01 respectively). In LY53857 pretreated rats, only the highest dose of RU 24969 produced an effect significantly above saline (P<0.01). PRC was significantly elevated at the highest dose of RU 24969 regardless of LY53857 treatment.

To determine cardiovascular mechanisms, mean arterial blood pressure was measured (fig. 4.3). ICV injections of RU 24969 caused a significant

elevation ($P<0.05$) in blood pressure at 5 minutes-post injection, compared to blood pressures at time 0 or 30 minutes post-injection. The same dose injected ia did not cause a significant elevation in mean blood pressure. By 30 minutes, the time at which blood was collected for the previous two experiments, blood pressure had normalized. There were no significant changes in heart rate between any of the groups during the 30 minute recording session. However, there was a reduction in heart rate following ICV administration of RU 24969, which mirrored the ICV BP elevation.

P-chloroamphetamine

Our working hypothesis is that PCA elevates plasma renin levels through a serotonergic mechanism. To confirm this, the ability of fluoxetine to inhibit the renin elevating effect of PCA was tested. As shown in figure 4.4, ip administration of PCA caused a significant increase in both PRA and PRC, compared to saline treated controls (Newman Keuls' test $P<0.01$). Pretreatment with the 5-HT uptake inhibitor fluoxetine blocked the PRA (interaction, $F_{(1,26)}=10.07$, $P<0.005$) and PRC (interaction, $F_{(1,26)}=21.87$, $P<0.0001$) response to PCA. Fluoxetine alone had no significant effect on renin secretion.

PCA was injected into the lateral cerebral ventricle (ICV), using doses below those peripherally effective. There was no significant change in either PRA or PRC at any dose of PCA (fig. 4.5). LY53857 had no effect on the renin response to PCA.

To determine if cardiovascular mechanisms were blunting the renin response, blood pressure and heart rate were measured following ICV injection of PCA. PCA caused a significant increase in blood pressure after ICV administration ($F_{(5,48)}=11.85$, $P<0.0001$). As shown in the top

panel of figure 4.6, there was a rapid and robust blood pressure elevation at 2 and 5 minutes (Newman Keuls' $P<0.01$). The maximal response was approximately 45 mm Hg above the baseline vehicle injected controls. When administered peripherally into the femoral artery the same dose of PCA elevated blood pressure only 10 mm Hg at 5 minutes. The lower panel of figure 4.6 shows changes in heart rate after PCA administration. When injected ICV, there was a rapid but non-significant increase in heart rate which paralleled the blood pressure response seen in the upper panel. Following this initial tachycardia, heart rate slowed at 15 minutes, then increased again by 60 minutes (Newman Keuls' test $P<0.05$, compared to 15 minutes). Vehicle (saline) injected ICV caused no changes in either blood pressure or heart rate.

To determine if vasoconstriction due to PCA was masking a stimulatory effect on renin secretion, the α_1 antagonist prazosin was administered (sc) prior to PCA injection (ICV). As shown in figure 4.7, PCA again produced a non-significant reduction in both PRA and PRC when administered alone into the brain ventricles. Prazosin alone caused a significant elevation (320%) in plasma renin activity compared to vehicle/saline controls ($P<0.05$). An increase was also observed in PRC (230%), although this did not reach statistical significance. The combination of prazosin pretreatment plus PCA caused an additional significant ($P<0.01$) increase (greater than 315% for both PRA and PRC) in the renin response above prazosin alone (PRA interaction, $F_{(1,32)}=9.86$, $P<0.005$; PRC interaction, $F_{(1,33)}=11.02$, $P<0.005$).

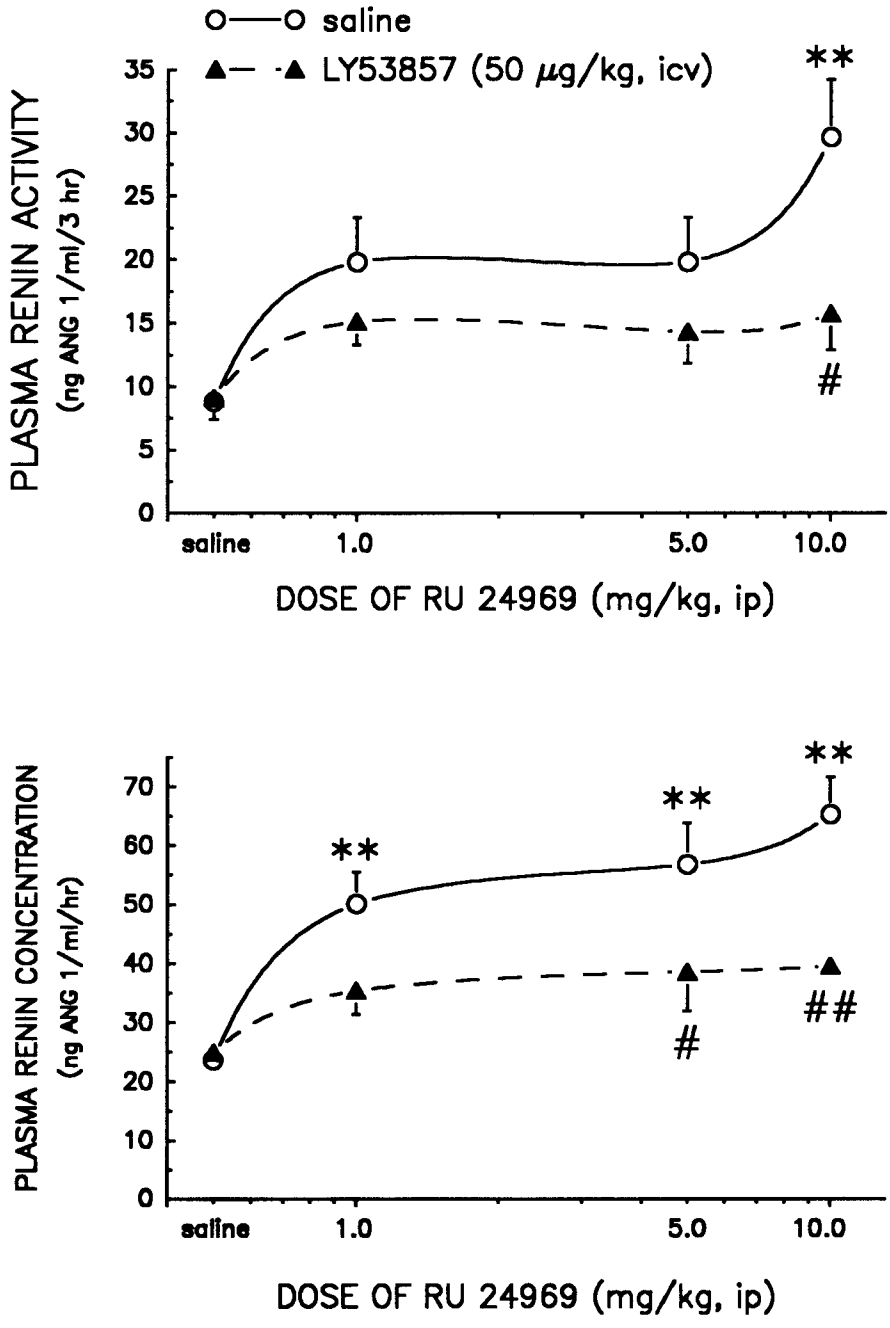

Figure 4.1. Effect of peripherally administered RU 24969 on plasma renin activity (top) and concentration (bottom) in rats pretreated with LY53857 (ICV). Data represent mean ± S.E.M. of 8 rats per group. ** Significant difference from corresponding saline (0 dose of RU 24969) group, $P<0.01$; # or ## significant difference from corresponding RU 24969-saline pretreated group, $P<0.05$ or $P<0.01$ respectively (2-way ANOVA and Newman Keuls' test).

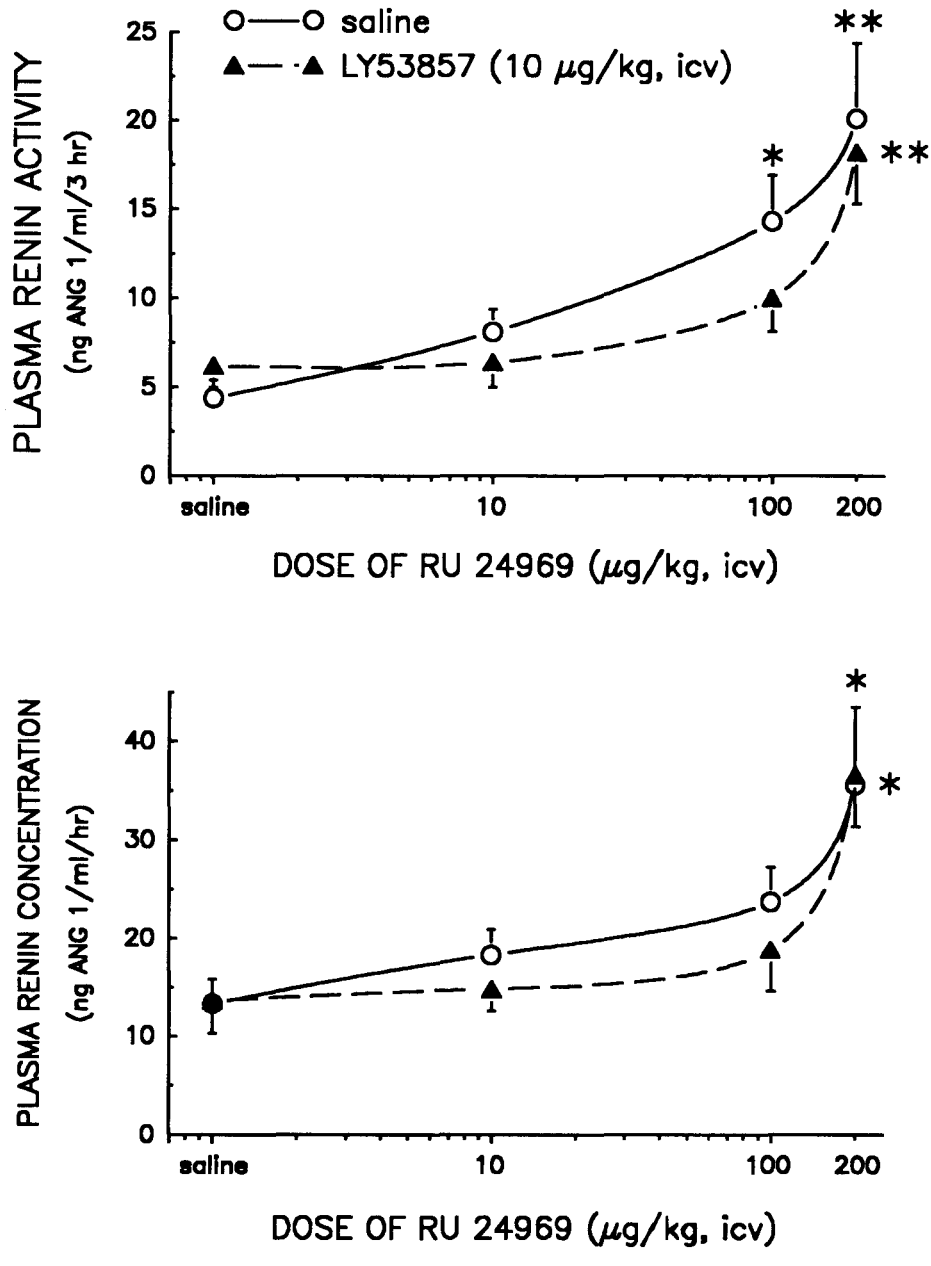

Figure 4.2. Effect of ICV administered RU 24969 on plasma renin activity (top) and concentration (bottom) in rats pretreated with LY53857 (ICV). Data represent mean ± S.E.M. of 8 rats per group. * or ** Significant difference from corresponding saline group, $P<0.05$ or $P<0.01$ respectively (2-way ANOVA and Newman Keuls' test).

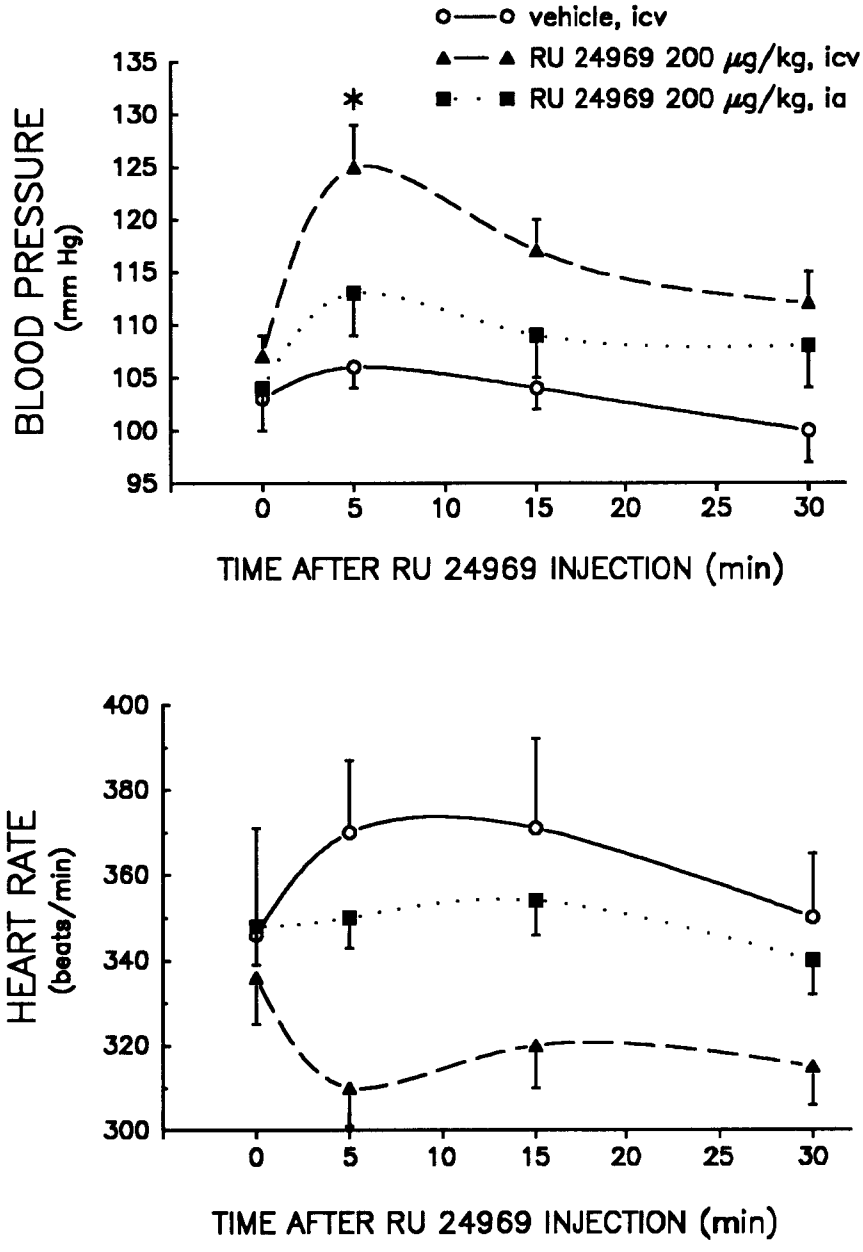

Figure 4.3. Cardiovascular effects of RU 24969 following central or peripheral administration. RU 24969 was injected either ICV or ia at time 0 to conscious male rats. Data represent mean ± S.E.M. of 8 rats per group. * Significant difference from corresponding vehicle (saline) group, $P<0.05$ (1-way ANOVA, repeated measures and Newman Keuls' test).

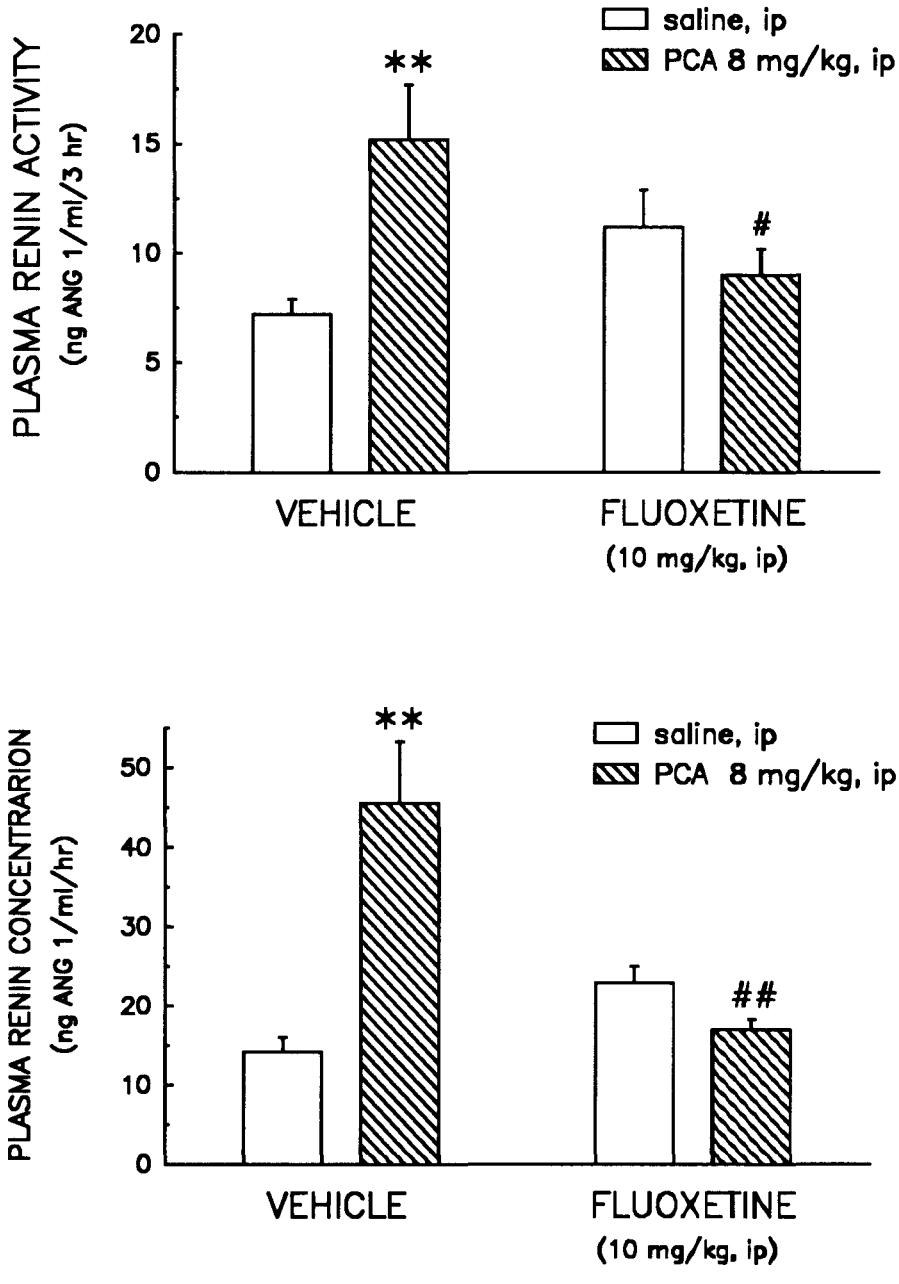

Figure 4.4. Fluoxetine blocks PCA stimulation of plasma renin activity (top) and concentration (bottom). Data represent mean ± S.E.M. of 8 rats per group. ** Significant difference from corresponding saline (0 dose of PCA) group, $P<0.01$. # or ## Significant difference from corresponding PCA-vehicle group, $P<0.05$ or $P<0.01$, respectively (2-way ANOVA and Newman Keuls' test).

