ENDOGENOUS ANGIOTENSIN II, SYMPATHETIC ACTIVITY, AND THE AREA POSTREMA

by

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Abstract

The mechanism by which normal arterial pressure is maintained during sodium deprivation is not completely understood. However, indirect evidence suggests that an interaction between endogenous angiotensin II (Ang II) and the sympathetic nervous system may participate in arterial pressure regulation during chronic changes in sodium intake, and plasma Ang II may have a central action to chronically increase or maintain sympathetic outflow to increase or maintain arterial pressure. The studies in this dissertation tested two closely related hypotheses: 1) in chronically sodium deprived rats, endogenous Ang II participates in long-term maintenance of arterial pressure, in part, by maintaining sympathetic outflow, and 2) this action of Ang II requires the area postrema in the brain.

To produce different circulating Ang II levels, male Sprague-Dawley rats were provided one of the three diets containing deficient (LS), control (CS), and high (HS) levels of sodium, respectively. Surgery was conducted to implant catheters for mean arterial pressure (MAP) measurement and drug delivery, and to implant nerve electrodes for multi-fiber nerve activity recording in each rat. Three studies were conducted in conscious rats to assess the effects of acute blockade of Ang II receptors on heart rate (HR), lumbar (LSNA) and renal (RSNA) sympathetic nerve activity, and baroreflex function.

In the first study, the hypothesis that endogenous Ang II chronically maintains LSNA, RSNA and HR at basal arterial pressure was tested. It was

determined if acute blockade of Ang II type 1 (AT1) receptor with losartan, a non-peptide AT1 antagonist, decreased LSNA, RSNA and HR more in LS rats with elevated circulating Ang II levels, compared to CS and HS rats with normal and low levels of circulating Ang II, respectively. In LS rats, losartan decreased MAP in two phases: an immediate rapid decrease (in minutes) and a slower further fall (up to 40 minutes). Corresponding to the rapid fall in MAP were increases in RSNA, LSNA and HR. Despite a further slow fall in MAP, the elevated RSNA and HR remained constant while LSNA decreased toward control. Following restoration of MAP with α1 agonists, RSNA, LSNA, and HR were suppressed to below pre-losartan levels. In CS rats, losartan caused similar, but smaller changes in MAP, RSNA and HR, compared with LS rats. In contrast, losartan did not change any observed variables in HS rats. These results suggest that endogenous Ang II chronically maintains LSNA, RSNA and HR in LS rats at basal arterial pressure, but this neurogenic action of Ang II can only be observed after hypotension was corrected and MAP was maintained at pre-losartan basal levels.

The second study tested the hypothesis that endogenous Ang II chronically maintains LSNA and HR at any given MAP levels of baroreflex range. The effects of either AT1 receptor blockade with losartan, or Ang II type 2 (AT2) receptor blockade with PD123319, on baroreflex function were examined in LS and HS rats. Baroreflex curves were produced before and 40 min after iv administration of losartan or PD123319. Post-losartan MAP was maintained at pre-losartan levels by infusion of methoxamine. In 40 min, losartan decreased LSNA and HR in LS but

not HS rats at pre-losartan basal MAP. At any other MAP levels, losartan decreased baroreflex-mediated LSNA more in LS than in HS rats. Losartan shifted the LSNA/MAP curve to lower MAP level in LS than in HS rats, without altering the maximal gain. Similarly, losartan reduced maximal HR and shifted the HR/MAP curve to lower MAP level in LS but not HS rats. In general, PD123319 did not change the observed variables in LS rats. These results suggest that in LS rats, endogenous Ang II chronically maintains LSNA and HR at any MAP levels of baroreflex range through AT1 but not AT2 receptors.

The last study tested the hypothesis that the area postrema is necessary for endogenous Ang II to chronically maintain LSNA and HR at basal arterial pressure and any other MAP levels of baroreflex range in LS rats. It was determined if the area postrema lesion (APx) abolished the effects of losartan to suppress LSNA and HR in LS rats. Before losartan administration, basal MAP, HR, and baroreflex function were similar between APx and sham-operated rats with or without food restriction, with the exception of lower baroreflex-mediated maximal LSNA and higher maximal gain of baroreflex control of HR in APx rats. In all groups, losartan similarly shifted the LSNA/MAP curve to lower MAP level without altering the maximal gain. Losartan decreased minimal LSNA in all groups and suppressed maximal LSNA only in sham, but not APx rats. In general, losartan had similar effects on the HR/MAP curve in all groups. These results suggest that in LS rats, the area postrema is not necessary for endogenous Ang II to chronically maintain LSNA and HR at most MAP levels, but is required for Ang II to maintain maximal

LSNA.