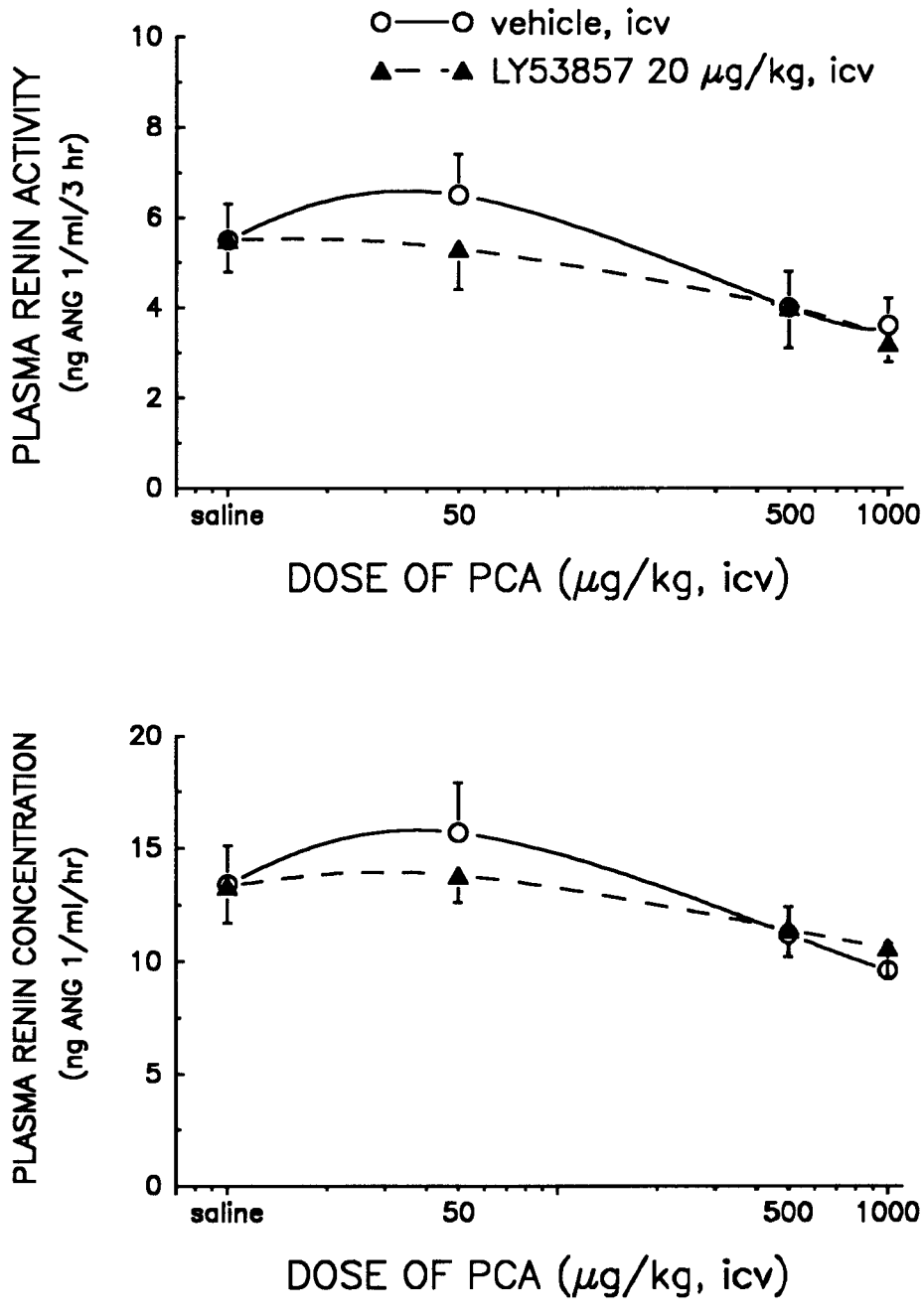

Figure 4.5. Effect of centrally administered PCA on plasma renin activity (top) and concentration (bottom). Data represent mean ± S.E.M. of 8 rats per group.

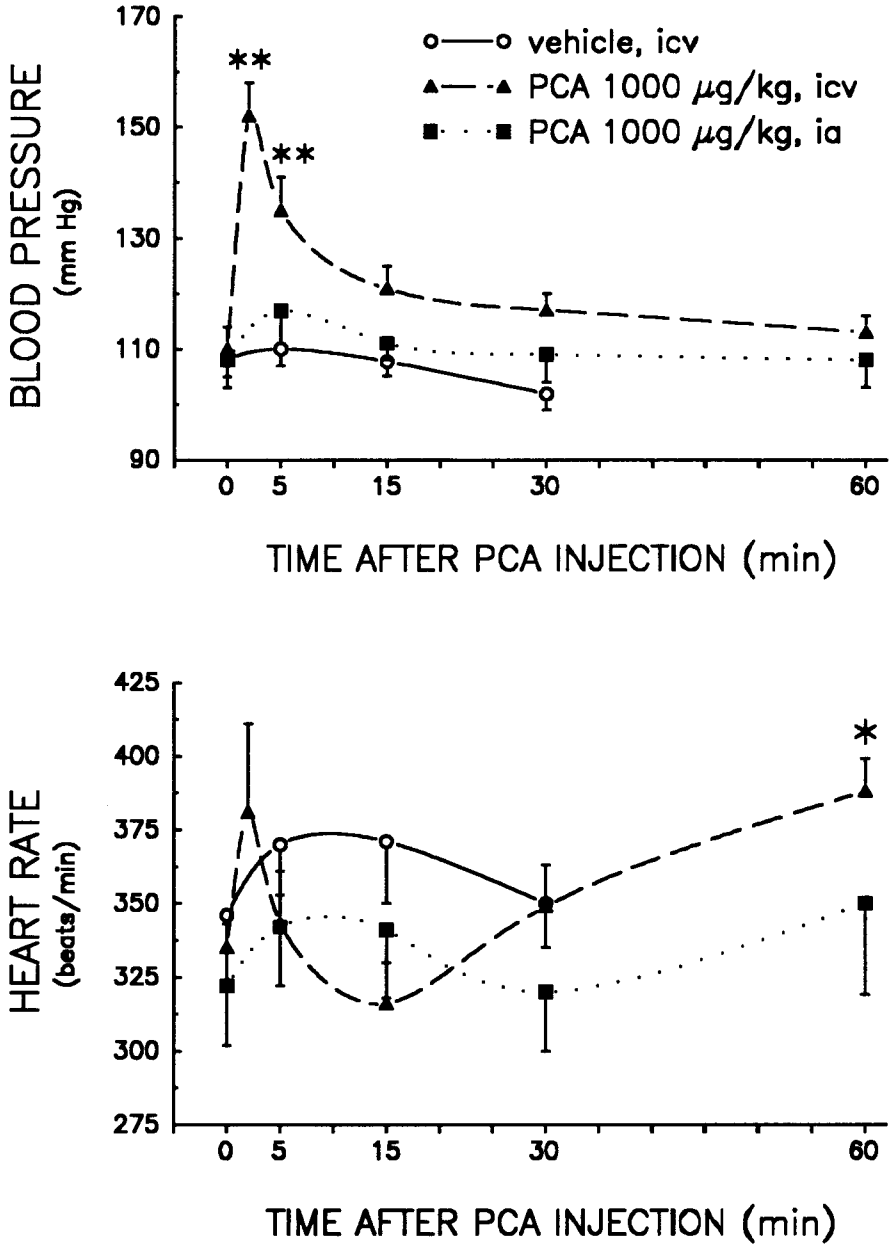

Figure 4.6. Cardiovascular effects of PCA following central or peripheral administration. PCA was injected either ICV or ia at time 0 to conscious male rats. Data represent mean ± S.E.M. of 8 rats per vehicle (saline) group, 4 rats per PCA-ia group, and 9 rats per PCA-ICV group. * or ** Significant difference from corresponding vehicle group, $P<0.05$ or $P<0.01$ respectively (1-way ANOVA, repeated measures and Newman Keuls' test).

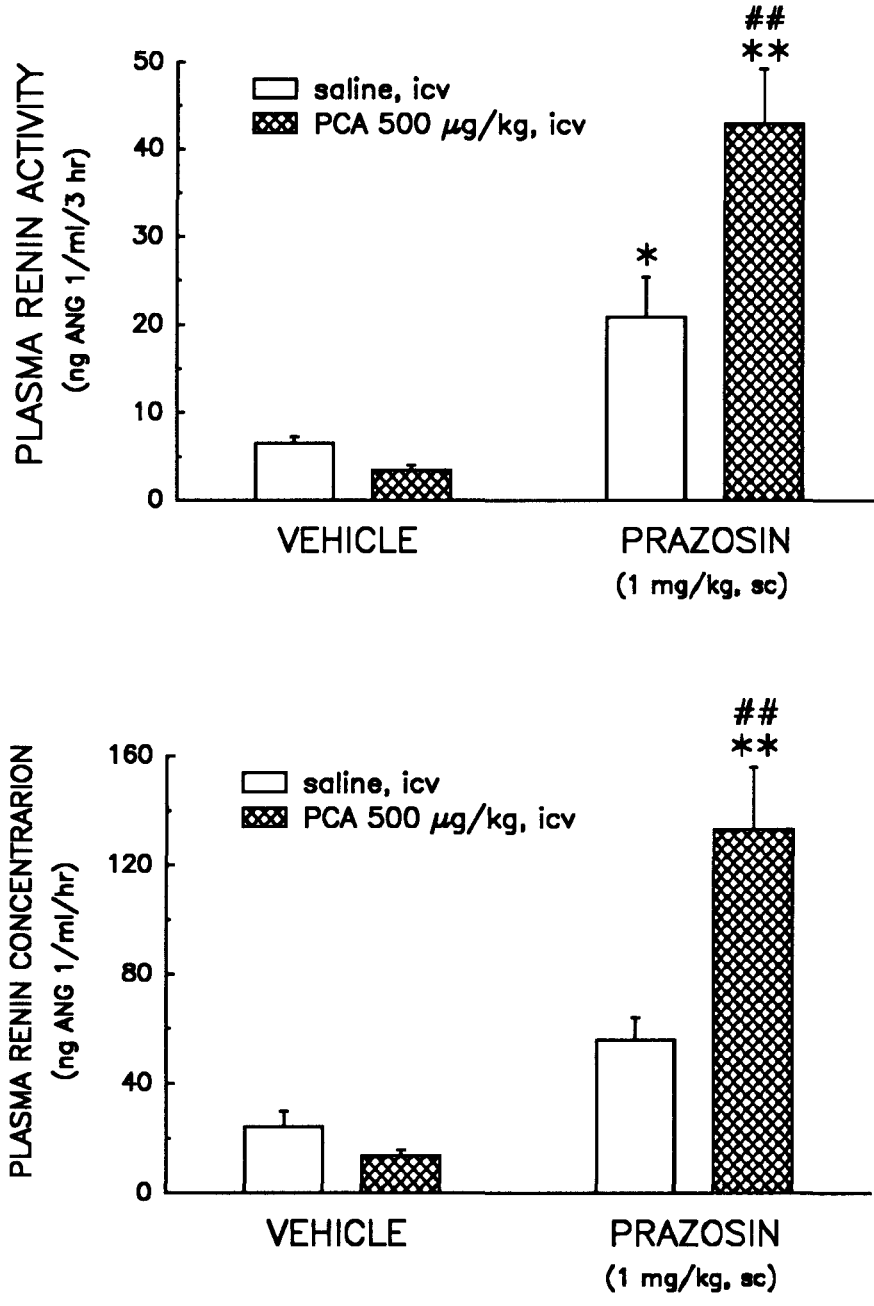

Figure 4.7. Prazosin exposes the stimulatory effect of PCA on plasma renin activity (top) and concentration (bottom). Data represent mean ± S.E.M. of 8 rats per group. * or ** Significant difference from corresponding vehicle (0 dose of PCA) group, $P<0.05$ or $P<0.01$ respectively. ## Significant difference from corresponding prazosin-saline group, $P<0.01$ (2-way ANOVA and Newman Keuls' test).

DISCUSSION

Evidence from this investigation indicates that brain serotonin pathways can stimulate renin release from the kidney. The results show that the 5-HT agonist RU 24969 increased renin secretion through activation of 5-HT receptors located in the brain. The results also demonstrate that the 5-HT releaser PCA acting centrally, can stimulate renin secretion, if its vasoconstrictive effects are inhibited with an α_1 antagonist.

The focus of this study was to investigate whether 5-HT in the brain is an important regulator of renin secretion. RU 24969 and PCA were used as tools to test this hypothesis. Both of these drugs are known to elevate plasma renin levels if injected peripherally (Van de Kar, 1991).

RU 24969

Central administration of the 5-HT_{1C}/5-HT_2 antagonist LY53857 produced a clear shift to the right in the renin dose-response curve, as well as a reduction in the maximum effect of ip injected RU 24969. These data suggest that RU 24969 injected peripherally stimulates renin secretion through 5-HT_2 receptors located in the brain. The possibility exists that at 50 μg/kg, LY53857 could have "leaked" out of the brain to antagonize peripheral 5-HT_2 receptors. However, in a previous study (Van de Kar et al., 1989) a dose of 1.0 mg/kg LY53857 (ip) inhibited, but did not completely block, the effect of RU 24969 on renin secretion. Since this dose was 20 times higher and injected peripherally, it seems unlikely that our dose of 50 μg/kg LY53857 injected ICV would be large enough to reach

peripheral targets.

When RU 24969 was injected directly into the brain ventricles, using doses below those which are peripherally effective, there was a dose-related increase in renin secretion (both PRA and PRC). These data also demonstrate that centrally located 5-HT receptors are mediating the renin response. A low dose of LY53857 was used to maintain receptor subtype selectivity. While there was a "suggestion" of a shift in the curve to the right, the antagonist dose used might not have been high enough.

The rationale for using a 5-HT_2 antagonist (LY53857) to inhibit the renin response to a 5-HT_{1A}/5-HT_{1B} agonist (RU 24969) was based on two lines of evidence. First, previous studies have verified that renin is stimulated by activation of 5-HT_2 receptors (Bagdy et al., 1992; Rittenhouse et al., 1991; Alper, 1990a). Secondly, Van de Kar et al. (1989) were able to inhibit the renin response to peripherally administered RU 24969 with two different 5-HT_2 antagonists, namely LY53857 and ritanserin. The explanation as to why the effect of RU 24969 can be blocked by 5-HT_2 antagonists was provided by Lyon et al. (1987) who showed that RU 24969 binds with 20 times higher affinity to the 5-HT_2 high affinity site (K_i=42nM; labeled with ^{3}H-DOB) compared to the 5-HT_2 low affinity site (K_i=777nM; labeled with ^{3}H-ketanserin). Thus RU 24969 should also be considered a 5-HT_2 agonist.

Auerbach et al. (1990) have proposed that RU 24969 functions as a presynaptic 5-HT releaser in the hippocampus. However, with regards to renin regulation, we have not observed this. Van de Kar et al. (1989) found that the 5-HT neurotoxin 5,7-DHT produced a leftward shift in the renin dose-response curve to RU 24969, indicating a sensitization of post-

synaptic 5-HT receptors or receptor mediated events. Those results also demonstrate that RU 24969 must be acting in the brain to stimulate renin secretion. The present results confirm the previous findings.

Elevated BP is a potent stimulus to inhibit renin secretion due to a reflex stretch receptor mechanism located in the kidney (Tobian et al., 1959; Hackenthal et al., 1990). RU 24969 elevated BP when administered ICV. Thus, it is probable that RU 24969 is stimulating renin secretion independently of a cardiovascular mechanism. While iv administration caused a decrease in BP (Cherqui et al., 1988), clearly in our hands RU 24969, administered centrally, caused an elevation. This discrepancy could be explained by the location or subtype of 5-HT receptors stimulating BP. A previous report suggested that 5-HT_1 agonists cause hypotension when administered onto the intermediate area of the ventrolateral surface of the medulla (Gillis et al., 1989), presumably by decreasing sympathetic output to the spinal cord (Saxena and Villalón, 1990). In contrast, 5-HT_2 agonists were reported to increase sympathetic nerve discharge and blood pressure (McCall and Harris, 1988; McCall et al., 1987). Rittenhouse et al. (1991) found that DOI, a potent 5-HT_2 agonist, increased both blood pressure and renin secretion when administered ICV, in agreement with the current findings. Thus, it is possible that ICV injected RU 24969 is increasing BP and renin through brain 5-HT_2 receptors, but located at a different sites. Regardless, the fact that both renin and blood pressure are elevated in the same direction suggests that 5-HT receptor mediated mechanisms are being activated which are able to overcome any baroreflex stimulation (as evidenced by the lower heart rate following ICV RU 24969 injection). If RU 24969 is stimulating

both BP and renin through activation of 5-HT_2 receptors in the brain, it may explain why there was not a reflex response to lower renin secretion. Alternatively, RU 24969 could well be producing a pressor response via a 5-HT independent mechanism.

P-chloroamphetamine

Fluoxetine selectively inhibits presynaptic 5-HT uptake (Fuller et al., 1991), and PCA is a presynaptic 5-HT releaser which enters the terminal through this uptake site. Previous studies have shown that fluoxetine blocks the PCA-induced release of brain 5-HT (Fuller et al., 1975; Marsden et al., 1979). Our control experiment revealed that fluoxetine also prevented PCA-induced renin secretion (fig. 4.4). These results demonstrate that the increase in renin secretion due to PCA is mediated via 5-HT and not other neurotransmitters.

Renin secretion was not stimulated by ICV administered PCA. Since PCA consistently stimulates renin secretion when injected peripherally (ip), we performed experiments to investigate whether elevated blood pressure could be masking a renin response to PCA. PCA is known to elevate blood pressure (Alper et al., 1987; Stein et al., 1987) which, as mentioned above, can cause inhibition of renin secretion through activation of the renal stretch receptor. Thus, after ICV injection of PCA, blood pressure and heart rate were measured to determine if a cardiovascular mechanism was inhibiting the renin response. Within 2 minutes, PCA caused a robust increase in BP if administered ICV, but caused no significant alteration when injected ia. This suggested to us that the increase in BP resulting from central administration of PCA could cause the inhibition of renin secretion. Additionally, since heart rate was elevated at 60 minutes

post-injection but blood pressure was not, there was an indication that the sympathetic nervous system had been activated.

The α_1 antagonist prazosin is recognized to cause hypotension via blockade of arteriolar α_1 receptors (Cavero and Roach, 1980). PCA causes an increase in sympathetic outflow (Alper and Ganong, 1984), and a pressor response which can be inhibited by prazosin pretreatment (Alper et al., 1987). Consequently, the pressor effect of PCA is thought to be mediated by the sympathetic nervous system. Alper et al. (1990) reported that if prazosin preceded a higher dose of iv injected PCA (6.0 mg/kg), then a substantial renin response was exposed. Therefore, we postulated that prazosin could also antagonize the hypertensive, and thus, the inhibitory effects of ICV administered PCA, using a dose 10-fold (500 μg/kg) lower than the iv dose used by Alper. The results shown in figure 4.4 support this conclusion. By masking the vasoconstrictive and pressor effects with the α_1 antagonist prazosin, PCA's central stimulatory effect on renin was revealed.

The studies performed here, while demonstrating that brain 5-HT mediates renin secretion from the kidney, do not characterize the mechanism of action. Centrally administered 5-HT has been shown to elevate BP and increase Na^+ excretion (Montes and Johnson, 1990; Stein et al., 1987). Pérgola and Alper (1991) postulated that the increase in BP caused by ICV 5-HT may be due to the release of vasopressin. Centrally administered PCA has also been shown to raise BP and stimulate Na^+ excretion (Stein et al., 1987), although it apparently does not cause neurodegeneration (Berger et al., 1990). Ikonomov et al. (1991) suggested that peripherally injected PCA produces increased urine excretion prior to

the increase in renin secretion. Collectively, these studies suggest that PCA's mechanism of action may be complicated by either direct or indirect action on the kidney, and that the increase in renin secretion may occur secondarily to elevated Na^+ excretion.

In the present investigation, with the exception of the fluoxetine control experiment, all of our studies entailed ICV injection of PCA well below peripherally effective doses. While this does not eliminate a direct action of PCA on the kidney, it argues in favor of a central site of action to increase renin release. Previous reports have provided evidence in support of a central site of action for PCA (Van de Kar et al., 1981; Karteszi et al., 1982; Van de Kar et al., 1982). Furthermore, Gotoh et al (1987) found that electrolytic lesions in either the paraventricular or ventromedial nucleus of the hypothalamus inhibited the renin response to ip injected PCA.

In conclusion, our results are consistent with the hypothesis that a serotonin agonist and releaser stimulate renin secretion by activating 5-HT receptors in the brain. We are currently investigating which hypothalamic nucleus is responsible for the renin response to RU 24969 and PCA.

CHAPTER V

NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS MEDIATE THE SEROTONERGIC STIMULATION OF RENIN SECRETION

INTRODUCTION

The regulation of renin release has a prominent central component. In the brain, different sites have been proposed which could influence renin secretion from the kidney. The hypothalamic PVN (Porter, 1988; Richardson Morton et al., 1989) and the VMN (Gotoh et al., 1987; 1988) have both been implicated. Dorsal raphé neurons innervate the hypothalamic PVN (Swanson and Sawchenko, 1983), where serotonin nerve terminals have been located (Liposits et al., 1987; Pazos and Palacios, 1985). Functions of the PVN include secretion of vasopressin and oxytocin (Swanson and Sawchenko, 1983), cardiovascular regulation (Ciriello et al., 1984; Zhang and Ciriello, 1985; Takeda et al., 1991; Herzig et al., 1991), feeding behavior/energy balance (Leibowitz, 1991) and mediation of the renin stress response (Richardson Morton et al., 1989). The VMN is an important regulator of weight/energy balance (Tokunaga et al. 1986; Parkinson and Weingarten, 1990), thermoregulation (Amir, 1990), lordosis (Schulze and Gorzalka, 1991) and has been implicated in cardiovascular function

(Valladao et al., 1990). The dorsomedial nucleus, located adjacent to the paraventricular and ventromedial nuclei, may also subserve feeding and growth regulation (Bernardis et al., 1988) as well as mediating cardiac stress responses (Anderson and DiMicco, 1990).

To determine which nucleus is responsible for regulation of renin secretion, we destroyed cells in each nucleus with ibotenic acid. We utilized ibotenic acid because it destroys cell bodies while leaving fibers of passage intact (Markowska et al., 1985). To limit surrounding tissue damage, very low concentrations of ibotenic acid were used (Fitzsimons and Ciriello, 1986; Richardson Morton et al., 1989). By making discrete lesions with a cell selective neurotoxin rather than ablating both fibers and cell bodies electrolytically, we aimed to resolve which nucleus contained the receptors necessary for this endocrine control.

Peripherally administered serotonin (5-HT) agonists and releasers reliably elevate plasma levels of renin (Van de Kar, 1991). As described previously, RU 24969 is an indole derivative considered to be a 5-HT_{1A}/5-HT_{1B} agonist (Glennon, 1987), but also appears to act at 5-HT_2 receptors. PCA releases both serotonin and catecholamines from nerve terminals (Marsden et al., 1979; Sharp et al., 1986). However, evidence suggests that both RU 24969 and PCA increase renin secretion through activation of central 5-HT receptors (Van de Kar et al., 1981; 1989). The increase in renin caused by PCA is mediated by 5-HT released from neurons originating in the dorsal raphé and terminating in the hypothalamus (Karteszi et al., 1982; Van de Kar et al., 1981 & 1982). These studies demonstrate that 5-HT neurons in the brain can increase renin secretion from the kidney.

Therefore, the purpose of these investigations was to determine if 5-HT receptors on cells in the hypothalamic PVN, or another adjacent site, mediate the renin response to RU 24969 and PCA in conscious male rats.

METHODS

Animals

Male Sprague-Dawley rats (275-300g) were purchased from Sasco-King Animal Laboratories (Oregon, WI). The rats were housed 2 per cage in a climate and illumination (12:12 hr light/dark cycle, lights on at 7:00 AM) controlled room. Water and food (Wayne Lab Blox, Lab Mills Inc., Chicago, IL) were available *ad libitum*. All experiments were conducted between 11:00 A.M. and 3:00 P.M. All protocols were approved by the Loyola University Animal Care and Use Committee, and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

Ibotenic acid injections into the hypothalamus were performed under pentobarbital (50 mg/kg, ip) anesthesia. Animals were pretreated with ampicillin (50 mg/kg, sc) to prevent infection and atropine methyl bromide (0.2 mg/kg, ip) to reduce excessive secretions. The vehicle used for ibotenic acid was 50 mM sodium phosphate buffer, pH 7.2.

Ibotenic acid was injected at a concentration of 10 $\mu g/\mu l$, in a volume of 0.3 μl, (Fitzsimons and Ciriello, 1986) bilaterally over a 10 minute period using a 33 gauge guide cannula mounted on a David Kopf stereotaxic apparatus. Lesions were made in three separate hypothalamic sites: 1.) the PVN, using the coordinates 0.6 mm lateral from lambda, 8.8 mm ventral from the skull surface, 5.7 mm rostral to lambda; 2.) the DMN, with coordinates 0.8 mm lateral from lambda, 9.2 mm ventral from the skull surface, 5.2 mm rostral to lambda; and 3.) the VMN, with coordinates 0.6

mm lateral from lambda, 10.2 mm ventral from the skull surface, 4.8 mm rostral to lambda (Paxinos and Watson, 1986). Rats were allowed two weeks recovery after the surgery.

Protocol

Following two weeks recovery from surgery, conscious rats were administered either RU 24969 (5.0 mg/kg, ip) 30 minutes prior to decapitation or PCA (8.0 mg/kg, ip) 60 minutes prior to decapitation. Control rats received saline injections. These time points were established with previous time-course studies (Van de Kar et al., 1981; 1989). Trunk blood was collected for radioimmunoassay of renin, and brains saved in buffered formalin for histological verification of lesions.

Histology

Coronal sections (40 μm) were cut on a vibratome, stained with cresyl violet and mounted on acid cleaned glass slides. All slides were inspected by two investigators who were kept unaware of the hormone data. Only animals with histologically confirmed bilateral lesions were used in the data analysis.

Drugs

PCA and ibotenic acid were purchased from Sigma Chemical Co. (St. Louis, MO). RU 24969 was donated by Roussel Uclaf (Romainville, France). Both PCA and RU 24969 were dissolved in 0.9% saline.

Radioimmunoassays

Plasma renin activity. Plasma renin activity (PRA) was measured by RIA for generated ANG I, as previously described (Richardson Morton et al., 1989). Briefly, 1.0 ml plasma samples were incubated for 3 hours at 37°C to generate ANG I. The antiserum against ANG I was used at a dilution of 1:16,000 with total binding of 30%. The sensitivity limit of the RIA is 10 pg ANG I per tube. Intra-assay variability was 4.4%, with inter-assay variability 12.6% (Richardson Morton et al., 1989). PRA is a reflection of the activity of the native enzyme renin in a given plasma sample to generate ANG I from endogenous substrate (angiotensinogen). It is therefore substrate dependent.

Plasma renin concentration. Plasma renin concentration (PRC) was determined by addition of exogenous renin substrate (0.1 ml of plasma from nephrectomized, dexamethasone injected rats) to 50 μl plasma samples and incubating at 37°C for 1 hour. The RIA of ANG I was the same as described above for plasma renin activity. PRC represents the maximal response possible since a saturating concentration of renin substrate is added to each plasma sample, and thus is substrate independent.

We run both PRA and PRC assays to distinguish substrate dependent from substrate independent ANG I production, and also to confirm and strengthen our renin results.

Statistics

The number of rats per group is described in each figure legend. RIA data were analyzed using RIA_AID software (Robert Maciel Associates, Arlington, MA). Statistical analysis of the data was performed using

computer software (STATPAC, NWA, Portland, OR) by 2-way analysis of variance (ANOVA). The group means were compared by Newman Keuls' multiple range test (Steel and Torrie, 1960).

RESULTS

These experiments were designed to establish which hypothalamic nucleus mediates the RU 24969 and PCA-induced stimulation of renin secretion. Discrete lesions were made in the PVN with ibotenic acid, a cell selective neurotoxin (Markowska et al., 1985). Figure 5.1A-D shows the extent of the PVN lesion. A brain section from a vehicle treated rat is shown in panel A; note the termination of the cannula tracts at the dorsal border of the PVN. Panel B shows the extent of an ibotenic acid lesion confined entirely within the PVN; note the very limited amount of damage to surrounding tissue. In panel C compare the large magnocellular neurons of a vehicle treated rat, to an ibotenic acid lesion in panel D where there is nearly a complete absence of cell bodies, but again no damage to the remaining tissue.

Figure 5.2 shows that RU 24969 caused a significant increase of both PRA ($F_{(1,34)}$=44.91, P<0.0001) and PRC ($F_{(1,33)}$=72.6, P<0.0001). Ibotenic acid also had a main effect, causing a reduction in PRA ($F_{(1,34)}$=4.47, P<0.05) and PRC ($F_{(1,33)}$=4.49, P<0.05). Post-hoc analysis revealed that the renin response to RU 24969 was significantly impaired (PRA 46% and PRC 37%) in rats with histologically verified PVN lesions compared to vehicle treated, RU 24969-injected controls (Newman Keuls' test P<0.05).

Figure 5.3 shows that PCA also produced a significant increase in PRA ($F_{(1,35)}$=4.87, P<0.05) and PRC ($F_{(1,34)}$=5.42, P<0.05). Ibotenic acid again caused a reduction in PRA ($F_{(1,35)}$=11.88, P<0.005) but not PRC. In rats with histologically verified PVN lesions, ibotenic acid caused a complete blockade of the PRA response to PCA (interaction: $F_{(1,35)}$=11.88, P<0.005).

Post-hoc analysis showed that the PRA and PRC responses to PCA were both significantly reduced (PRA >100% and PRC 77%) in animals with PVN lesions, compared to vehicle treated controls (Newman Keuls' test $P<0.01$ and $P<0.05$, respectively).

The success rate of ibotenic acid lesions in the PVN varied with each group. In the RU 24969 experiment, 13 of 19 RU 24969 treated, and 6 of 14 saline treated rats had confirmed ibotenic acid lesions in the PVN. In the PCA experiment, just 6 of 22 PCA treated, and 8 of 22 saline treated rats had confirmed ibotenic acid lesions in the PVN. The primary reason for the low rate of confirmed lesions was that we used such small volumes in attempting to limit damage within the PVN boundaries, that only a precise hit resulted in a complete lesion. Only renin data from those rats with confirmed lesions in the PVN are shown in figures 5.2 and 5.3. The remaining data from rats with incomplete PVN lesions are summarized in Table I. These were considered to have only partial or unilateral damage in the PVN. There was no significant inhibition of the renin response to RU 24969 or PCA if PVN lesions were incomplete (Table I).

Figure 5.4 shows coronal sections through the dorsomedial and ventromedial hypothalamus. A brain from a vehicle treated rat is shown in Panel A. An ibotenic acid lesion in the DMN of the hypothalamus is shown in figure 5.4B, with a lesion in the VMN shown in figure 5.4C. Note that cell damage is confined to each specific nucleus, while the surrounding tissue remains intact.

As shown in figure 5.5, PCA caused a significant elevation in PRA ($F_{(1,31)}=9.05$, $P<0.005$) and PRC ($F_{(1,30)}=10.41$, $P<0.005$) in both vehicle treated rats and rats with histologically confirmed DMN lesions. There

was no difference between either group of animals that received PCA injections. Thus, lesions in this nucleus did not change the PCA-induced stimulation of renin secretion. There was actually a potentiation of the PRC response to PCA in rats with ibotenic acid lesions in this nucleus ($F_{(1,30)}$=5.05, $P<0.05$).

Finally, as with the DMN lesions, the PCA-induced stimulation of renin secretion remained unaltered in rats with VMN lesions. Ibotenic acid had no main effect, and there was no significant interaction between ibotenic acid and PCA. As shown in figure 5.6, PCA caused a significant elevation in PRA and PRC in both vehicle treated rats, and rats with histologically confirmed VMN lesions (PRA: $F_{(1,34)}$=20.30, $P<0.0001$; PRC: $F_{(1,34)}$=14.64, $P<0.0005$). Thus, there was no difference between either group of animals that received PCA injections.

The success rate for DMN and VMN ibotenic acid lesions was somewhat better than for the PVN. In the DMN experiment, 10 of 20 PCA treated and 9 of 16 saline treated rats had confirmed ibotenic acid lesions in the DMN. In the VMN experiment, 12 of 18 PCA injected rats and 10 of 16 saline injected rats had confirmed lesions in the VMN. The PRA and PRC data resulting from incomplete ibotenic acid lesions in the VMN or DMN were not statistically different from histologically confirmed VMN or DMN lesions. Since the two sets of data are virtually identical to each other, the PRA and PRC data from incomplete lesions in the DMN or VMN are not shown.

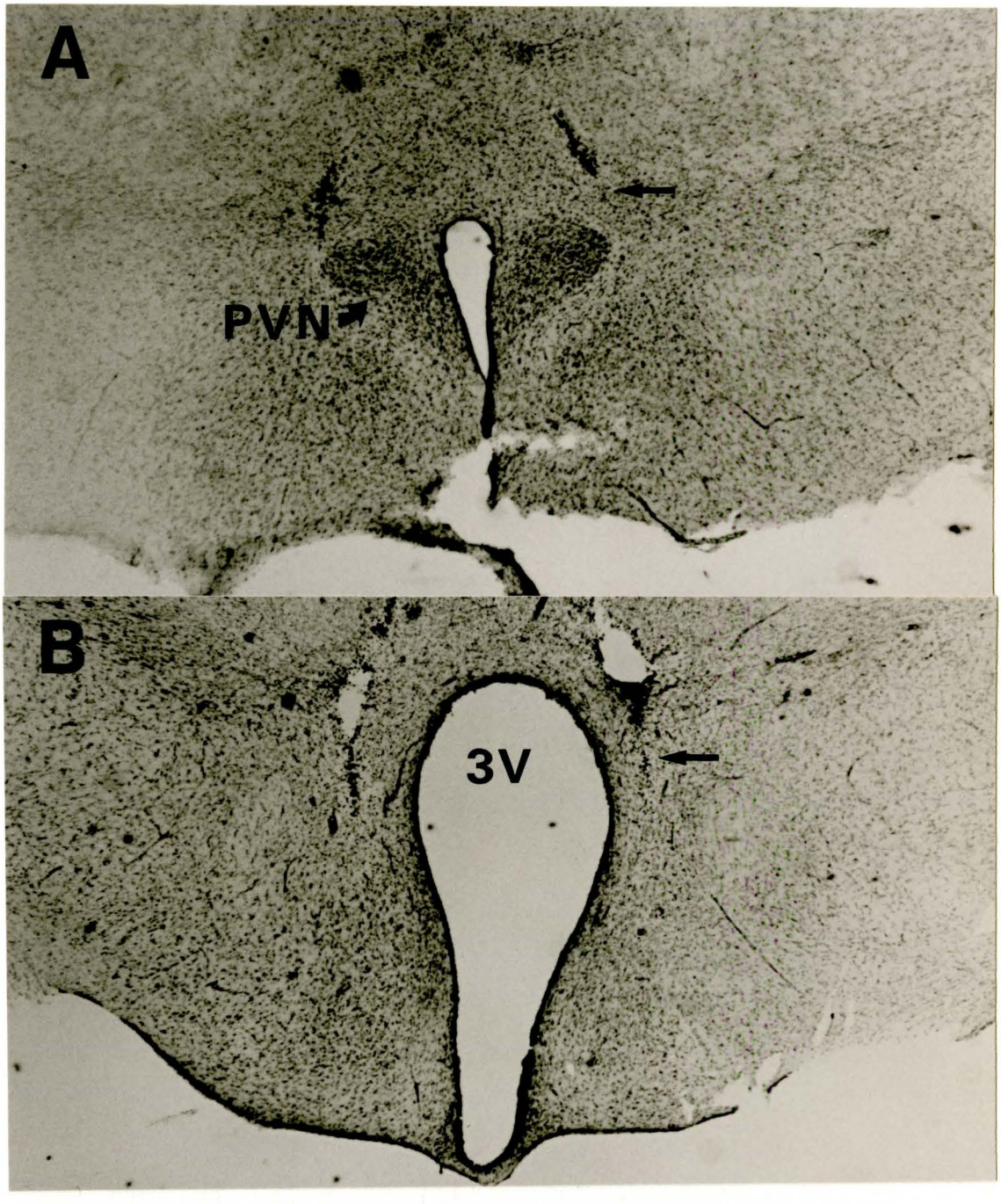

Figure 5.1. Histological verification of ibotenic acid lesions in the hypothalamic paraventricular nucleus. 40 μm coronal sections were stained with cresyl violet.

Panel A: PVN of the hypothalamus, vehicle treated. Cannula tips are located on dorsal border of PVN (straight arrow).

Panel B: Bilateral ibotenic lesion in PVN. Cannula tips are within PVN boundaries (arrow). 3V, third ventricle.

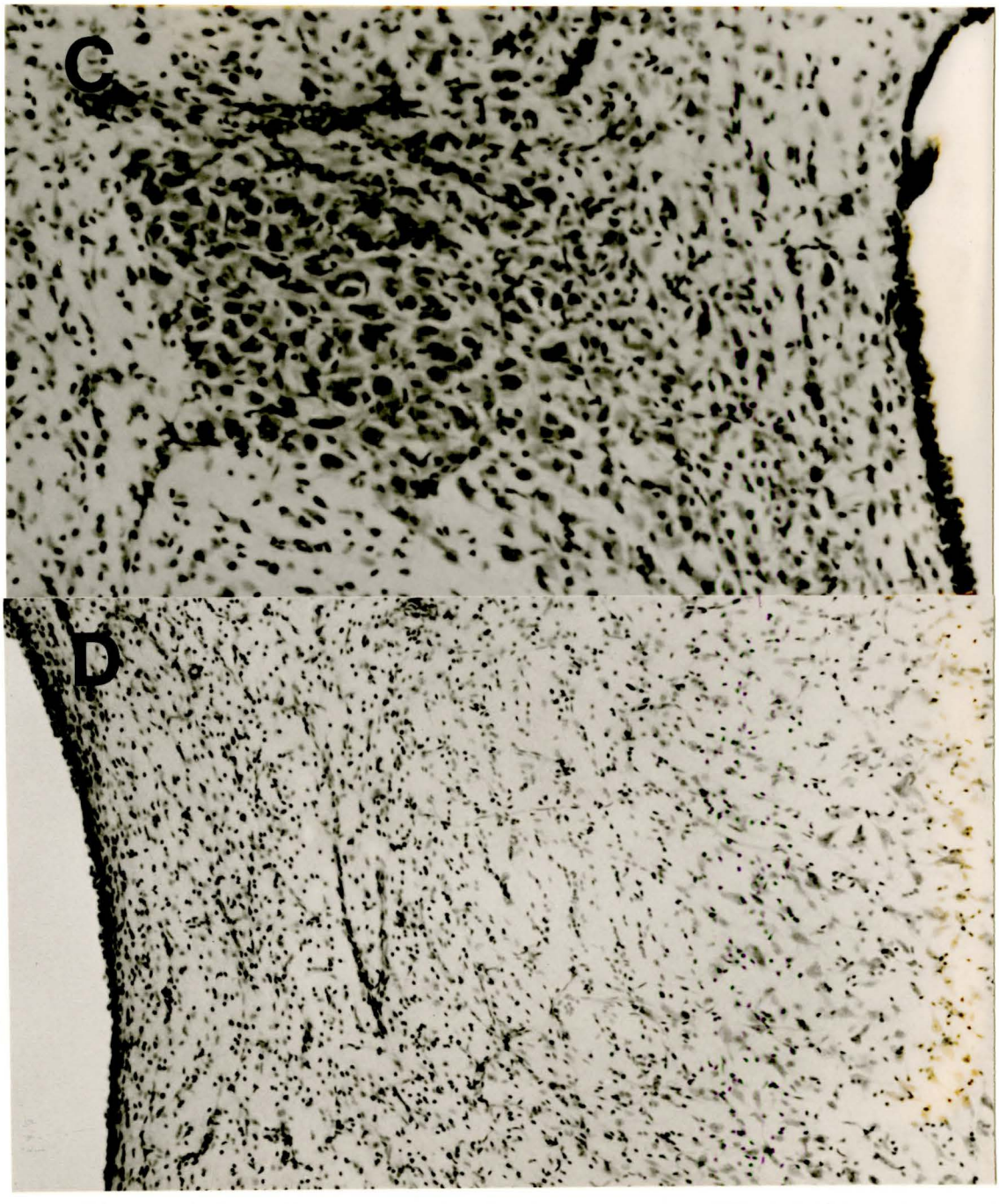

Figure 5.1. Histological verification of ibotenic acid lesions in the hypothalamic paraventricular nucleus. 40 μm coronal sections were stained with cresyl violet.
Panel C: Magnocellular neurons of PVN, vehicle treated.
Panel D: Ibotenic acid lesion in PVN: note absence of neurons.

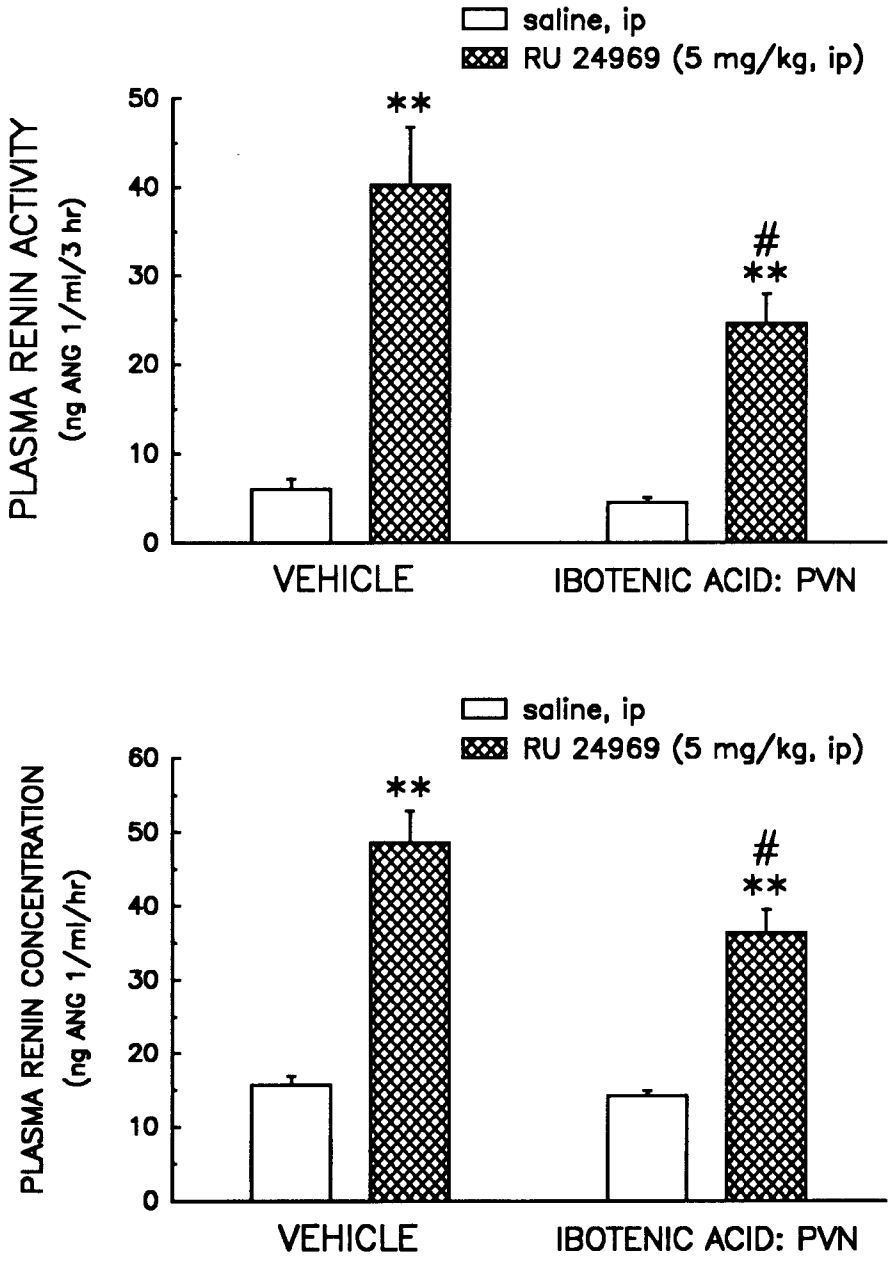

Figure 5.2. Ibotenic acid lesions in the PVN inhibit the renin response to RU 24969 (ip). Data represent mean ± S.E.M. of 8 rats per vehicle/saline and vehicle/RU groups, 6 rats in IBO/saline group, and 13 rats in IBO/RU group. ** Significant difference from corresponding saline (0 dose of RU) group, $P<0.01$. # Significant difference from corresponding vehicle (non-lesion) group, $P<0.05$ (2-way ANOVA and Newman Keuls' test).