In conclusion, the studies in this dissertation provided direct evidence that endogenous Ang II chronically maintains sympathetic outflow and heart rate at any given levels of MAP within baroreflex range as well as baroreflex function in conscious, sodium deprived rats through AT1 but not AT2 receptors. This action of Ang II does not require the area postrema in the brain. The results suggest that an interaction between endogenous Ang II and sympathetic nervous system is important in long-term regulation of arterial pressure during chronic changes in sodium intake.

Chapter 1

Introduction

The renin-angiotensin system (RAS) is a major endocrine system regulating arterial pressure and body fluids homeostasis. Angiotensin II (Ang II) is the effective, principal end product of the RAS. Regulations of the production of endogenous Ang II have been extensively reviewed (31,48,83,131,133). Briefly, circulating Ang II is formed mainly from angiotensin I by angiotensin converting enzyme (ACE). Angiotensin I, a substrate of renin, is produced from angiotensinogen from the liver. Renin, the rate limiting enzyme in the RAS, is produced by the kidneys. The production or secretion of renin is controlled by changes in sodium intake, arterial pressure, and sympathetic drive to the kidneys (33). Decreases in sodium intake, blood volume, arterial pressure, and increases in sympathetic drive to the kidneys stimulate renin release. Increases in sodium intake, blood volume, arterial pressure and decreases in sympathetic drive to the kidneys inhibit renin release. Therefore, a change in sodium intake is a major factor in the regulation of plasma concentrations of Ang II.

Ang II has broad actions in the regulation of cardiovascular function. It directly constricts blood vessels, stimulates aldosterone secretion, increases renal sodium and water retention, facilitates norepinephrine release from sympathetic nerve endings and stimulates vasopressin secretion. However, it is not clear if

endogenous Ang II participates in long-term regulation of arterial pressure, in part, by increasing or maintaining sympathetic nervous system outflow. In this introduction, the evidence for Ang II and the sympathetic nervous system to participate in long-term regulation of arterial pressure will be reviewed. Then, the hypotheses will be presented. A brief description of the methods as well as the specific aims for each of the three studies presented in this dissertation will also be presented.

Ang II and long-term arterial pressure regulation

Abundant evidence shows that Ang II participates in long-term arterial pressure regulation, as reviewed before (27,48). A typical study showing this role of Ang II was conducted in conscious dogs (67). In this study (67), Hall et al demonstrated that arterial pressure increased with chronic step-increases of sodium intake when the RAS was not allowed to function properly, by inhibiting Ang II formation with captopril, an ACE inhibitor, or by chronic iv infusion of Ang II at suppressor dose. In contrast, arterial pressure remained unchanged in dogs with a functional RAS, regardless of changes in sodium intake (67). The authors suggested that the RAS helps to maintain arterial pressure during chronic decreases in sodium intake by increasing renal sodium reabsorption, independent of changes in plasma aldosterone concentration, arterial pressure and glomerular filtration rate. Chronic Ang II infusion can induce hypertension not only in dogs (26,67), but also in other species including rabbits (11,12,37) and rats

(39,53,62,157). The mechanism for Ang II induced hypertension is not clear. In contrast to Ang II infusion, blockade of Ang II action lowers arterial pressure. Either ACE inhibitors or Ang II receptor antagonists decrease arterial pressure in both animal models and humans with hypertension (35,102,125,135,155), or without hypertension but with elevated plasma Ang II levels due to low sodium intake (6,24,32). These studies demonstrate that endogenous Ang II is involved in long-term arterial pressure regulation.