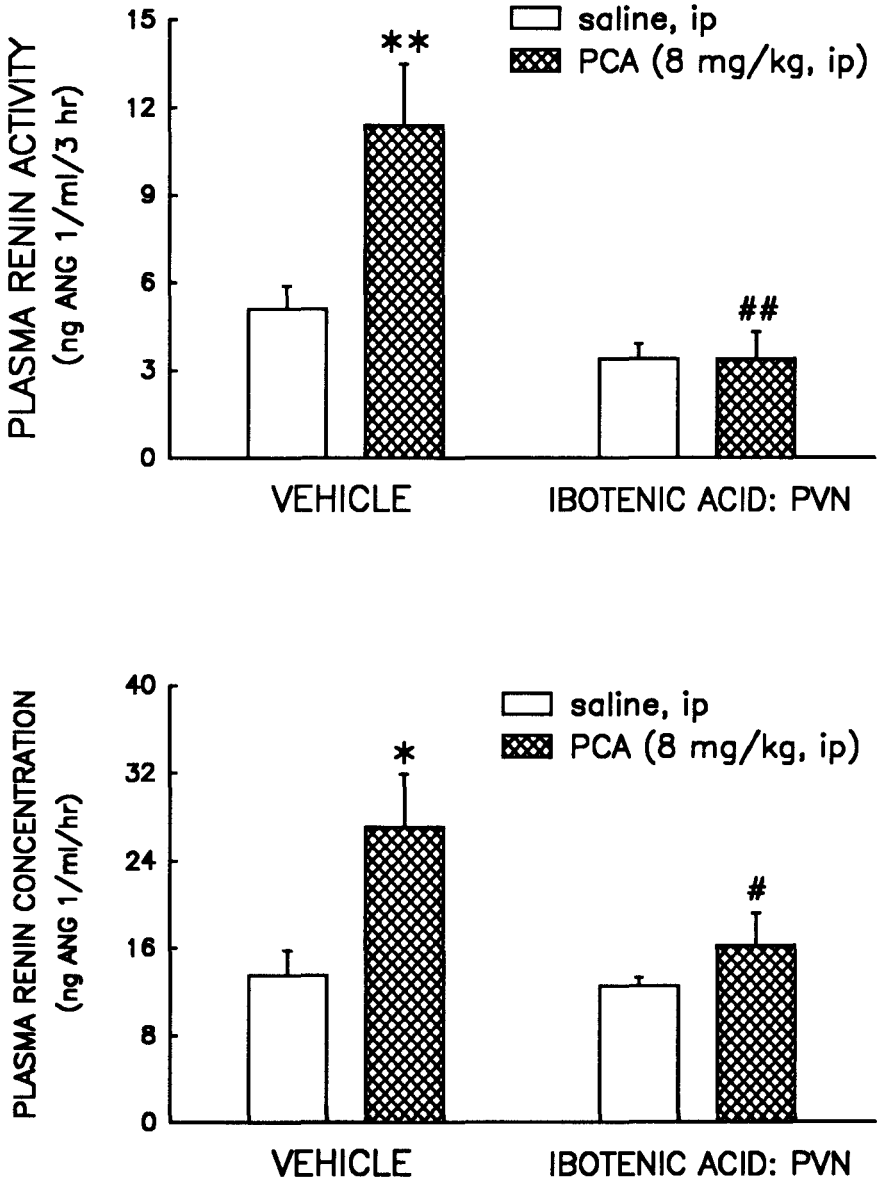

Figure 5.3. Ibotenic acid lesions in the PVN block the renin response to PCA (ip). Data represent mean ± S.E.M. of 13 rats per vehicle/saline group, 11 rats per vehicle/PCA group, 8 rats per IBO/saline group, and 6 rats per IBO/PCA group. * or ** Significant difference from corresponding saline (0 dose of PCA) group, $P<0.05$ or $P<0.01$, respectively. # or ## Significant difference from corresponding vehicle (non-lesion), PCA injected group, $P<0.05$ or $P<0.01$, respectively (2-way ANOVA and Newman Keuls' test).

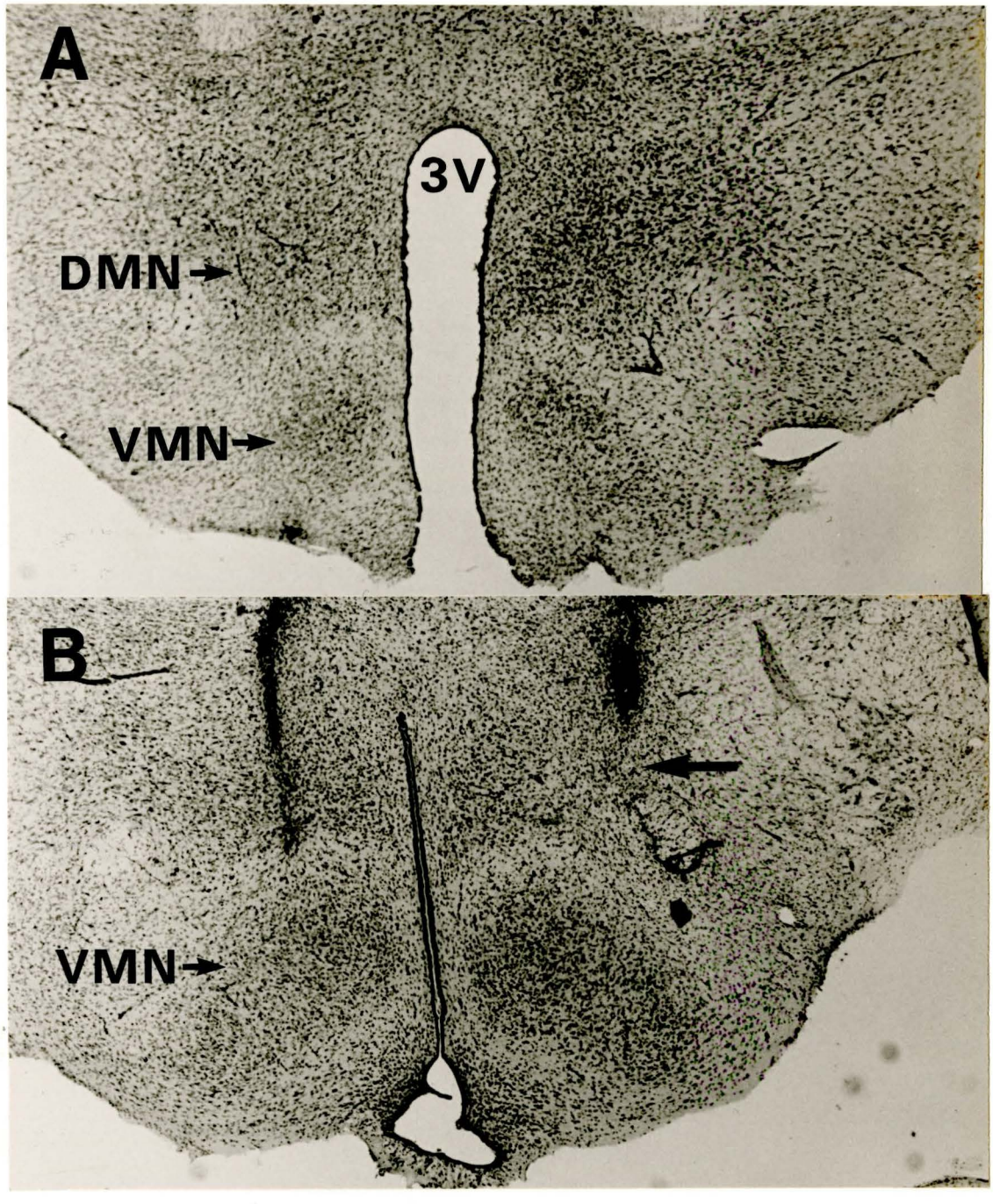

Figure 5.4. Histological verification of ibotenic acid lesions in the hypothalamic DMN and VMN. 40 μm coronal sections were stained with cresyl violet.

Panel A: DMN and VMN of the hypothalamus, vehicle treated. 3V, third ventricle.

Panel B: Bilateral ibotenic acid lesion in DMN. Cannula tips are within DMN boundaries (arrow).

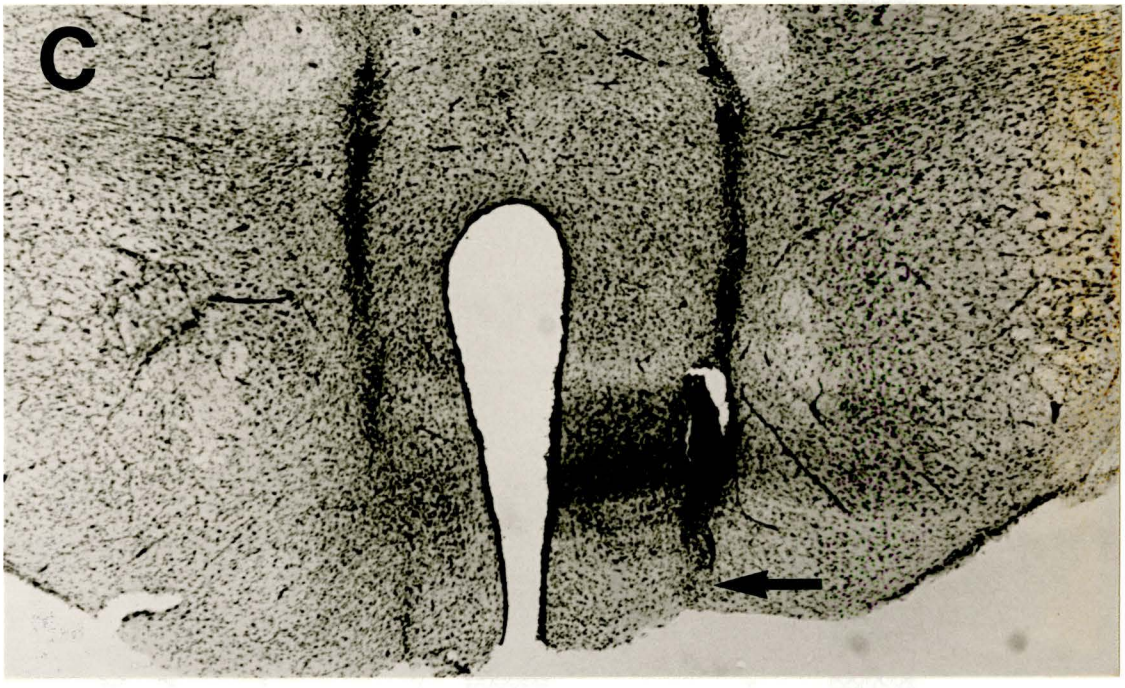

Figure 5.4. Histological verification of ibotenic acid lesions in the hypothalamic DMN and VMN. 40 μm coronal sections were stained with cresyl violet.

Panel C: Bilateral ibotenic acid lesion in VMN. Cannula tips are within VMN boundaries (arrow).

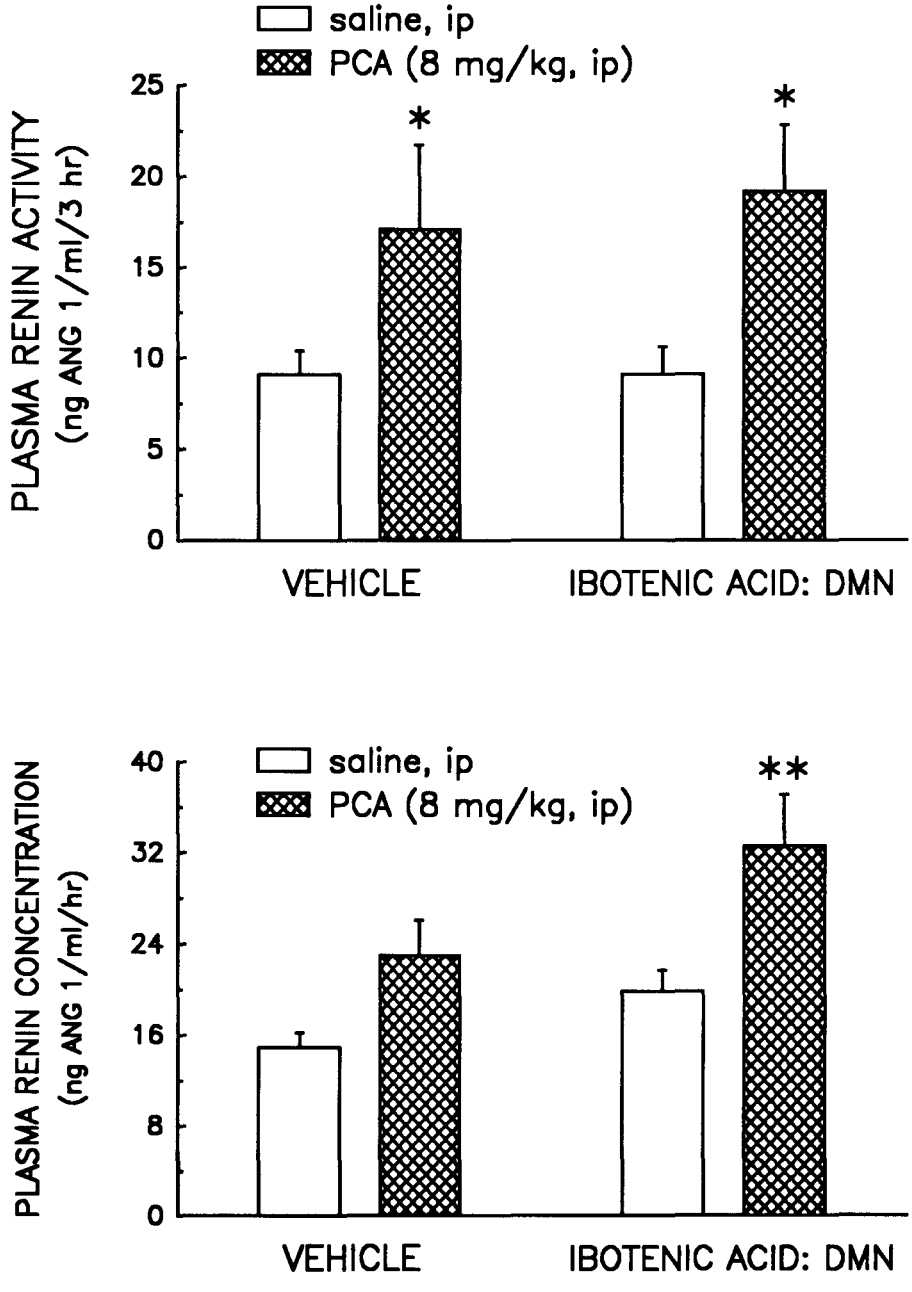

Figure 5.5. Ibotenic acid lesions in the hypothalamic DMN have no effect on the renin response to PCA (ip). Data represent mean ± S.E.M. of 8 rats per vehicle/saline group, 7 rats per vehicle/PCA group, 9 rats per IBO/saline group, and 10 rats per IBO/PCA group. * or ** Significant difference from corresponding saline (0 dose of PCA) group, $P<0.05$ or $P<0.01$, respectively (2-way ANOVA and Newman Keuls' test).

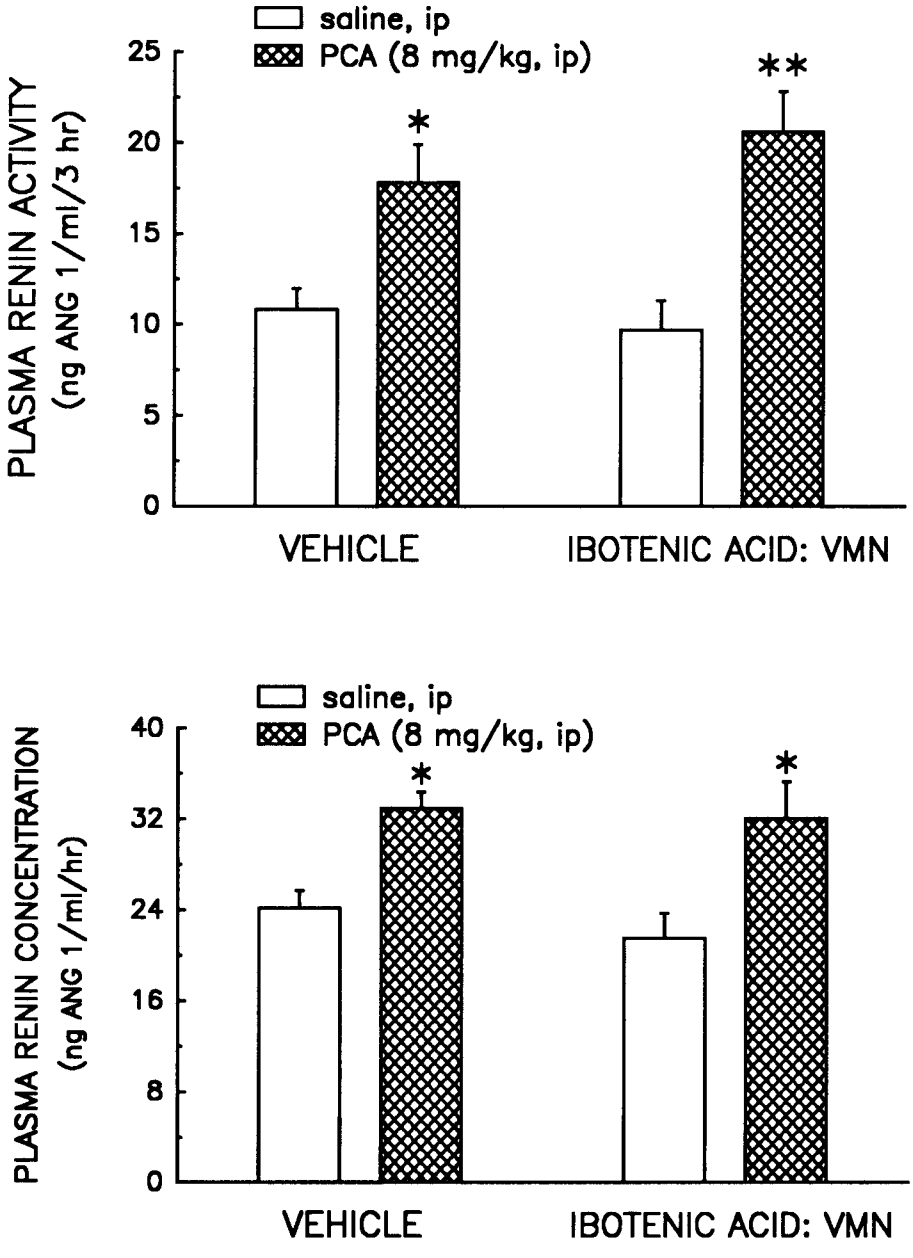

Figure 5.6. Ibotenic acid lesions in the hypothalamic VMN have no effect on the renin response to PCA (ip). Data represent mean ± S.E.M. of 8 rats per vehicle/saline group, 8 rats per vehicle/PCA group, 10 rats per IBO/saline group, and 12 rats per IBO/PCA group. * or ** Significant difference from corresponding saline group, $P<0.05$ or $P<0.01$, respectively (2-way ANOVA and Newman Keuls' test).

TABLE I

PRA and PRC responses in rats with incomplete (partial or unilateral) lesions in the paraventricular nucleus.

	PRA ng ANG I/ml/3 hr	*PRC* ng ANG I/ml/hr
Vehicle/saline	5.1 ± 0.8 (12)	13.5 ± 2.3 (12)
Ibotenic/saline	4.9 ± 0.6 (14)	14.3 ± 2.5 (14)
Vehicle/PCA	11.4 ± 2.1 (11)*	28.3 ± 5.1 (11)*
Ibotenic/PCA	6.8 ± 1.7 (16)	23.4 ± 3.3 (16)
Vehicle/saline	6.0 ± 1.2 (8)	15.7 ± 1.2 (8)
Ibotenic/saline	7.7 ± 1.7 (8)	15.6 ± 1.7 (8)
Vehicle/RU 24969	41.9 ± 7.2 (7)**	48.6 ± 4.4 (7)**
Ibotenic/RU 24969	35.6 ± 4.3 (6)**	47.5 ± 6.9 (6)**

Data represent mean ± S.E.M. * or ** $P<0.05$ and $P<0.01$ respectively, from Vehicle/saline group (two-way ANOVA, Newman Keuls' multiple range test). Figure in parentheses are numbers of animals. Ibotenic: ibotenic acid lesions.