The mechanisms by which Ang II maintains arterial pressure during sodium deprivation are not completely understood. Although Ang II can increase renal sodium retention and this action of Ang II has been suggested to be a major mechanism in the regulation of arterial pressure during changes in sodium intake (27,67), other possible mechanisms cannot be ruled out. Indeed, the action of Ang II on renal sodium retention takes time (days) and loss of this action after acute blockade of Ang II can hardly explain the rapid (minutes) decrease in arterial pressure observed in chronic sodium deprived animals (32). Furthermore, this type of pressure reduction is greater in lower sodium intake animals with higher circulating Ang II levels (32). Clearly, this type of hypotension cannot be due to withdrawal of the renal action of Ang II, since arterial pressure decreases so quickly (within minutes) after AT1 receptor blockade.

It is true that loss of the direct vasoconstrictor action of Ang II can cause arterial pressure to fall. It could also be argued that the greater fall of arterial pressure after Ang II blockade in lower sodium intake animals is due to a greater

prior occupancy of vascular receptor sites by circulating Ang II, because pressor response to iv injected Ang II is blunted during sodium deprivation (146,149,150,152). However, the direct action on the vascular receptors may not be the only mechanism. First, vascular Ang II receptors are down-regulated by sodium deprivation (2), and this mechanism could explain the blunted Ang II pressor effect during low sodium intake (146,149,150,152). Thus, a greater percentage occupancy of vascular Ang II receptors during lower sodium intake does not necessarily mean a greater number of the receptor sites that are occupied, because the total receptor number is decreased. Second, a recent study showed that losartan decreased arterial pressure in two phases in Ang II induced hypertension: an initial rapid fall (5 min) and a further slow fall (from 5 min to 2 hr) (62). The slow fall in arterial pressure was not due to slow blockade of vasculature AT1 receptors, because arterial pressure did not increase in response to iv Ang II injection at either 5 min or 24 hr post-losartan, indicating complete receptor blockade (62). The slow depressor action of losartan could be due to slow blockade of a neurogenic action of Ang II, causing slow decrease in the sympathetic drive to the vasculature to decrease total peripheral resistance, but this possibility has not been directly tested.

There is a line of indirect evidence suggesting that Ang II may have a neurogenic action to maintain sympathetic system activity. Decreases in arterial pressure with Ang II blockade do not elicit baroreflex increases in heart rate (HR) (40,69,77,159). Normally, decreases in arterial pressure reduce the firing of

baroreceptors that are located in the aortic arch and carotid sinus. The decrease in the baroreceptor firing to the nucleus tractus solitarius (NTS) in the brain decreases the inhibitory action on sympathetic system outflow. Therefore, decrease in arterial pressure increases sympathetic outflow through the baroreflex pathway. Since sympathetic drive to the heart increases heart rate, hypotension after Ang II blockade is expected to increase heart rate. Lack of increase in heart rate after Ang II blockade suggests that a direct neurogenic action of Ang II is blocked to counteract or balance the excitatory baroreflex effects on heart rate by hypotension. Thus, Ang II may maintain the sympathetic drive to the heart to maintain heart rate. However, it is also possible that Ang II suppresses parasympathetic drive to the heart. Thus, an increased parasympathetic drive to the heart after Ang II blockade may also counteract the baroreflex effect of hypotension. Taking together, endogenous Ang II may chronically maintain sympathetic outflow, but this possibility has not been directly investigated.

The hypothesis that endogenous Ang II maintains sympathetic system activity has been tested in studies of this dissertation. It should be made clear that the proposed mechanism in the hypothesis is one of many involved in long-term arterial pressure maintenance. As we know, arterial pressure is the product of cardiac output and total peripheral resistance. Cardiac output is in proportion to effective blood volume. During increase in sodium intake, arterial pressure is maintained at normal levels, because circulating Ang II level decreases to reduce total peripheral resistance to counteract the effect of increased cardiac output (93).

If circulating Ang II level is not allowed to decrease in response to increases in sodium intake with iv Ang II infusion, both total peripheral resistance and cardiac output increase, causing arterial pressure to increase (93). These results indicate that before increases in sodium intake, circulating Ang II maintains the total peripheral resistance. During sodium deprivation, this action of Ang II could be important in maintaining normal arterial pressure. Decreases in sodium intake lower effective blood volume, hence cardiac output decreases. At the same time, decreases in sodium intake activate the RAS, hence circulating Ang II increases. Elevated circulating levels of Ang II can increase total peripheral resistance to counteract the effect of decreased cardiac output. Thus, arterial pressure can be maintained at or close to normal levels.