DISCUSSION

The results from these studies demonstrate that cell bodies in the PVN are necessary to mediate the renin response to the 5-HT agonist RU 24969 and the 5-HT releaser PCA. In contrast to either DMN or VMN lesions, PVN lesions were clearly proficient at inhibiting the renin response to either drug.

We utilized two forms of selectivity in our approach . First, we strived to establish discrete lesions within each specific hypothalamic nucleus, the paraventricular, dorsomedial or ventromedial, while limiting surrounding tissue damage. There are currently no selective 5-HT post-synaptic receptor toxins. Therefore, we elected to eliminate cell bodies thought to contain 5-HT receptors, and maintain our second form of selectivity through the use of a post-synaptic 5-HT agonist and a pre-synaptic 5-HT releaser. If the renin response to these drugs were diminished, it would indicate that the neurons responsible (and presumably containing the responsible 5-HT receptor subtypes), had been eliminated. We only observed this pattern with lesions in the PVN, which argues in favor of this nucleus mediating serotonergic control of renin release. Consistent with our data, Appel et al. (1990) established that 5-HT_2 binding sites exist in the PVN, and activation of 5-HT_2 receptors is known to stimulate renin secretion (Van de Kar, 1991).

It is clear from both the PRA and PRC data that the renin response to PCA and RU 24969 was not entirely abolished by PVN lesions. Since our lesions were small and confined entirely within the PVN, enough neurons could have remained to mediate a partial renin response. This would

explain why the renin response to both PCA and RU 24969 was not reduced further. These results also suggest a lack of nonspecific tissue damage, since larger electrolytic lesions have been shown to more completely eliminate the renin response to PCA (Gotoh et al., 1987). These PCA data also confirm the predominant central effect of PCA to increase renin secretion. It is unclear why the effect of PCA on renin secretion was abolished by PVN lesions, whereas the RU 24969 effect was only attenuated.

The reasons for choosing RU 24969 and PCA as pharmacological tools are as follows. Binding studies have characterized RU 24969 as a 5-HT_{1A}/5-HT_{1B} agonist with 100 fold lower affinity for 5-HT_{1C} or 5-HT_2 receptors (Glennon, 1987; Hoyer and Schoeffter, 1991). However, it has been demonstrated that RU 24969 increases renin via post-synaptic 5-HT_2 receptors located in the brain (Van de Kar et al., 1989). The present studies confirm the location of the 5-HT receptors in the brain. PCA has been characterized as a releaser of 5-HT and catecholamines (Sanders-Bush and Steranka, 1978). Evidence that PCA is increasing renin via release of 5-HT was provided in studies utilizing the 5-HT synthesis inhibitor PCPA, which inhibited PCA's stimulation of renin release (Van de Kar et al., 1981).

Previous investigations have sought to clarify serotonin's role in the brain to regulate renin secretion from the kidney. Dorsal raphé lesions with the 5-HT neurotoxin 5,7-DHT, attenuated the renin response to PCA (Van de Kar et al., 1982). Efferent connections have been shown to exist between the dorsal raphé and the PVN (Swanson and Sawchenko, 1983). Karteszi et al. (1982) found that a knife cut severing the midbrain from the hypothalamus inhibited the PCA-induced increase in PRA. Furthermore,

a large lesion in the mediobasal hypothalamus (the area containing the PVN, DMN and VMN) also inhibited the effect of PCA on PRA (Karteszi et al., 1982). Thus, the circuitry influencing renin regulation involves serotonergic neurons in the dorsal raphé, which terminate in the hypothalamus.

In studies comparable to the present one, Gotoh et al. (1987; 1988) made sizable electrolytic lesions in the PVN, DMN, VMN and dorsal raphé nucleus. They tested several paradigms which increase renin secretion: PCA, immobilization stress, head-up tilt, and a low Na^+ diet. The only paradigm that PVN and dorsal raphé lesions inhibited was PCA. This suggests a serotonergic connection between the dorsal raphé and the PVN, as well as differential processing depending on the renin releasing stimulus. However, VMN lesions inhibited renin secretion in all of their paradigms. Thus, since there was no differential response following VMN lesions, it suggests that nonspecific damage may have resulted from destroying both neurons and all surrounding fibers of passage. In addition, if the VMN were an important regulatory site, then dorsal raphé lesions should have inhibited the renin response to the other paradigms, which did not occur. We were unable to replicate inhibition of PCA-stimulated renin secretion with our cell selective VMN lesions.

In the present study, ibotenic acid lesions in the PVN significantly impaired the renin response to RU 24969 and PCA. In contrast, neither DMN nor VMN lesions reduced this serotonergic mediated response. Thus, our results strengthen the hypothesis that neurons in the PVN, rather than the DMN or VMN, mediate the RU 24969 and PCA-induced increase in renin secretion. Furthermore the results suggest that fibers of passage, or

connections running between the PVN and the VMN, rather than cells in the VMN, are mediating the PCA-induced renin elevations seen by Gotoh et al. (1987). Since our lesions were confined to each designated nucleus, the evidence presented here supports the PVN as the serotonergic integrator for renin secretion.

It is quite likely that different renin-releasing stimuli (such as immobilization stress, head-up tilt or low Na^+ diet) would converge on the hypothalamus, to form a common final output pathway to the kidney. The stimuli would not necessarily have to be mediated by 5-HT, but only synapse in the hypothalamus. The VMN may lie along the output pathway, which would explain why nonspecific electrolytic lesions blocked the renin response to so many different stimuli. Evidence for this anatomical arrangement suggests that connections from forebrain structures such as the subfornical organ may traverse through the VMN to the brain stem where they influence cardiovascular function and body fluid homeostasis (Hartle and Brody, 1984; Knuepfer et al., 1984; Ciriello and Gutman, 1991).

Two possible functions are proposed for PVN regulation of renin release. We have shown repeatedly that a variety of stressors increase renin (Van de Kar et al., 1991). For example, the renin response to conditioned fear stress is mediated by PVN cells (Richardson Morton et al., 1989). In addition, since the paraventricular nucleus clearly plays a vital role in integrating body fluid homeostasis, it would not be surprising if renin release from the kidney were also regulated from this site. The mechanism may be either hormonal (Van de Kar et al., 1987) or involve the sympathetic nervous system (Ciriello and Calaresu, 1980; Alper and Ganong, 1984; Hosoya et al., 1991).

In summary, these studies provide evidence that cell bodies in the PVN mediate the renin response to a serotonin agonist and releaser. It appears that cells within the VMN or DMN are not required for this action. These results also confirm previous reports suggesting 5-HT regulates renin secretion through central 5-HT receptors.

CHAPTER VI

DISCUSSION

Summary. The hypothesis to be tested by this dissertation was that serotonergic receptors on hypothalamic paraventricular neurons mediate renin secretion from the kidney. To answer this, two questions were asked. First, are the 5-HT receptors located in the brain, and two, if they are, is the site in the PVN or some other site. The present data provide answers to these two questions, given the limitations of the pharmacological tools available. The 5-HT agonists DOI and RU 24969, and the 5-HT releaser PCA were all found to stimulate renin secretion by activating 5-HT receptors in the brain. DOI had an additional mechanism of action peripherally, and was therefore not used in the subsequent studies. Upon creating chemical, cell-selective lesions in three different hypothalamic nuclei, only those lesions in the paraventricular nucleus inhibited the renin response to RU 24969 and PCA. These data suggest that cell bodies in the PVN are necessary to mediate the renin response to a 5-HT agonist and 5-HT releaser. The remainder of this discussion will summarize the data, analyze issues that are unclear, and frame the results into a more global picture. An attempt will be made not to rediscuss issues already explored in each of the three papers presented as chapters.

Pharmacology of drugs used for these studies. The three drugs used to study the serotonergic stimulation of renin secretion were DOI, RU 24969 and PCA. They were chosen because each have been shown to increase renin secretion dose-dependently when administered peripherally (Van de Kar et al., 1981; 1989). They represented different drug classes with different mechanisms of action.

RU 24969 is an indole compound resembling 5-HT, initially classified by binding studies as a 5-HT_{1A}/5-HT_{1B} agonist (Glennon, 1987). However, other data suggest that Ru 24969 can bind with higher affinity to the 5-HT_2 "high-affinity" site (defined by ^{3}H-DOB binding) than to the 5-HT_2 "low-affinity" site (defined by ^{3}H-ketanserin binding, Lyon et al., 1987), and may now be considered to have 5-HT_2 agonist properties. Clearly, it is capable of stimulating renin secretion through a 5-HT_2 mediated mechanism (Van de Kar et al., 1989).

DOI is an aminopropane with 5-HT agonist characteristics and an extremely high affinity for 5-HT_{1C}/5-HT_2 binding sites (Shannon et al., 1984). Previous evidence using high affinity 5-HT_2 antagonists suggested that renin was stimulated by 5-HT_2 receptor activation (Lorens and Van de Kar, 1987). Until the development of DOI and agents similar to it, no high affinity agonists existed which could be tested for 5-HT_2 mediated effects. DOI was chosen for these reasons.

PCA is an amphetamine derivative, shown to release 5-HT (Sanders-Bush and Steranka, 1978; Marsden et al., 1979). PCA has been used repeatedly to stimulate renin secretion. As a 5-HT releaser, it was originally used to provide evidence that brain serotonergic systems could be involved in renin regulation (Van de Kar et al., 1981; Karteszi et al., 1982). A

possible drawback to the use of PCA was the issue raised by Ikonomov et al. (1991), who maintain that peripherally administered PCA damages the kidney, and any subsequent renin data are questionable. Three of my experiments (ICV dose response, cardiovascular parameters, and prazosin) used doses of PCA below those peripherally effective, and were injected into the brain ICV. The possibility of kidney damage cannot be ruled out, but is unlikely, in these experiments. The other PCA experiments (fluoxetine and the ibotenic acid studies), did involve peripheral injections. Of more importance is the issue that renin secretion may be occurring secondarily to elevated NA^+ excretion (Stein et al., 1987). Further tests would have to be carried out to determine if centrally administered PCA was increasing renin through a direct or indirect mechanism.

Chapter III: DOI experiments. The purpose of the experiments in this chapter was to determine the identity and location of the 5-HT receptor subtype(s) mediating the renin and blood pressure responses to DOI. DOI injected ip into conscious male rats elevated plasma renin activity in a dose-dependent manner. The 5-HT_{1C}/5-HT_2 antagonist ritanserin completely blocked the DOI-induced increase in plasma renin activity and concentration. In order to distinguish the 5-HT_2 from the 5-HT_{1C} mediated effect of DOI, spiperone was administered prior to DOI. Low doses of spiperone (sc) significantly reduced the renin response to DOI. Since spiperone has a higher affinity for 5-HT_2 than 5-HT_{1C} receptors, these data suggest that DOI stimulates renin secretion through 5-HT_2 receptors. To separate central from peripheral 5-HT receptors, DOI was injected in rats

pretreated with saline or xylamidine, a 5-HT_2 antagonist which does not cross the blood brain barrier. Xylamidine produced a shift to the right and suppression of the maximal effect of DOI on plasma renin activity and concentration, suggesting a role for peripheral 5-HT_2 receptors in the effect of DOI. On the other hand, ICV administration of DOI, using doses lower than the peripherally effective doses, caused a significant elevation of plasma renin activity. These experiments suggest that DOI's elevation of plasma renin activity has both peripheral and central sites of action. To determine if BP changes were responsible for DOI's effects, BP was measured after injection of DOI (ICV). A dose-dependent rise in BP occurred within 10 minutes post-injection, in contrast to ia injection of DOI which produced a slower and lower BP rise. Heart rate (HR) was significantly reduced after ICV but not after ia injection of DOI. The increase in plasma renin concentration after ICV injection of DOI was significantly higher than the corresponding value after ia injection of the same dose of DOI. To summarize, these data suggest that DOI increases renin secretion and elevates BP by activating both peripheral and brain 5-HT receptors.

Several issues raised by the data need to be addressed. The data from this study show that the renin response to DOI was clearly blocked by ritanserin (ip). However, the renin response was only attenuated, but not abolished by spiperone. To conclude that renin is regulated by 5-HT_2 receptors, it would have been more convincing to see a separation of the renin response between the two doses of spiperone. That is, if spiperone were only antagonizing 5-HT_2 receptors at the low dose, but antagonized both 5-HT_2 and 5-HT_{1C} receptors at the high dose, we might expect to see

an even greater inhibition of the DOI effect on renin release at the higher dose. Alternatively, a separation could be interpreted as meaning that more 5-HT_2 receptors were antagonized, especially if the hypothesis is that renin is regulated by only 5-HT_2 and not 5-HT_{1C} receptors. It could be that the higher dose of spiperone used, which was only 10 fold higher than the low dose, simply was not great enough to discern a receptor differentiation since the two receptors are so similar. A final interpretation could be that both 5-HT_2 and 5-HT_{1C} receptors contribute to renin regulation. Thus, at each dose, spiperone would antagonize a portion of the renin response mediated by 5-HT_2 receptors, and a portion mediated by 5-HT_{1C} receptors, with no differentiation between spiperone doses distinguishable. In any event, the fact that the low dose of spiperone inhibited the renin response to DOI, suggests that 5-HT_2 receptors are likely involved in renin regulation.

Another question is why did the same doses of ritanserin completely eliminate the renin response to DOI, compared to spiperone and xylamidine. The simplest explanation is that ritanserin was antagonizing non-competitively, while spiperone and xylamidine were antagonizing competitively. Ritanserin has been shown to noncompetitively inhibit [^{3}H]ketanserin binding (Leysen et al., 1985). Considering how low a dose of ritanserin was used, the renin response blockade was impressive. This suggests that, in contrast to either spiperone or xylamidine, ritanserin could be blocking both peripheral and central 5-HT_2 receptors, or some other receptors involved in renin regulation. Ritanserin appears to remove receptors from availability, making any dose of DOI ineffective. In support of this, Bond et al. (1989) found that 5-HT_2 antagonists were

producing renal vasoconstriction by a proposed "pseudoirreversible inhibition" of 5-HT receptors, resulting in the appearance of unsurmountable (or noncompetitive) antagonism. Although the maximal renin response to DOI was blunted by both spiperone and xylamidine, the case that they are also inhibiting in a noncompetitive manner is not as strong as with ritanserin, but should still be considered.

Since the renin and blood pressure responses were not attenuated by a centrally administered 5-HT_2 antagonist, a strong case cannot be made that 5-HT_2 receptors in the brain mediate these responses to DOI. The explanation for why ICV ritanserin did not inhibit the renin response to DOI is puzzling. The dose used (2.0 μg/kg, ICV) should have been high enough considering that 10 μg/kg (sc) completely abolished the response peripherally. One possibility is that at the doses used, DOI was capable of stimulating both central and peripheral receptors, while ritanserin could only antagonize the central sites. Another explanation is that ritanserin could be metabolized peripherally to a compound that interacts non-competitively with 5-HT receptors. The ICV renin dose-response to DOI does suggest, however, that central receptors were activated. And clearly, the BP response to ICV injected DOI is faster and more robust than peripherally injected DOI, again suggesting that both central and peripheral sites are activated by DOI. A recent report agrees that DOI stimulates BP through separate mechanisms centrally compared to peripherally (Dedeoglu and Fisher, 1991). This is not surprising since 5-HT_2 receptors are located on a number of important cardiovascular sites, including vascular smooth muscle and platelets (Saxena and Villalón, 1990). However, the argument could be made that since DOI is such a

selective 5-HT_{1C}/5-HT_2 agonist (Shannon et al., 1984), that presumably any response that is occurring in the brain must be mediated by those receptor subtypes. Other evidence agrees with this conclusion. 5-HT_2 receptors have been localized in the PVN (Appel et al., 1990). DOI applied to the ventrolateral medulla increases blood pressure and sympathetic output (McCall and Harris, 1988; Mandal et al., 1990a). DOI-induced renin secretion could be inhibited by the peripherally and centrally acting 5-HT_2/5-HT_{1C} antagonist LY53857 (Alper, 1990a).

A final point, is that the peripheral (ip) dose of DOI which significantly and maximally increased renin secretion was 2.0 mg/kg (fig. 3.1). In contrast, the ICV dose needed to significantly increase renin secretion was 0.2 mg/kg (fig. 3.5), which is 10 times lower. This suggests that 0.2 mg/kg injected into the brain would be too low a dose to stimulate renin secretion even if it diffused out of the brain and reached peripheral targets. Taken together, the results presented here agree with previous reports that DOI can stimulate renin secretion through central 5-HT_2 receptors, but do not exclude the contribution of peripheral receptors.

<u>Chapter IV: ICV experiments with RU 24969 and PCA</u>. The next set of experiments examined whether RU 24969 or PCA have a central site of action to increase renin release. The mechanism of action of fluoxetine is to block the high affinity 5-HT uptake transporter (Fuller et al., 1991). PCA enters 5-HT nerve terminals by this same mechanism. Thus, since fluoxetine blocked the renin response to PCA, these results confirm that the renin response to PCA is mediated by nerve terminals containing 5-HT, rather than some other neurotransmitter. ICV injection of a low dose of

the 5-HT_{1C}/5-HT_2 antagonist LY53857 inhibited the renin response to peripherally injected RU 24969. These data suggest that ip injected RU 24969 activates brain 5-HT_2 receptors to increase renin secretion. Subsequently, RU 24969 or PCA were injected ICV at doses below those peripherally effective. While RU 24969 dose-dependently increased plasma levels of renin, PCA did not. To determine if a cardiovascular mechanism was preventing PCA from stimulating renin, BP and HR were recorded. RU 24969 (ICV) induced a mild but significant BP elevation of 18 mm Hg, 5 minutes after injection. PCA (ICV) produced a larger rise of 44 mm Hg at 2 and 5 minutes after injection. Neither drug administered peripherally (ia) had a significant effect on BP. These data suggest that the large elevation in BP, resulting from ICV administration of PCA, could be causing the inhibition of renin secretion. Indeed, PCA injected ICV significantly increased renin secretion if preceded by the α_1 antagonist prazosin. Thus, by inhibiting the cardiovascular effects with prazosin, PCA's central stimulation of renin secretion was exposed. In conclusion, these data suggest that both RU 24969 and PCA increase renin secretion through central 5-HT receptors, rather than through a cardiovascular mechanism.

PCA stimulation of renin occurs after ip administration (Van de Kar et al., 1981). When injected iv (Alper et al., 1990) or ICV as in these experiments, PCA does not increase renin secretion unless preceded by prazosin. Prazosin is an α_1 antagonist known to cause vasodilation, hypotension and inhibition of sympathetic nerve activity (Cavero and Roach, 1980). ICV administration of PCA produced a rapid and sizable elevation in BP. An increase in renal arterial pressure activates renal

stretch receptors, which inhibit renin secretion (Hackenthal et al., 1990). While blood pressure was not measured in these experiments after prazosin treatment, a previous study showed that prazosin does inhibit the blood pressure response to PCA (Alper et al., 1987). Additionally, paradigms designed to inhibit 5-HT (5,7-DHT; fluoxetine), did not reduce the BP response to PCA (Alper et al., 1987), suggesting PCA's pressor response is not mediated by serotonergic systems. These studies, plus the combined data from the fluoxetine and prazosin experiments, demonstrate that PCA is stimulating renin secretion via a serotonergic mechanism, rather than a cardiovascular mechanism.

It could be argued that the ICV administered drugs "leaked" from the brain to the periphery. While the time course of drug administration does allow for this possibility, it is unlikely for one primary reason, which is dosage. The doses were all well below peripherally effective concentrations. Consider that LY53857 was injected ICV at 50 μg/kg compared to 1.0 mg/kg used by Van de Kar et al. (1989). Their dose of LY53857 was 20 times higher than the dose used here and still did not completely eliminate the renin response to RU 24969. This suggests that the dose of 50 μg/kg was low enough to ensure that inhibition of 5-HT receptors was occurring in the brain and not in the periphery. The highest dose of RU 24969 injected ICV was 200 μg/kg, compared to the lowest peripherally effective dose of 1.0 mg/kg, which is 5-fold higher. In addition, it should be noted that the lowest effective dose of RU 24969 injected ICV was 100 μg/kg, 10-fold lower than the peripheral dose of 1.0 mg/kg. The highest dose of PCA injected ICV was 1.0 mg/kg compared to the lowest peripherally effective dose of 5.0 mg/kg (Van de Kar et al., 1981).

However, since this dose (1.0 mg/kg) did not increase renin due to PCA's potent pressor effects, the proper comparison is between the 0.5 mg/kg dose of PCA injected ICV following prazosin, and the 6.0 mg/kg (iv) dose used by Alper et al. (1990) following prazosin. Their dose was over 10-fold higher than the dose used here. Yet their 10-fold higher dose could not produce the same increase in renin as seen after 0.5 mg/kg ICV. Taken together, these comparisons do not rule out peripheral effects of these drugs once they have been injected into the ventricles. However, these doses do suggest that the most likely explanation for a renin response following ICV administration RU 24969 and PCA is that it is due to activation of brain 5-HT receptors.