Besides its direct vasoconstriction, Ang II could have an indirect neurogenic vasoconstriction to maintain or increase total peripheral resistance. In other words, Ang II could maintain or increase sympathetic drive to arterial blood vessels. If this idea is correct, it is expected that higher level of circulating Ang II maintains greater proportion of sympathetic activity. Thus, the higher the endogenous Ang II level, the greater the decrease in sympathetic activity after Ang II blockade. Studies in this dissertation have been designed based on this rationale.

Whether Ang II maintains sympathetic outflow has been difficult to determine. First, direct nerve activity recording, the only way to accurately measure sympathetic nerve activity, is difficult technically, especially in conscious animals. Second, loss of Ang II's direct vasoconstriction induces hypotension. This

hypotension increases sympathetic outflow through the baroreflex pathway. The baroreflexly increased sympathetic outflow may counteract possible decrease in sympathetic activity caused by withdrawal of neurogenic action of Ang II. Therefore, the net sympathetic activity after Ang II blockade may not decrease if the hypotension is not corrected. However, if arterial pressure after Ang II blockade is maintained at pre-blockade levels, decreases in the sympathetic activity due to blockade of neurogenic action of Ang II should be revealed.

Sympathetic nervous system and long-term arterial pressure regulation

Although it remains controversial, accumulating indirect evidence suggests that sympathetic nervous system participates in long-term regulation of arterial pressure (16). Elevated sympathetic activity is related to the pathogenesis of, at least, some forms of hypertension (9,16,49,61). Plasma norepinephrine concentration, an indirect index of the overall sympathetic activity, is above normal in both clinical and experimental models of renal vascular hypertension (30,63,119) and essential hypertension (61,119). In chronic heart failure, either increased norepinephrine levels (46,55,60), or norepinephrine spill-over, an indirect index of sympathetic nervous system activity considered to be free from affects of changes in blood volume (43,136), has been observed. During sodium deprivation, increased total and renal norepinephrine spill-over have also been observed in humans (56,160). In addition, decreasing sympathetic activity by using sympatholytic agents, such as clonidine, lowers arterial pressure chronically in

hypertension (119). Therefore, a chronically overactive sympathetic nervous system is correlated with hypertension, and a chronically suppressed sympathetic nervous system leads to lower arterial pressure.

The integrity of the sympathetic nervous system seems necessary for long-term arterial pressure regulation during changes in sodium intake. Specifically, a sympathetic nervous system that is unable to adjust its outflow in response to sodium loading may cause an increase in arterial pressure, or salt-dependent hypertension. Blocking afferent information on arterial pressure to the central nervous system by denervating arterial baroreceptor or destroying the NTS also causes salt-dependent hypertension (73,123,158). Pre-blockade of the output of the sympathetic system with prazosin, an α1 adrenergic antagonist before sodium loading prevents the sympathetic activity from further decrease during the sodium loading, and this manipulation renders rats susceptible to salt-dependent hypertension (124). Chronic intrarenal norepinephrine infusion, an attempt to mimic an elevated sympathetic outflow to the kidneys, also produces salt-induced hypertension (28). Although chronic iv infusion of norepinephrine does not cause hypertension during normal sodium intake (86), chronic intrarenal norepinephrine infusion does, either at doses sufficient (28,81) or not sufficient (120,121) to decrease renal blood flow. This type of hypertension is a result of increased total peripheral resistance, a phenomenon not observed when the same doses are infused iv (28,81). Therefore, a responsive sympathetic nervous system seems to be required for normal long-term regulation of arterial pressure during changes in

sodium intake. However, how changes in sodium intake influence sympathetic activity is not clear. One hypothesis is that changes in endogenous Ang II levels due to changes in sodium intake mediate sympathetic activity (16).