Chapter V: Hypothalamic lesion experiments. The final group of experiments were designed to resolve which hypothalamic nucleus is necessary for the serotonergic control of renin secretion. Previous studies suggest that serotonergic neurons, projecting to the hypothalamus, mediate the effect of PCA on renin secretion. Discrete cell-selective lesions were made with ibotenic acid in three hypothalamic sites: the paraventricular, the dorsomedial or the ventromedial nuclei. The renin response to both RU 24969 (ip) and PCA (ip) was significantly reduced in rats with histologically verified PVN lesions compared to vehicle treated controls. In contrast, the renin response to PCA remained unchanged in rats with either DMN or VMN hypothalamic lesions. Thus, these results are consistent with the hypothesis that 5-HT receptors located on cell bodies in the PVN mediate the renin response to a serotonin agonist and releaser. Furthermore, these lesion studies confirm previous studies that suggest

that 5-HT neurons regulate renin secretion through central receptors (Van de Kar et al., 1981; 1982; 1989; 1990; Karteszi et al., 1982).

These experiments, combined with previous studies (Van de Kar et al., 1990; Appel et al., 1990), suggest that the 5-HT receptor subtype in the PVN mediating the renin response may be 5-HT_2. RU 24969 is a 5-HT_{1A}/5-HT_{1B} agonist, but clearly has 5-HT_2 agonist properties. However, these data cannot be considered conclusive evidence that 5-HT_2 receptors in the PVN mediate the renin response to 5-HT. A single 10 μg/kg dose of RU 24969 has been injected directly into the PVN, and caused a significant increase in plasma renin concentration (Van de Kar et al., 1990). Even more conclusive would be a renin dose-response experiment with a selective agonist such as DOI injected directly into the PVN, and inhibited with a selective 5-HT_2 antagonist.

The rationale for the choice of ibotenic acid as a method for destroying cell bodies and their complement of receptors is as follows. Electrolytic lesions have already been successfully used to destroy both the PVN and VMN (Gotoh et al., 1987; 1988). The electrolytic technique, while certainly complete, has the disadvantage of usually leaving large holes in the brain encompassing areas of tissue in excess of the designated target site. Two other toxins could have been used, EEDQ or 5,7-DHT. The irreversible receptor inactivator EEDQ, eliminates all 5-HT receptor subtypes (Battaglia, personal communication). This procedure would not selectively differentiate 5-HT receptor types. This could be overcome by use of selective agonists. However, EEDQ receptor inactivation could not easily be verified by histology, and would require extensive autoradiographic verification procedures. A second possibility

would have been to use the serotonin neurotoxin 5,7-DHT. This toxin is taken up presynaptically to destroy 5-HT nerve terminals and cell bodies (Van de Kar et al., 1982). Thus, all post-synaptic receptors would remain. The only paradigm this lesion would inhibit is one utilizing the presynaptic 5-HT releaser PCA. Elimination of nerve terminals may cause up-regulation of post-synaptic receptors, and a subsequent shift to the left of dose-response curves. Presumably, this is due to the development of post-synaptic supersensitivity. This toxin also requires a difficult verification procedures such as 5-HT assay of brain tissue "punches" or autoradiography of [^{3}H]-citalopram binding to 5-HT uptake sites located on presynaptic 5-HT terminals. The toxin used in my studies, ibotenic acid, has been verified to destroy cell bodies and presumably their receptors (Markowska et al., 1985). However, the advantage over electrolytic lesions is that fibers of passage are left intact. Receptor selectivity is maintained by use of specific direct or indirect agonists, in this case, RU 24969 and PCA. Verification of ibotenic acid lesions entails simple histology with cresyl violet staining. None of the above mentioned procedures is perfect, but they are currently the best available. Ibotenic acid lesions were previously made in the PVN, and inhibited the renin response to a conditioned fear procedure (Richardson Morton et al., 1989). In addition, the excitotoxin *N*-methyl-D-aspartic acid (NMDA) has been used to produce lesions in the PVN (Rockhold et al., 1990). NMDA also destroys cell bodies and leaves fibers of passage intact. These lesions altered blood pressure acutely (1-2 hours), and produced some apparent myocardial necrosis, but renin was not measured.

Receptor selectivity, as just mentioned, was maintained through the

use of selective 5-HT agonists. Why not inject 5-HT directly into the brain and measure renin secretion? First of all, evidence discussed earlier indicates that renin is regulated by 5-HT_2 receptors. Secondly, 5-HT has, by definition, high affinity for 5-HT_1 receptors and low affinity for 5-HT_2 receptors. Although, it has become clear recently, that the 5-HT_2 receptor also exists in a high affinity state (Lyon et al, 1987). Thus, dissecting out which receptor was responsible for which response would be nearly impossible without highly effective antagonists. The simpler approach would be to use selective agonists known to stimulate renin secretion, which was the method employed here. Finally, 5-HT would be rapidly taken up by the high affinity presynaptic uptake mechanism or metabolized by monoamine oxidase. Therefore, quantifying how much 5-HT reaches a particular receptor site would be difficult to estimate.

Comparison of results with the literature. The inspiration for these experiments came from two sets of data in the literature. The first being from Alper's laboratory, who carried out studies suggesting that 5-HT_2 agonists such as quipazine and DOI were increasing renin secretion by decreasing renal blood flow, not by acting in the brain (Zink et al., 1990; Alper, 1990a). The data presented here support the hypothesis that 5-HT receptors in the brain can stimulate renin secretion, but do not contradict Alper's earlier findings of possible peripheral mechanisms.

The second source came from Ganong's group, who performed studies suggesting that the VMN, PVN or both might regulate renin secretion from the kidney (Gotoh et al., 1987; 1988). Their findings demonstrated that electrolytic lesions in the PVN inhibited the renin response to PCA, while

electrolytic lesions in the VMN inhibited the renin response to a variety of stimuli, including PCA. The results reported here do not support the VMN as the likely site for the serotonergic receptors influencing renin secretion, but do support the PVN. Since the electrolytic lesions used by Gotoh also destroyed fibers of passage, it is probable that the VMN lies along a pathway important for fluid homeostasis. Evidence for such a pathway exists (Hartle and Brody, 1984; Ciriello and Gutman, 1991).

Where do these results fit in with overall regulation of kidney function? The renin-angiotensin system (RAS) is designed to respond to hypotension, blood loss, and Na^+ loss. The RAS is also necessary to regulate homeostatic fluid and electrolyte balance. If the kidney is the ultimate "manager" of the arterial pressure setpoint, as postulated by Guyton (1972; 1990), then why would the brain be involved in control of renin secretion? There are several credible answers to this question.

The first explanation is that renin as a neuroendocrine factor, is very responsive to stress (Van de Kar et al., 1991). The 5-HT_{1A} anxiolytic ipsapirone has attenuated the effects of immobilization and conditioned fear on renin secretion (Rittenhouse et al., 1992). Lesions in the PVN inhibited the renin response to conditioned fear (Richardson Morton et al., 1989). These studies suggest that renin is a "stress hormone" or marker, as reactive to stress as ACTH, corticosterone and prolactin (Van de Kar et al., 1991). Since the physical stressor of hemorrhage potently releases renin, it is not surprising that other physical stressors (immobilization) or more "emotional" stressors (conditioned fear) would also stimulate renin. In each situation, the organism is preparing to deal with a real or perceived environmental insult which could threaten

fluid balance. This notion is based fundamentally on Cannon's hypothesis of stress: that an organism will prepare itself for "fight or flight". Mobilization of the renin-angiotensin system as part of the generalized alarm response, is an appropriate response in anticipation of possible injury or wounds in a fight/flight situation. ANG II has the important responsibility of redirecting blood from enteric pools to vital organs (brain and heart) in response to serious injury (Keeton and Campbell, 1980). In addition, it is not surprising that renin secretion would increase in response to stress, since the hallmark of the stress-response is activation of the sympathetic nervous system, which also stimulates renin release through β receptors in, and outside the kidney. Perceived stress, as occurs during conditioned fear, would obviously have to be mediated through the brain, as no physical element is involved. Nonetheless, stress in any form, may be capable of activating the generalized stress response, and with it, the renin-angiotensin system. Given the previous work by Richardson Morton et al. (1989), and the data presented here, the PVN is very likely an integrative center for stress-induced renin release.

The second explanation of why the brain would be regulating renin secretion is based on known autonomic and endocrine functions of the PVN (Swanson and Sawchenko, 1983). The anatomy and connections of the PVN certainly suggest an additional integrative role. There are a reported 30 neuroactive substances within the PVN, a bilateral structure bordering the third ventricle, with each side comprising only 0.3 mm^2 of tissue. The PVN has been estimated to contain less than 10,000 cells, approximately 1700 large magnocellular neurons and approximately 7000 smaller parvocellular

neurons (Swanson and Sawchenko, 1983).

The PVN sends efferent projections in several directions, for example rostrally, to the subfornical organ, the OVLT, and preoptic area (Larsen et al., 1991). Caudally, efferents extend to the spinal cord where direct connections are made with preganglionic sympathetic cell groups (Hosoya et al., 1991). Ventrally, the PVN sends major projections to the median eminence and neurohypophysis along the hypothalamo-pituitary tract (Swanson and Sawchenko, 1983). And finally there are dorsomedial projections to midline structures such as the thalamus, periaquaductal gray and area postrema (Larsen et al., 1991; Shapiro and Miselis, 1985).

The PVN receives afferent noradrenergic and adrenergic inputs from the ventrolateral medulla (A_1 and C_1) the nucleus of the solitary tract (NTS, A_2 and C_2), and the locus coeruleus (Cunningham et al., 1990; Weiss and Hatton, 1990a). The dorsal raphé sends limited projections to the PVN (Swanson and Sawchenko, 1983). Interconnections exist within the hypothalamus from the DMN, VMN, preoptic area, anterior and lateral hypothalamus (Swanson and Sawchenko, 1983). The PVN also receives a prominent ANG II input pathway from the subfornical organ (Weiss and Hatton, 1990b; Lind et al., 1985). Lastly, limbic structures including the central nucleus of the amygdala, hippocampal formation, and septal region send afferents to the PVN (Swanson and Sawchenko, 1983). These anatomical connections, both afferent and efferent, suggest that the PVN is in a central position to act as an integrator of cardiovascular and visceral information.

The kidney receives direct CNS input via the sympathetic nerves. It could be speculated, that the brain may be vital in establishing the

"setpoint" discussed by Guyton (1972, 1990). If this is the case, then dysfunction anywhere along the integrative network which includes the PVN, could contribute or even be causative for the development of hypertension. Electrolytic lesions in the subfornical organ (Hartle and Brody, 1984), area postrema (Mangiapane et al., 1989), and PVN (Ciriello et al., 1984; Takeda et al., 1991; Herzig et al., 1991) attenuated either development or maintenance of hypertension in experimental models of hypertension. Thus, dysfunction of central pressor pathways containing the PVN could contribute to essential hypertension (Ferrario and Averill, 1991).

Finally, if the PVN is a central integrator or at least modulator of renal function, it is in an ideal position to monitor feedback signals from the kidney, presumably via blood borne ANG II. ANG II receptors are present in both the subfornical organ and PVN (Lind et al., 1985), and the PVN receives and sends projections to all the circumventricular organs (Larsen et al., 1991), which lie outside the blood-brain barrier. An established mechanism whereby the PVN could have a direct impact on renal function, is via the sympathetic nerves (Brody, 1988). Alternatively, the PVN may be stimulating renin secretion by a putative renin releasing factor (Urban et al., 1985; Van de Kar et al., 1987).

The dorsal and median raphé nuclei send efferent 5-HT projections to most of the structures in the brain. Six major ascending pathways have been identified, with substantial overlap between them. The dorsal raphé sends projections to the frontal cortex (basal ganglia and amygdala), striatum (caudate/putamen), and via the periventricular tract to the thalamus and hypothalamus. The median raphé sends projections to frontal cortex (cingulate, hippocampus and septum), and along with the dorsal

raphé, to the mammillary bodies (Azmitia and Segal, 1978; Molliver, 1987). 5-HT nerve terminals originating from the dorsal raphé have been identified in the subfornical organ (Lind, 1986) and the OVLT (Bosler and Descarries, 1988). The area postrema also contains 5-HT immunoreactive cells and fibers (Newton et al., 1985). Each of these circumventricular organs have afferent and efferent connections with the PVN (Larsen et al., 1991).

Conclusion. Two conclusions can be made from the results presented in this dissertation. First, 5-HT can act both in the brain and periphery to stimulate renin secretion. A central response was elicited by the 5-HT agonists DOI, RU 24969, and PCA to stimulate renin secretion. Second, neurons in the PVN mediate renin secretion to two serotonin agonists, RU 24969 and PCA. Therefore the receptors are most likely serotonergic.

The PVN is ideally situated to be a central integrator for cardiovascular and fluid homeostasis, which are closely linked functions. The PVN receives serotonergic input from the dorsal raphé and ANG II input from the subfornical organ, in addition to having efferent projections to all of the circumventricular organs. In this capacity it may also act as a feedback site for ANG II produced by the kidney, mediated in part by 5-HT from the dorsal raphé. Thus, by whatever pathway or mechanism the PVN receives its information, the net output signal results in altered renin secretion from the kidney. Since baseline renin secretion did not change following PVN lesions, it is unlikely that the PVN exerts a tonic input to the kidney, but rather responds to environmental stimuli to increase renin secretion. Some of these environmental stimuli must be mediated by 5-HT,

since PVN lesions retarded the renin response to 5-HT agonists. Thus, 5-HT may play an important role in the PVN as a regulator of renin secretion.

REFERENCES

Aghajanian, G.K., Sprouse, J.S., Sheldon, P. and Rasmussen, K. Electrophysiology of the central serotonin system: Receptor subtypes and transducer mechanisms. Ann. NY Acad. Sci. **600**:93-103, 1990.

Alderman, M.H., Madhavan, S., Ooi, W.L., Cohen, H., Sealey, J.E. and Laragh, J.H. Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension. New Eng. J. Med. **324**:1098-1130, 1991.

Alper, R.H. Hemodynamic and renin response to (±)-DOI, a selective 5-HT_2 receptor agonist, in conscious rats. Eur. J. Pharmacol. **175**:323-332, 1990a.

Alper, R.H. Evidence for central and peripheral serotonergic control of corticosterone secretion in the conscious rat. Neuroendocrinology **51**:255-260, 1990b.

Alper, R.H. and Ganong, W.F. Pharmacological evidence that the sympathetic nervous system mediates the increase in secretion of renin produced by p-chloroamphetamine. Neuropharmacology **23**:1237-1240, 1984.

Alper, R.H. and Snider, J.M. Activation of serotonin2 (5-HT_2) receptors by quipazine increases arterial pressure and renin secretion in conscious rats. J. Pharmacol. Exp. Ther. **243**:829-833, 1987.

Alper, R.H., Lee, C. and Brody, M.J. Hemodynamic pharmacology of p-chloroamphetamine, a serotonin agonist, in conscious rats. J. Pharmacol. Exp. Ther. **243**:446-454, 1987.

Alper, R.H., Pergola, P.E., Sippy, B.D. and Zink, M.H.,III Prazosin unmasks a renin response to intravenous *para*-chloroamphetamine. Life Sci. **47**:1661-1666, 1990.

Amir, S. Intra-ventromedial hypothalamic injection of glutamate stimulates brown adipose tissue thermogenesis in the rat. Brain Res. **511**:341-344, 1990.

Anderson, J.J. and DiMicco, J.A. Effect of local inhibition of γ-aminobutyric acid uptake in the dorsomedial hypothalamus on extracellular levels of γ-aminobutyric acid and on stress-induced tachycardia: a study using microdialysis. J. Pharmacol. Exp. Ther. **255**:1399-1407, 1990.

Appel, N.M., Mitchell, W.M., Garlick, R.K., Glennon, R.A., Teitler, M. and de Souza, E.B. Autoradiographic characterization of (±)-1-(2,5 -dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]DOI) binding to 5-HT_2 and 5-HT_{1C} receptors in rat brain. J. Pharmacol. Exp. Ther. 255:843-857, 1990.

Atlas, S.A., Volpe, M., Sosa, R.E., Laragh, J.H., Camargo, M.J.F. and Maack, T. Effects of atrial natriuretic factor on blood pressure and the renin-angiotensin-aldosterone system. Federation Proc. 45:2115-2121, 1986.

Auerbach, S.B., Kamalakannan, N. and Rutter, J.J. TFMPP and RU 24969 enhance serotonin release from rat hippocampus. Eur. J. Pharmacol. 190:51-57, 1990.

Azmitia, E.C. and Segal, M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphé nuclei in the rat. J. Comp. Neurol. 179:641-668, 1978.

Bagdy, G., Sved, A.F., Murphy, D.L. and Szemeredi, K. Pharmacological characterization of serotonin receptor subtypes involved in vasopressin and plasma renin activity responses to serotonin agonists. Eur. J. Pharmacol. 210:285-289, 1992.

Becker, R.H.A., Linz, W. and Scholken, B.A. Pharmacological interference with the cardiac renin-angiotensin system. J. Cardiovasc. Pharmacol. 4:S10-S15, 1989.

Berger, U.V., Molliver, M.E. and Grzanna, R. Unlike systemic administration of *p*-chloroamphetamine, direct intracerebral injection does not produce degeneration of 5-HT axons. Exp. Neurol. 109:257-268, 1990.

Bernardis, L.L., Bellinger, L.L., McEwen, G., Kodis, M. and Feldman, M.J. Further evidence for the existence of an "organismic" set point in rats with dorsomedial hypothalamic nucleus lesions (DMNL rats): normal catch-up growth. Physiol. Behav. 44:561-568, 1988.

Blair, M.L., Hisa, H., Sladek, C.D., Radke, K.J. and Gengo, F.M. Dual adrenergic control of renin during nonhypotensive hemorrhage in conscious dogs. Am. J. Physiol. 260:E910-E919, 1991.

Bockaert, J., Sebben, M. and Dumuis, A. Pharmacological characterization of 5-hydroxytryptamine 4 (5-HT_4) receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes: effect of substituted benzamide derivatives. Mol. Pharmacol. 37:408-411, 1990.

Boden, P.R., Woodruff, G.N. and Pinnock, R.D. Pharmacology of a cholecystokinin receptor on 5-hydroxytryptamine neurons in the dorsal raphe of the rat brain. Br. J. Pharmacol. 102:635-638, 1991.

Bond, R.A., Ornstein, A.G. and Clarke, D.E. Unsurmountable antagonism to 5-hydroxytryptamine in rat kidney results from pseudoirreversible inhibition rather than multiple receptors or allosteric receptor modulation. J. Pharm. Exp. Ther. **249**:401-410, 1989.

Bosler, O. and Descarries, L. Monoamine innervation of the organum vasculosum laminae terminalis (OVLT): a high resolution radioautographic study in the rat. J. Comp. Neurol. **272**:545-561, 1988.

Brody, M.J. Central nervous system and mechanisms of hypertension. Clin. Physiol. Biochem. **6**:230-239, 1988.

Brosnihan, K.B. and Ferrario, C.M. Central regulation of renin release. In: *Hypertension and the Brain*, edited by Guthrie, GP,Jr. and Kotchen, T.A. Mt. Kisco, NY: Futura Publ. Co., 1984, p. 83-112.

Brownfield, M.S., Reid, I.A., Ganten, D. and Ganong, W.F. Differential distribution of immunoreactive angiotensin and angiotensin-converting enzyme in rat brain. Neuroscience **7**:1759-1769, 1982.

Brownfield, M.S., Greathouse, J., Lorens, S.A., Armstrong, J., Urban, J.H. and Van de Kar, L.D. Neuropharmacological characterization of serotonergic stimulation of vasopressin secretion in conscious rats. Neuroendocrinology **47**:277-283, 1988.

Calogero, A.E., Bernardini, R., Margioris, A.N., Bagdy, G., Gallucci, W.T., Tamarkin, L. and Tomai, T.P. Effect of serotonergic agonists and antagonists on corticotropin-releasing hormone secretion by explanted rat hypothalami. Peptides **10**:189-200, 1989.

Castren, E. and Saavedra, J.M. Repeated stress increases the density of angiotensin II binding sites in rat paraventricular nucleus and subfornical organ. Endocrinology **122**:370-372, 1988.

Cavero, I. and Roach, A.G. The pharmacology of prazosin, a novel antihypertensive agent. Life Sci. **27**:1525-1540, 1980.

Cherqui, C., Dabiré, H., Fournier, B. and Schmitt, H. Cardiovascular properties of a 5-HT_1-like receptor agonist, 5-methoxy-3(1,2,3,6-tetrahydro- 4-pyridinyl)-1H-indole (RU 24969) in normotensive anaesthetized rats. Arch. int. Pharmacodyn. **295**:94-108, 1988.

Chiu, A.T., Herblin, W.F., McCall, D.E., Ardecky, R.J., Carini, D.J., Duncia, J.V., Pease, L.J., Wong, P.C., Wexler, R.R., Johnson, A.L. and Timmermans, P.B.M.W.M. Identification of angiotensin II receptor subtypes. Biochem. Biophys. Res. Commun. **165**:196-203, 1989.

Chopin, P. and Briley, M. Animal models of anxiety: The effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. **8**:383-388, 1987.

Churchill, P.C. Cellular mechanism of renin release. Clin. Exper. Hyper. **6**:1189-1202, 1988.

Ciriello, J. and Calaresu, F.R. Role of paraventricular and supraoptic nuclei in central cardiovascular regulation in the cat. Am. J. Physiol. **239**:R137-R142, 1980.