Circulating Ang II and sympathetic nervous system outflow

Acute action of Ang II. No consistent actions of Ang II has been found in the past studies. Acute application of Ang II was found to increase, decrease or have no effects on sympathetic activity. Matsukswa et al (109) found that Ang II increased muscle sympathetic nerve activity in humans. Stein et al (148) reported that in chloralose-anesthetized cats, intracarotid Ang II infusion did not consistently affect splanchnic sympathetic nerve activity, but equal-pressor dose of phenylephrine or iv Ang II always decreased splanchnic sympathetic nerve activity, suggesting that Ang II acts centrally to oppose the baroreceptor-induced sympathoinhibition (148). Indirect evidence supporting a role of Ang II to increase sympathetic outflow was also found. Ferrario et al (47) showed indirectly that acute exogenous Ang II can act in dog brain to increase sympathetic outflow. In a recent study, Fujii et al (57) observed that, in sinoaortic baroreceptor denervated (SAD) dogs, increases in arterial pressure and total peripheral resistance in response to iv Ang II bolus after ganglionic blockade were about 50% of those before ganglionic blockade, indicating that about half of the Ang II pressor effect was due to a neurogenic action of Ang II to increase sympathetic outflow. However, there are also reports for an inhibitory action of Ang II on sympathetic activity. Aars and Akre

(1) observed that iv infusion of Ang II decreased cervical and renal nerve activity in chloralose-anesthetized rabbits. Guo and Abboud (66) demonstrated that Ang II infused iv decreased lumbar sympathetic nerve activity (LSNA) in chloralose-anesthetized rabbits. Matsumura et al (110) found that Ang II decreased renal sympathetic nerve activity (RSNA) in conscious rabbits.

It should be noted that in most of these studies, the concomitant increase in arterial pressure after Ang II injection was not considered. This increase in arterial pressure may, by baroreflex pathway, counteract or offset the excitatory action of Ang II on sympathetic outflow. Thus, a decreased net sympathetic outflow may be observed. In other words, if the baroreflex affect on sympathetic outflow had been taken away, an increase in sympathetic activity after Ang II may have been observed in these studies. For example, in the study of Guo and Abboud (66), iv injected Ang II was less potent in suppression of LSNA than equal pressor dose of iv injected phenylephrine, suggesting that Ang II has a direct excitatory action on LSNA and this action counteracts the baroreflex induced inhibition. This view is also supported by studies of Stein et al (148) and Matsukawa et al (109), in which the effect of direct vasoconstriction of Ang II on sympathetic outflow through baroreflex actions has been considered. Another piece of indirect evidence supporting this view is that in SAD dogs in which baroreflex pathway were blocked anatomically, Ang II produced larger pressor response, compared with intact animals in which baroreflex pathway was in function (25,57). Other factors that can affect sympathetic activity also need to be noted. Chloralose anesthesia can

depress the central excitatory action of Ang II (139). Different anesthetics may differently affect the same sympathetic nerve branch (68,72). Ang II may have different effects on different sympathetic nerve branches. Taken together, it seems more likely that acute iv Ang II acts centrally to increase sympathetic outflow, at least to some branches, but this action can only be revealed after the inhibitory effect of the baroreflex is taken away.

Chronic action of Ang II. Previous studies also provide controversial results concerning the chronic action of Ang II on the sympathetic nervous system. Ang II infused iv causes neurogenic hypertension starting 10 hr-24 hr after Ang II infusion (101), and shifts baroreflex control of sympathetic nerve activity to a higher arterial pressure level (18,105). Chronic central Ang II infusion also causes hypertension (7,18,20). Increases in plasma norepinephrine levels or norepinephrine spill-over are found in humans and animals with elevated plasma Ang II levels such as in renovascular hypertension (30,63,119), chronic cardiac failure (43,46,55,60,136) and sodium deprivation (56,118,160). However, no changes in norepinephrine spill-over have also been observed during sodium deprivation (21) or during chronic iv infusion of Ang II (87).

Blockade of endogenous Ang II, when its level is elevated, also produces inconsistent results on sympathetic activity. In patients with stable congestive heart failure, Ang II blockade caused no change in norepinephrine spill-over (59). However, this blockade decreased splanchnic and renal sympathetic nerve activity in renal hypertension and chronic heart failure (34,71,117). Since Ang II can also

act at the sympathetic nerve endings to facilitate norepinephrine release (168), norepinephrine spill-over may not accurately reflect the sympathetic nerve activity. In comparison with norepinephrine methods, directly measured nerve activity is more reliable to assess changes of sympathetic nerve activity. In other words, the results from studies using direct nerve recording method are probably more reliable than those from studies using norepinephrine methods. Thus, it is likely that circulating Ang II could act centrally to chronically maintain sympathetic outflow in diseased states including heart failure and renal hypertension. However, it is not clear whether or not endogenous Ang II during normal physiology, such as under sodium deprived state, also chronically maintains sympathetic outflow by a central action.