Ciriello, J., Kline, R.L., Zhang, T.X. and Caverson, M.M. Lesions of the paraventricular nucleus alter the development of spontaneous hypertension in the rat. Brain Res. **310**:355-359, 1984.

Ciriello, J. and Gutman, M.B. Functional identification of central pressor pathways originating in the subfornical organ. Can. J. Physiol. Pharmacol. **69**:1035-1045, 1991.

Clement, M.E. and McCall, R.B. Studies on the site and mechanism of the sympathoexcitatory action of 5-HT_2 agonists. Brain Res. **515**:299-302, 1990.

Conn, P.J. and Sanders-Bush, E. Central serotonin receptors: Effector systems, physiological roles and regulation. Psychopharmacology **92**:267-277, 1987.

Connor, H.E. and Higgins, G.A. Cardiovascular effects of 5-HT_{1A} receptor agonists injected into the dorsal raphé nucleus of conscious rats. Eur. J. Pharmacol. **182**:63-72, 1990.

Cooper, J.R., Bloom, F.E. and Roth, R.H. *The Biochemical Basis of Neuropharmacology*, New York: Oxford University Press, 1991.

Cooper, S.J. and Abbott, A. Clinical psychopharmacology may benefit new advances in 5-HT pharmacology. Trends Pharmacol. Sci. **9**:1988.

Copp, F.C., Green, A.F., Hodson, H.F., Randall, A.W. and Sim, M.F. New peripheral antagonists of 5-hydroxytryptamine. Nature **214**:200-201, 1967.

Corvol, P., Chauveau, D., Jeunemaître, X. and Ménard, J. Human renin inhibitor peptides. Hypertension **16**:1-11, 1990.

Cowen, P.J., Anderson, I.M. and Gartside, S.E. Endocrinological responses to 5-HT. Ann. NY Acad. Sci. **600**:250-259, 1990.

Cunningham, E.T., Bohn, M.C. and Sawchenko, P.E. Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J. Comp. Neurol. **292**:651-667, 1990.

Dabiré, H., Chaouche-Teyara, K., Cherqui, C., Fournier, B., Laubie, M. and Schmitt, H. Characterization of DOI, a putative 5-HT_2 receptor agonist in the rat. Eur. J. Pharmacol. **168**:369-374, 1989.

Dabiré, H., Cherqui, C., Safar, M. and Schmitt, H. Haemodynamic aspects and serotonin. Clin. Physiol. Biochem. **8**:56-63, 1990.

Dabiré, H. and Richer, C. Implication of the central nervous system in the systemic and regional hemodynamics of two centrally acting hypotensive drugs, flesinoxan and clonidine, in the rat. J. Cardiovasc. Pharmacol. **18**:605-613, 1991.

Dahlström, A. and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system IV. Acta Physiol. Scand. **64(247)**:1-36, 1965.

De Clerck, F. Effect of serotonin on platelets and blood vessels. J. Cardiovasc. Pharmacol. **17**:S1-S5, 1991.

Dedeoglu, A. and Fisher, L.A. Central and peripheral injections of the 5-HT_2 agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, modify cardiovascular function through different mechanisms. J. Pharmacol. Exp. Ther. **259**:1027-1033, 1991.

Della Bruna, R., Pinet, F., Corvol, P. and Kurtz, A. Regulation of renin secretion and renin synthesis by second messengers in isolated mouse juxtaglomerular cells. Cell. Physiol. Biochem. **1**:98-110, 1991.

Den Boer, J.A. and Westenberg, H.G.M. Serotonin function in panic disorder: A double blind placebo controlled study with fluvoxamine and ritanserin. Psychopharmacology **102**:85-94, 1990.

Dzau, V.J., Ingelfinger, J., Pratt, R.E. and Ellison, K.E. Identification of renin and angiotensinogen messenger RNA sequences in mouse and rat brains. Hypertension **8**:544-548, 1986.

Dzau, V.J. and Pratt, R.R. Renin-Angiotensin System: Biology, Physiology and Pharmacology. In: *The Heart and Cardiovascular System*, edited by Fozzard, H.A. New York: Raven Press, 1986,

Feldman, S., Conforti, N. and Melamed, E. Paraventricular nucleus serotonin mediates neurally stimulated adrenocortical secretion. Brain Res. Bull. **18**:165-168, 1987.

Ferrario, C.M. The renin-angiotensin system: importance in physiology and pathology. J. Cardiovasc. Pharmacol. **15**:S1-S5, 1990.

Ferrario, C.M., Barnes, K.L., Block, C.H., Brosnihan, K.B., Diz, D.I., Khosla, M.C. and Santos, R.A.S. Pathways of angiotensin formation and function in the brain. Hypertension **15**:I-13-I-19, 1990.

Ferrario, C.M. and Averill, D.B. Do primary dysfunctions in neural control of arterial pressure contribute to hypertension. Hypertension **18**: I38-I51, 1991.

Filaretov, A.A. and Filaretov, L.P. Role of the paraventricular and ventromedial hypothalamic nuclear areas in the regulation of the pituitary-adrenocortical system. Brain Res. **342**:135-140, 1985.

Fitzsimons, C.L. and Ciriello, J. Selective destruction of parvocellular neurons in the paraventricular nucleus (PVN) decreases norepinephrine (NE) turnover rates in kidney of spontaneously hypertensive rats (SHR). Fed. Proc. **45**:4188, 1986.

Fray, J.C.S. Control of renin secretion by extracellular calcium. Cell Calcium 11:339-341, 1990.

Frazer, A., Maayani, S. and Wolfe, B.B. Subtypes of receptors for serotonin. Ann. Rev. Pharmacol. Toxicol. **30**:307-348, 1990.

Fuller, R.W. Serotonin receptors and neuroendocrine responses. Neuropsychopharmacology 3:495-502, 1990.

Fuller, R.W., Perry, K.W. and Molloy, B.B. Effect of 3-(p-trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. J. Pharmacol. Exp. Ther. **193**:796-803, 1975.

Fuller, R.W. and Snoddy, H.D. Serotonin receptor subtypes involved in the elevation of serum corticosterone concentration in rats by direct- and indirect-acting serotonin agonists. Neuroendocrinology **52**:206-211, 1990.

Fuller, R.W., Wong, D.T. and Robertson, D.W. Fluoxetine, a selective inhibitor of serotonin uptake. Med. Res. Rev. **11**:17-34, 1991.

Gillis, R.A., Hill, K.J., Kirby, J.S., Quest, J.A., Hamosh, P., Norman, W.P. and Kellar, K.J. Effect of activation of central nervous system serotonin 1A receptors on cardiorespiratory function. J. Pharmacol. Exp. Ther. **248**:851-857, 1989.

Glennon, R.A., Young, G., Benington, F. and Morin, R.D. Behavioral and serotonin receptor properties of 4-substituted derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane. J. Med. Chem. **25**:1163-1168, 1982.

Glennon, R.A. Central serotonin receptors as targets for drug research. J. Med. Chem. **30**:1-12, 1987.

Goffinet, A.M., Leysen, J. and Labar, D. In vitro pharmacological profile of 3-*N*-(2-fluoroethyl)spiperone. J. Cereb. Blood Flow Metab. **10**:140-142, 1990.

Gomez, R.A., Chevalier, R.L., Everett, A.D., Elwood, J.P., Peach, M.J., Lynch, K.R. and Carey, R.M. Recruitment of renin gene-expressing cells in adult rat kidneys. Am. J. Physiol. **259**:F660-F665, 1990.

Göthert, M. and Schlicker, E. Identification and classification of 5-HT_1 receptor subtypes. J. Cardiovasc. Pharmacol. **15**:S1-S7, 1990.

Gotoh, E., Murakami, K., Bahnson, T.D. and Ganong, W.F. Role of brain serotonergic pathways and hypothalamus in regulation of renin secretion. Am. J. Physiol. **253**:R179-R185, 1987.

Gotoh, E., Golin, R.M.A. and Ganong, W.F. Relation of the ventromedial nuclei of the hypothalamus to the regulation of renin secretion. Neuroendocrinology **47**:518-522, 1988.

Grahame-Smith, D.G. Serotonin (5-hydroxytryptamine, 5-HT). Quart. J. Med. **67**:459-466, 1988.

Guyton, A.C., Coleman, T.G., Cowley, A.W., Scheel, K.W., Manning, R.D. and Norman, R.A. Arterial pressure regulation: overriding dominance of the kidneys in long-term regulation and in hypertension. Am. J. Med. **52**:584-594, 1972.

Guyton, A.C. Long-term arterial pressure control: An analysis from animal experiments and computer and graphic models. Am. J. Physiol. **259**: 865-R877, 1990.

Hackenthal, E., Paul, M., Ganten, D. and Taugner, R. Morphology, physiology, and molecular biology of renin secretion. Physiol. Rev. **70**:1067-1116, 1990.

Hall, J.E., Mizelle, H.L. and Woods, L.L. The renin-angiotensin system and long-term regulation of arterial pressure. J. Hypertension **4**:387-397, 1986.

Hamon, M., Gozlan, H., El Mestikawy, S., Emerit, M.B., Bolaños, F. and Schechter, L. The central 5-HT_{1A} receptors: Pharmacological, biochemical, functional, and regulatory properties. Ann. NY Acad. Sci. **600**:114-131, 1990.

Hartig, P., Kao, H.-T., Macchi, M., Adham, N., Zgombick, J., Weinshank, R. and Branchek, T. The molecular biology of serotonin receptors: An overview. Neuropsychopharmacology **3**:335-347, 1990.

Hartle, D.K. and Brody, M.J. The angiotensin II pressor system of the rat forebrain. Circ. Res. **54**:355-366, 1984.

Healy, D.P. and Printz, M.P. Distribution of immunoreactive angiotensin II, angiotensin I, angiotensinogen, and renin in the central nervous system of intact and nephrectomized rats. Hypertension **6**:I130-I136, 1984.

Herzig, T.C., Buchholz, R.A. and Haywood, J.R. Effects of paraventricular nucleus lesions on chronic renal hypertension. Am. J. Physiol. **261**: H860-H867, 1991.

Hirsch, A.T., Pinto, Y.M., Schunkert, H. and Dzau, V.J. Potential role of the tissue renin-angiotensin system in the pathophysiology of congestive heart failure. Am. J. Cardio. **66**:22D-32D, 1990.

Hodge, R.L., Lowe, R.D. and Vane, J.R. The effects of alteration of blood-volume on the concentration of circulating angiotensin in anaesthetized dogs. Physiol. **185**:613-626, 1966.

Hosoya, Y., Sugiura, Y., Okado, N., Loewy, A.D. and Kohno, K. Descending input from the hypothalamic paraventricular nucleus to sympathetic preganglionic neurons in the rat. Exp. Brain Res. **85**:10-20, 1991.

Hoyer, D. Molecular pharmacology and biology of 5-HT_{1C} receptors. Trends Pharmacol. Sci. **9**:89-94, 1988.

Hoyer, D. and Schoeffter, P. 5-HT receptors: Subtypes and second messengers. J. Receptor Res. **11**:197-214, 1991.

Humphrey, P.P.A., Feniuk, W., Perren, M.J., Beresford, J.M. and Skingle, M. Serotonin and migraine. Ann. NY Acad. Sci. **600**:587-600, 1990.

Ikonomov, O.C., Shisheva, A.C., Stoynev, A.G. and Kurtev, I.V. Peripheral p-chloroamphetamine is an unsuitable probe for investigation of central serotonergic control on renal renin secretion in the rat. Physiol. Behav. **49**:1119-1128, 1991.

Jackson, E.K. Adenosine: A physiological brake on renin release. Ann. Rev. Pharmacol. Toxicol. **31**:1-35, 1991.

Johns, D.W., Peach, M.J., Gomez, R.A., Inagami, T. and Carey, R.M. Angiotensin II regulates renin gene expression. Am. J. Physiol. **259**:F882-F887, 1990.

Karteszi, M., Van de Kar, L.D., Makara, G., Stark, E. and Ganong, W.F. Evidence that the mediobasal hypothalamus is involved in serotonergic stimulation of renin secretion. Neuroendocrinology **34**:323-326, 1982.

Keeton, T.K. and Campbell, W.B. The pharmacologic alteration of renin release. Pharmacol. Rev. **32(2)**:81-227, 1980.

Kim, S., Hosoi, M., Shimamoto, K., Takada, T. and Yamamoto, K. Increased production of angiotensin II in the adrenal gland of stroke-prone spontaneously hypertensive rats with malignant hypertension. Biochem. Biophys. Res. Comm. **178**:151-157, 1991.

King, B.H., Brazell, C., Dourish, C.T. and Middlemiss, D.N. MK-212 increases rat plasma ACTH concentration by activation of the 5-HT_{1C} receptor subtype. Neurosci. Lett. **105**:174-176, 1989.

King, K.A. and Holtman, J.R.,Jr. Characterization of the effects of activation of ventral medullary serotonin receptor subtypes on cardiovascular activity and respiratory motor outflow to the diaphragm and larynx. J. Pharmacol. Exp. Ther. **252**:665-674, 1990.

Kivlighn, S.D., Lohmeier, T.E., Yang, H.M. and Shin, Y. Chronic effects of a physiological dose of ANP on arterial pressure and renin release. Am. J. Physiol. **258**:H1491-H1497, 1990.

Kjos, T., Gotoh, E., Tkacs, N., Shackelford, R. and Ganong, W.F. Neuroendocrine regulation of plasma angiotensinogen. Endocrinology **129**:901-906, 1991.

Knuepfer, M.M., Johnson, A.K. and Brody, M.J. Vasomotor projections from the anteroventral third ventricle (AV3V) region. Am. J. Physiol. **247**:H139-H145, 1984.

Koenig, J.I., Gudelsky, G.A. and Meltzer, H.Y. Stimulation of corticosterone and beta-endorphin secretion in the rat by selective 5-HT receptor subtype activation. Eur. J. Pharmacol. **137**:1-8, 1987.

Koss, M.C. Mechanism of ketanserin-induced sympatho-inhibition. Eur. J. Pharmacol. **194**:161-166, 1991.

Koulu, M., Saavedra, J.M., Bjelogrlic, N., Niwa, M., Agren, H. and Linnoila, M. Serotonin turnover in discrete hypothalamic nuclei and mesencephalic raphé nuclei of young and adult spontaneously hypertensive rats. Brain Res. **379**:257-263, 1986a.

Koulu, M., Saavedra, J.M., Niwa, M., Scheinin, M. and Linnoila, M. Association between increased serotonin metabolism in rat brainstem nuclei and development of spontaneous hypertension. Brain Res. **371**:177-181, 1986b.

Kubo, R., Ito, T., Shibanoki, S., Kogure, M. and Ishikawa, K. Role of central serotonergic systems in the development of hypertension in spontaneously hypertensive rats. Brain Res. **516**:151-154, 1990.

Kuhn, D.M., Wolf, W.A. and Lovenberg, W. Review of role of the central serotonergic neuronal system in blood pressure regulation. Hypertension **2**:243-255, 1980.

Kurtz, A. Do calcium-activated chloride channels control renin secretion? News Pysiol. Sci. **5**:43-46, 1990.

Kurtz, A., Scholz, H. and Della Bruna, R. Molecular mechanisms of renin release. J. Cardiovasc. Pharmacol. **16**:S1-S7, 1990.

Kushiro, T., Kurumatani, H., Ishii, T., Yokoyama, H., Koike, J., Hatayama, Y., Kobayashi, Y. and Kajiwara, N. Role of central serotonergic (5-HT_2) receptor in blood pressure regulation in rats. Clin. Exp. -Theory & Prac. **A 10**:339-345, 1988.

Laragh, J.H. The renin system in high blood pressure, from disbelief to reality: converting-enzyme blockade for analysis and treatment. Prog. Cardiovasc. Dis. **21**:159-166, 1978.

Larsen, P.J., Moller, M. and Mikkelsen, J.D. Efferent projections from the periventricular and medial parvicellular subnuclei of the hypothalamic paraventricular nucleus to circumventricular organs of the rat: A *Phaseolus vulgaris*-leucoagglutinin (PHA-L) tracing study. J. Comp. Neurol. **306**:462-479, 1991.

Leibowitz, S.F. The role of serotonin in eating disorders. Drugs **393**: 33-48, 1990.

Leibowitz, S.F. Brain neuropeptide Y: An integrator of endocrine, metabolic and behavioral processes. Brain Res. Bull. **27**:333-337.

Leysen, J.E. Gaps and peculiarities in 5-HT_2 receptor studies. Neuropsychopharmacology **3**:361-369, 1990.

Leysen, J.E., Gommeren, W., Van Gompel, P., Wynants, J., Janssen, P.F.M. and Lauduron, P.M. Receptor-binding properties in vitro and in vivo of ritanserin. A very potent and long acting serotonin-S_2 antagonist. Mol. Pharmacol. **27**:600-611, 1985.

Leysen, J.E. and Pauwels, P.J. 5-HT_2 receptors, roles and regulation. Ann. NY Acad. Sci. **600**:183-193, 1990.

Lind, R.W. Bi-directional, chemically specified neural connections between the subfornical organ and the midbrain raphé system. Brain Res.

Lind, R.W., Swanson, L.W. and Ganten, D. Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. Neuroendocrinology **40**:2-24, 1985.
384:250-261, 1986.

Liposits, Z., Phelix, C. and Paull, W.K. Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. A light and electron microscopic immunocytochemical study. Histochemistry **86**:541-549, 1987.

Lorens, S.A. and Van de Kar, L.D. Differential effects of serotonin (5-HT_{1A} and 5-HT_2) agonists and antagonists on renin and corticosterone secretion. Neuroendocrinology **45**:305-310, 1987.

Lou, Y., Smith, D.L., Robinson, B.G. and Morris, B.J. Renin gene expression in various tissues determined by single-step polymerase chain reaction. Clin. Exp. Pharmacol. Physiol. **18**:357-362, 1991.

Lyon, R.A., Davis, K.H. and Titeler, M. ^{3}H-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of cortical 5-HT_2 receptors. Mol. Pharmacol. **31**:194-199, 1987.

Mandal, A.K., Kellar, K.J., Norman, W.P. and Gillis, R.A. Stimulation of serotonin$_2$ receptors in the ventrolateral medulla of the cat results in nonuniform increases in sympathetic outflow. Circ. Res. **67**:1267-1280, 1990a.

Mandal, A.K., Zhong, P., Kellar, K.J. and Gillis, R.A. Ventrolateral medulla: an important site of action for the hypotensive effect of drugs that activate serotonin-1A receptors. J. Cardiovasc. Pharmacol. **15**: S49-S60, 1990b.

Mangiapane, M.L. and Simpson, J.B. Subfornical organ: forebrain site of pressor and dipsogenic action of angiotensin II. Am. J. Physiol. **239**:R382-R389, 1980.

Mangiapane, M.L., Skoog, K.M., Rittenhouse, P., Blair, M.L. and Sladek, C.D. Lesion of the area postrema region attenuates hypertension in spontaneously hypertensive rats. Circ. Res. **64**:129-135, 1989.

Markowska, A., Bakke, H.K., Walther, B. and Ursin, H. Comparison of electrolytic and ibotenic acid lesions in the lateral hypothalamus. Brain Res. **328**:313-323, 1985.

Marsden, C.A., Conti, J., Strope, E., Curzon, G. and Adams, R.N. Monitoring 5-hydroxytryptamine release in the brain of the freely moving unanesthetized rat using in vivo voltammetry. Brain Res. **171**:85-99, 1979.

Matzen, S., Knigge, U. and Warberg, J. Brain regulation of renin secretion involves central histaminergic neurons. Neuroendocrinology **52**:175-180, 1990.

Mayhan, W.G. and Faraci, F.M. Cerebral vasoconstrictor responses to serotonin during chronic hypertension. Hypertension **15**:872-876, 1990.

McCall, R.B., Patel, B.N. and Harris, L.T. Effect of serotonin1 and serotonin2 receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. J. Pharmacol. Exp. Ther. **242**:1152-1159, 1987.

McCall, R.B. and Harris, L.T. 5-HT_2 receptor agonists increase spontaneous sympathetic nerve discharge. Eur. J. Pharmacol. **151**:113-116, 1988.

McCall, R.B., Clement, M.E. and Harris, L.T. Studies on the mechanism of the sympatholytic effect of 8-OH-DPAT: lack of correlation between inhibition of serotonin neuronal firing and sympathetic activity. Brain Res. **501**:73-83, 1989.

McEntee, W.J. and Crook, T.H. Serotonin, memory, and the aging brain. Psychopharmacology **103**:143-149, 1991.

Meltzer, H.Y. Role of serotonin in depression. Ann. NY Acad. Sci. **600**: 486-500, 1990.

Mendelsohn, F.A.O., Allen, A.M., Chai, S.Y., McKinley, M.J., Oldfield, B.J. and Paxinos, G. The brain angiotensin system: Insights from mapping its components. Trends Endocrinol. Metabol. 189-198, 1990.

Mengod, G., Nguyen, H., Le, H., Waeber, C., Lübbert, H. and Palacios, J.M. The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by *in situ* hybridization histochemistry. Comparison with receptor binding distribution. Neuroscience **35**:577-591, 1990.

Mikulic, L.E., Kurnjek, M.L., Russo, R., Trolliet, M.R. and Basso, N. Effect of central serotonin depletion on blood pressure and the renin system in rats. Hypertension **11**:I-190-I-193, 1988.

Miselis, R.R. The efferent projections of the subfornical organ of the rat: a circumventricular organ within a neural network subserving water balance. Brain Res. **230**:1-23, 1981.

Moffett, R.B., Bumpus, F.M. and Husain, A. Minireview: Cellular organization of the brain renin-angiotensin system. Life Sci. **41**:1867-1879, 1987.

Molliver, M.E. Serotonergic neuronal systems: what their anatomic organization tells us about function. Journal of Clinical Psychopharmacology **7**:3S-23S, 1987.

Montes, R. and Johnson, A.K. Efferent mechanisms mediating renal sodium and water excretion induced by centrally administered serotonin. Am. J. Physiol. **259**:R1267-R1273, 1990.

Morris, B.J. Molecular genetics links renin to hypertension. Mol. Cell. Endocrinol. **75**:C13-C18, 1991.

Murphy, D.L. Neuropsychiatric disorders and the multiple human brain serotonin receptor subtypes and subsystems. Neuropsychopharmacology **3**:457-471, 1990.

Nakamura, A., Iwao, H., Fukui, K., Kimura, S., Tamaki, T., Nakanishi, S. and Abe, Y. Regulation of liver angiotensinogen and kidney renin mRNA levels by angiotensin II. Am. J. Physiol. **258**:E1-E6, 1990.

Navar, L.G., Inscho, E.W., Ibarrola, M. and Carmines, P.K. Communication between the macula densa cells and the afferent arteriole. Kidney Int. **39**:S78-S82, 1991.

Newton, B.W., Maley, B. and Traurig, H. The distribution of substance P, enkephalin, and serotonin immunoreactivities in the area postrema of the rat and cat. J. Comp. Neurol. **234**:87-104, 1985.

Okura, T., Kitami, Y., Iwata, T. and Hiwada, K. Quantitative measurement of extra-renal renin mRNA by polymerase chain reaction. Biochem. Biophys. Res. Commun. **179**:25-31, 1991.

Palacios, J.M., Waeber, C., Hoyer, D. and Mengod, G. Distribution of serotonin receptors. Ann. NY Acad. Sci. **600**:36-52, 1990.

Pardy, K., Williams, B.C. and Noble, A.R. Inhibitory role of Ca^{2+} in the control of renin secretion: A study using superfused dispersed rat renal cortical cells. Clin. Sci. **77**:273-279, 1989.

Paris, J.M. and Lorens, S.M. A dose-response analysis of intra-raphe tachykinin-induced hyperactivity. J. Pharmacol. Exp. Ther. **251**:388-393.

Parkinson, W.L. and Weingarten, H.P. Dissociation analysis of ventromedial hypothalamic obesity syndrome. Am. J. Physiol. **259**:R829-R835, 1990.

Paxinos, G. and Watson, C. *The Rat Brain in Stereotaxic Coordinates*, Sydney: Academic Press Australia, 1986. Ed. 2nd

Pazos, A., Cortes, R. and Palacios, J.M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res. **346**:231-249, 1985.

Pazos, A. and Palacios, J.M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. Brain. Res. **346**:205-230, 1985.

Peroutka, S.J. and Snyder, S.H. Multiple serotonin receptors: differential binding of 3H-serotonin, 3H-lysergic acid diethylamide and 3H-spiroperidol. Mol. Pharmacol. **16**:689-699, 1979.

Persson, A.E.G., Salomonsson, M., Westerlund, P., Greger, R., Schlatter, E. and Gonzalez, E. Macula densa cell function. Kidney Int. **39**:S39-S44, 1991.

Pérgola, P.E. and Alper, R.H. Vasopressin and autonomic mechanisms mediate cardiovascular actions of central serotonin. Am. J. Physiol. **260**: R1188-R1193, 1991.

Porter, J.P. Electrical stimulation of paraventricular nucleus increases plasma renin activity. Am. J. Physiol. **254**:R325-R330, 1988.

Radja, F., Laporte, A.-M., Daval, G., Vergé, D., Gozlan, H., Hamon, M., Palacios, J.M., Waeber, C., Mengod, G. and Hoyer, D. Autoradiography of serotonin receptor subtypes in the central nervous system. Neurochem. Int. **18**:1-15, 1991.

Ramage, A.G. Examination of the effects of some 5-HT-2 receptor antagonists on central sympathetic outflow and blood pressure in anaesthetized cats. Arch. Pharmacol. **338**:601-607, 1988.

Rapport, M.M., Green, M.M. and Page, I.H. Purification of the substance which is responsible for vasoconstrictor activity of serum. Fed. Proc. **6**:184-185, 1947.

Reid, I.A. Actions of angiotensin II on the brain: Mechanisms and physiologic role. Am. J. Physiol. **246**:F533-F543, 1984.

Richardson Morton, K.D., Van de Kar, L.D., Brownfield, M.S. and Bethea, C.L. Neuronal cell bodies in the hypothalamic paraventricular nucleus mediate stress-induced renin and corticosterone secretion. Neuroendocrinology **50**:73-80, 1989.

Richardson Morton, K.D., Van de Kar, L.D., Brownfield, M.S., Lorens, S.A., Napier, T.C. and Urban, J.H. Stress-induced renin and corticosterone secretion is mediated by catecholaminergic nerve terminals in the hypothalamic paraventricular nucleus. Neuroendocrinology **51**:320-327, 1990.

Rittenhouse, P.A., Bakkum, E.A. and Van de Kar, L.D. Evidence that the serotonin agonist, DOI, increases renin secretion and blood pressure through both central and peripheral 5-HT_2 receptors. J. Pharmacol. Exp. Ther. **259**:58-65, 1991.

Rittenhouse, P.A., Bakkum, E.A., O'Connor, P.A., Carnes, M., Bethea, C.L. and Van de Kar, L.D. Comparison of neuroendocrine and behavioral effects of ipsapirone, a 5-HT_{1A} agonist, in three stress paradigms: Immobilization, forced swim and conditioned fear. Brain Res. 1992. In Press.

Robinson, S.E., Austin, M.J.F. and Gibbens, D.M. The role of serotonergic neurons in dorsal raphe, median raphe and anterior hypothalamic pressor mechanisms. Neuropharmacology **24**:51-58, 1985.

Rockhold, R.W., Acuff, C.G. and Clower, B.R. Excitotoxic lesions of the paraventricular hypothalamus: Metabolic and cardiac effects. Neuropharmacology **29**:663-673, 1990.

Sanders-Bush, E. and Steranka, L.R. Immediate and long-term effects of p-chloroamphetamine on brain amines. Ann. NY Acad. Sci. **305**: 208-221,1978.

Sasaki, S. and Dampney, R.A.L. Tonic cardiovascular effects of angiotensin II in the ventrolateral medulla. Hypertension **15**:274-283, 1990.

Saxena, P.R. and Villalón, C.M. Cardiovascular effects of serotonin agonists and antagonists. J. Cardiovasc. Pharm. **15**:S17-S34, 1990.

Saxena, P.R. and Villalón, C.M. 5-Hydroxytryptamine: A chameleon in the heart. Trends Pharmacol. Sci, **12**:223-227, 1991.

Saydoff, J.A., Rittenhouse, P.A., Carnes, M., Brownfield, M.S. and Van de Kar, L.D. Neuroendocrine effect of m-chlorophenylpiperazine (m-CPP), a relatively selective 5-HT_{1B} agonist. Soc. Neurosci. **15**:429.13, 1989. Abstract.

Saydoff, J.A., Rittenhouse, P.A., Van de Kar, L.D. and Brownfield, M.S. Enhanced serotonergic transmission stimulates oxytocin secretion in conscious male rats. J. Pharmacol. Exp. Ther. **257**:95-99, 1991.

Schotte, A., De Bruyckere, K., Janssen, P.F.M. and Leysen, J.E. Receptor occupancy by ritanserin and risperidone measured using ex vivo autoradiography. Brain Res. **500**:295-301, 1989.

Schulze, H.G. and Gorzalka, B.B. Oxytocin effects on lordosis frequency and lordosis duration following infusion into the medial pre-optic area and ventromedial hypothalamus of female rats. Neuropeptides **18**:99-106, 1991.

Seuwen, K. and Pouyssegur, J. Serotonin as a growth factor. Biochem. Pharmacol. **39**:985-990, 1990.

Shannon, M., Battaglia, G., Glennon, R.A. and Titeler, M. 5-HT_1 and 5-HT_2 binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). Eur. J. Pharmacol. **102**:23-29, 1984.

Shapiro, R.E. and Miselis, R.R. The central neural connections of the area postrema of the rat. J. Comp. Neurol. **234**:344-364, 1985.

Sharp, T., Zetterstrom, T., Christmanson, L. and Ungerstedt, U. p-Chloroamphetamine releases both serotonin and dopamine into rat brain dialysates in vivo. Neurosci. Lett. **72**:320-324, 1986.

Shibutani, Y., Sakamoto, K., Katsuno, S., Yoshimoto, S. and Matsuura, T. An epidemiological study of plasma renin activity in school children in Japan: distribution and its relation with family history of hypertension. J. Hypertension **6**:489-493, 1988.

Shoji, T., Tamaki, T., Fukui, K., Iwao, H. and Abe, Y. Renal hemodynamic responses to 5-hydroxytryptamine (5-HT): involvement of the 5-HT receptor subtypes in the canine kidney. Eur. J. Pharmacol. **171**:219-228, 1989.

Shoji, T., Kiyomoto, H., Tamaki, T., Iwao, H. and Abe, Y. Renal vasodilatation induced by DOI, a 5-HT_2 receptor agonist, in the canine kidney. Eur. J. Pharmacol. **190**:247-250, 1990.

Sjoerdsma, A. and Palfreyman, M.G. History of serotonin and serotonin disorders. Ann. NY Acad. Sci. **600**:1-8, 1990.

Smits, J.R.M., Van Essen, H. and Struyker-Boudier, H.A.J. Serotonin-mediated cardiovascular responses to electrical stimulation of the raphé nuclei in the rat. Life Sci. **23**:173-178, 1978.

Sole, M.J., Van Loon, G.R., Shum, A., Lixfield, W. and MacGregor, D.C. Left ventricular receptors inhibit brain serotonin neurons during coronary artery occlusion. Science. **201**:620-622, 1978.

Sole, M.J., Versteeg, D.H.G., De Kloet, E.R., Hussain, N. and Lixfeld, W. The identification of specific serotonergic nuclei inhibited by cardiac vagal afferents during acute myocardial ischemia in the rat. Brain Res. **265**:55-61, 1983.

Steel, R.G.D. and Torrie, J.H. *Principles and Procedures of Statistics With Special Reference to the Biological Sciences*, New York: McGraw-Hill, 1960.

Stein, J.M., Lind, R.W. and Johnson, A.K. Central serotonergic influences on renal electrolyte and water excretion. Neuropharmacology **26**: 1685-1692, 1987.

Sternbach, H. The serotonin syndrome. Am. J. Psychiatry **148**:705-713, 1991.

Swanson, L.W. and Sawchenko, P.E. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Ann. Rev. Neurosci. **6**:269-324, 1983.

Takahashi, T., Hisa, H. and Satoh, S. Serotonin-induced renin release in the dog kidney. Eur. J. Pharmacol. **193**:315-320, 1991.

Takeda, K., Nakata, T., Takesako, T., Itoh, H., Hirata, M., Kawasaki, S., Hayashi, J., Oguro, M., Sasaki, S. and Nakagawa, M. Sympathetic inhibition and attenuation of spontaneous hypertension by PVN lesions in rats. Brain Res. **543**:296-300, 1991.

Taylor, D.P. Serotonin agents in anxiety. Ann. NY Acad. Sci. **600**:545-557, 1990.

Teitler, M., Leonhardt, S., Weisberg, E.L. and Hoffman, B.J. 4-[^{125}I]Iodo-(2,5-dimethoxy)phenylisopropylamine and [^{3}H]ketanserin labeling of 5-hydroxytryptamine$_2$ ($5HT_2$) receptors in mammalian cells transfected with a rat $5HT_2$ cDNA: Evidence for multiple states and not multiple $5HT_2$ receptor subtypes. Mol. Pharmacol. **38**:594-598, 1990.

Tobian, L., Tomboulian, A. and Janacek, J. The effect of high perfusion pressures on the granulation of juxtaglomerular cells in an isolated kidney. J. Clin. Invest. **38**:605-610, 1959.

Tokunaga, K., Fukushima, M., Kemnitz, J.W. and Bray, G.A. Comparison of ventromedial and paraventricular lesions in rats that become obese. Am. J. Physiol. **251**:R1221-1227, 1986.

Tork, I. Anatomy of the serotonergic system. Ann. NY Acad. Sci. **600**:9-35, 1990.

Tsutsumi, K. and Saavedra, J.M. Characterization and development of angiotensin II receptor subtypes (AT_1 and AT_2) in rat brain. Am. J. Physiol. **261**:R209-R216, 1991.

Tyce, G.M. Origin and metabolism of serotonin. J. Cardiovasc. Pharmacol. **16**:S1-S7, 1990.

Ueda, H., Yasuda, H., Takabatake, Y., Iizuka, M., Iizuka, T., Ihori, M., Yamamoto, M. and Yoshiyuki, S. Increased renin release evoked by mesencephalic stimulation in the dog. Jap. Heart J. **8**:498-506, 1967.

Urban, J.H., Van de Kar, L.D., Schmitt, S.L. and Brownfield, M.S. In vitro evidence for a blood-borne renin-releasing factor. Life Sci. **37**: 1335-1342, 1985.

Valladao, A.S., Saad, W.A., De Arruda Camargo, L.A., Renzi, A., De Luca, L.A.,Jr. and Menani, J.V. Interaction between cholinergic and adrenergic pathways of the hypothalamic ventromedial nucleus on cardiovascular regulation. J. Auton. Nerv. Sys. **30**:239-246, 1990.

Vallotton, M.B. The renin-angiotensin system. Trends Pharmacol. Sci. **8**:69-74, 1987.

Van de Kar, L.D. Neuroendocrine aspects of the serotonergic hypothesis of depression. Neurosci. Biobehav. Rev. **13**:237-246, 1989.

Van de Kar, L.D. Neuroendocrine pharmacology of serotonergic (5-HT) neurons. Ann. Rev. Pharmacol. Toxicol. 31:289-320, 1991.

Van de Kar, L.D., Wilkinson, C.W. and Ganong, W.F. Pharmacological evidence for a role of brain serotonin in the maintenance of plasma renin activity in unanesthetized rats. J. Pharmacol. Exp. Ther. **219**:85-90, 1981.

Van de Kar, L.D., Wilkinson, C.W., Skrobik, Y., Brownfield, M.S. and Ganong, W.F. Evidence that serotonergic neurons in the dorsal raphé nucleus exert a stimulatory effect on the secretion of renin but not of corticosterone. Brain Res. **235**:233-243, 1982.

Van de Kar, L.D. and Bethea, C.L. Pharmacological evidence that serotonergic stimulation of prolactin secretion is mediated via the dorsal raphe nucleus. Neuroendocrinology **35**:225-230, 1982.

Van de Kar, L.D., Lorens, S.A., McWilliams, C.R., Kunimoto, K., Urban, J.H. and Bethea, C.L. Role of midbrain raphe in stress-induced renin and prolactin secretion. Brain Res. **311**:333-341, 1984.

Van de Kar, L.D. and Richardson-Morton, K.D. Serotonergic regulation of renin release is not mediated by the autonomic nervous system, but involves beta adrenoceptors. Neuropharmacology **25**:487-492, 1986.

Van de Kar, L.D., Urban, J.H., Brownfield, M.S. and Simmons, W.H. Partial characterization of a renin-releasing factor from plasma and hypothalamus. Hypertension **9**:598-606, 1987.

Van de Kar, L.D., Carnes, M., Maslowski, R.J., Bonadonna, A.M., Rittenhouse, P.A., Kunimoto, K., Piechowski, R.A. and Bethea, C.L. Neuroendocrine evidence for denervation supersensitivity of serotonin receptors: effects of the 5-HT agonist RU 24969 on corticotropin, corticosterone, prolactin and renin secretion. J. Pharmacol. Exp. Ther. **251**:428-434, 1989.

Van de Kar, L.D., Urban, J.H. and Brownfield, M.S. Serotonergic regulation of renin and vasopressin secretion. In: *Serotonin. From Cell Biology to Pharmacology and Therapeutics*, edited by Paoletti, R., Vanhoutte, P.M., Brunello, N. and Maggi, F.M. Dordrecht Netherlands: Kluwer Academic Publishers, 1990, p. 123-129.

Van de Kar, L.D., Richardson Morton, K.D. and Rittenhouse, P.A. Stress: neuroendocrine and pharmacological mechanisms. In: *Stress Revisited. 1. Neuroendocrinology of Stress. Methods and Achievements in Experimental Pathology*, edited by Jasmin, G. and Cantin, M. Basel: S. Karger, 1991, p. 133-173.

Van Heuven-Nolsen, D. 5-HT receptor subtype-specific drugs and the cardiovascular system. Trends Pharmacol. Sci. **9**:423-425, 1988.

van Houten, M., Schiffrin, E.L., Mann, J.F.E., Posner, B.I. and Boucher, R. Radioautographic localization of specific binding sites for blood-borne angiotensin II in the rat brain. Brain Res. **186**:480-485, 1980.

Van Neuten, J.M., Janssens, W.J. and Vanhoutte, P.M. Serotonin and vascular smooth muscle. In: *Serotonin and the Cardiovascular System*, edited by Vanhoutte, P.M. New York: Raven Press, 1985, p. 95-103.

Van Nueten, J.M., Schuurkes, J.A.J., De Ridder, W.J.E., Kuyps, J.J.M.D. and Janssens, W.J. Comparative pharmacological profile of ritanserin and ketanserin. Drug Dev. Res. **8**:187-195, 1986.

Van Zwieten, P.A., Blauw, G.J. and Van Brummelen, P. Serotonin and serotonin antagonists. Pathophysiological and pharmacotherapeutic aspects of serotonin and serotonergic drugs. Clin. Physiol. Biochem. **8**:1-18, 1990.

Vander, A.J. Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs. Am. J. Physiol. **209**:659-662, 1965.

Vayssettes-Courchay, C., Bouysset, F., Verbeuren, T.J., Laubie, M. and Schmitt, H. The cardiovascular effects of quipazine are mediated by peripheral 5-HT_2 and 5-HT_3 receptors in anaesthetized rats. Eur. J. Pharmacol. **184**:75-85, 1990.

Vayssettes-Courchay, C., Bouysset, F., Verbeuren, T.J., Laubie, M. and Schmitt, H. Quipazine-induced hypertension in anaesthetized cats is mediated by central and peripheral 5-HT_2 receptors: role of the ventrolateral pressor area. Eur. J. Pharmacol. **192**:389-395, 1991.

Waeber, B., Nussberger, J. and Brunner, H.R. The renin-angiotensin system: role in experimental and human hypertension. In: *Handbook of Hypertension Vol.8: Pathophysiology of Hypertension-Regulatory Mechanisms*, edited by Zanchetti, A. and Tarazi, R.C. Elsevier Science Publishers B.V., 1986, p. 489-519.

Waeber, C., Dietl, M.M., Hoyer, D. and Palacios, J.M. 5-HT_1 receptors in the vertebrate brain: regional distribution examined by autoradiography. Arch. Pharmacol. **340**:486-494, 1989.

Weber, M.A., Neutel, J.M., Essinger, I., Glassman, H.M., Boger, R.S. and Luther, R. Assessment of renin dependency of hypertensin with a dipeptide renin inhibitor. Circulation **81**:1768-1774, 1990.

Weiss, M.L. and Hatton, G.I. Collateral input to the paraventricular and supraoptic nuclei in rat. II. Afferents from the ventral lateral medulla and nucleus tractus solitarius. Brain Res. Bull. **25**:561-567, 1990a.

Weiss, M.L. and Hatton, G.I. Collateral input to the paraventricular and supraoptic nuclei in rat. I. afferents from the subfornical organ and the anteroventral third ventricle region. Brain Res. Bull. **24**:231-238, 1990b.

Whitebread, S., Mele, M., Kamber, B. and De Gasparo, M. Preliminary biochemical characterization of two angiotensin II receptor subtypes. Biochem. Biophys. Res. Commun. **163**:284-291, 1989.

Wolf, W.A., Kuhn, D.M. and Lovenberg, W. Pressor effect of dorsal raphe stimulation and intrahypothalamic application of serotonin in the spontaneously hypertensive rat. Brain Res. **208**:192-197, 1981.

Zhang, T.X. and Ciriello, J. Effect of paraventricular nucleus lesions on arterial pressure and heart rate after aortic baroreceptor denervation in the rat. Brain Res. **341**:101-109, 1985.

Zimmerman, H. and Ganong, W.F. Pharmacological evidence that stimulation of central serotonergic pathways increases renin secretion. Neuroendocrinology 30:101-107, 1980.

Zimmerman, B.G., Sybertz, E.J. and Wong, P.C. Interaction between sympathetic and renin-angiotensin system. J. Hypertension 2:581-587, 1984.

Zink, M.H.,III, Pergola, P.E., Doane, J.F., Sved, A.F. and Alper, R.H. Quipazine increases renin release by a peripheral hemodynamic mechanism. J. Cardiovasc. Pharmacol. 15:1-9, 1990.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

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

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