In conclusion, previous studies have not provided consistent results regarding either acute or chronic effects of circulating Ang II on sympathetic outflow. Excitatory effects, no effects or even inhibitory effects have been reported. There may be several reasons for the controversy. Plasma norepinephrine level or norepinephrine spill-over has been frequently used as an indirect index of sympathetic activity. Since sympathetic outflow is not equally distributed, the overall sympathetic outflow indicated by plasma norepinephrine levels can not reflect regional changes in sympathetic activity that may be specifically important in the regulation of arterial pressure. Norepinephrine spill-over is better than plasma norepinephrine levels in studies of sympathetic activity because norepinephrine spill-over can reflect regional sympathetic activity. However, the

accuracy of norepinephrine methods remain questionable since Ang II can act at the sympathetic adrenergic nerve endings to facilitate norepinephrine release (168). Therefore, directly measured nerve activity is more reliable than norepinephrine methods in study of sympathetic nerve activity. Unfortunately, in most studies using direct nerve recording, animals were studied under anesthesia. Anesthetics such as chloralose can depress the central excitatory action of Ang II (139) and different anesthetics may differently affect the same sympathetic nerve branch (68,72). The use of ACE inhibitors or partial Ang II antagonists can also contribute to the controversy, because ACE inhibitor can block bradykinin degradation besides blocking Ang II formation, and the partial antagonist may have an agonist action when Ang II level is low. Finally, studying a direct action of Ang II on sympathetic outflow without considering its direct vasoconstriction on sympathetic outflow through the baroreflex pathway is another important factor causing inconsistent results.

The area postrema and the central action of circulating Ang II

Considering the above mentioned evidence, it is possible that endogenous circulating Ang II participates in long-term regulation of arterial pressure, in part, by maintaining sympathetic outflow. If so, where could it act? The circumventricular organs (CVOs) in the brain becomes an immediate candidate, because many studies suggest that circulating Ang II can act at this structure (44,45,47,128,132).

The area postrema is one of the CVOs. It functions as a chemoreceptor

Purpose of studies presented in this dissertation

It has been many years since Ang II was first proposed to act in the brain to increase sympathetic activity, and there have already been several studies concerning the action of exogenous Ang II to support this hypothesis (47,50,80). However, controversy exists, partly because of different methods used to evaluate sympathetic activity. In addition, no study has been done to examine the action of endogenous Ang II on sympathetic nervous system regarding long-term regulation of arterial pressure. The role of endogenous Ang II on baroreflex control of sympathetic outflow during sodium deprivation has neither been investigated.

It is known that during sodium deprivation normal arterial pressure is maintained, with blood volume being decreased and circulating Ang II levels being elevated. However, it is not known how arterial pressure is maintained during sodium deprivation. It is also not clear whether endogenous circulating Ang II contributes to the maintenance of normal arterial pressure, in part, by maintaining sympathetic outflow or whether the baroreflex function is maintained by Ang II during sodium deprivation. Considering that an action of Ang II on renal sodium retention cannot explain the large rapid fall of arterial pressure after acute blockade of Ang II during sodium deprivation and Ang II may act centrally to influence sympathetic outflow, the studies presented in this dissertation were conducted to test the hypotheses that endogenous Ang II chronically maintains sympathetic nerve activity in sodium deprived rats and the area postrema is necessary for this action of Ang II. Direct nerve activity recordings and conscious animals were used

to avoid the disadvantage of using plasma norepinephrine levels as the indirect index of sympathetic nerve activity and to avoid the side effect of anesthetics.

Rats were fed diets containing different amounts of sodium: sodium deficient (LS, Na<0.02%), control sodium (CS, Na =0.5%), and high sodium (HS, Na=4%), for 2 to 3 weeks to induce different circulating Ang II levels. Catheters were implanted for drug delivery and mean arterial pressure (MAP) measurement. Electrodes were implanted around either renal or lumbar nerve for direct multi-fiber nerve activity recording. Ang II antagonists, mainly losartan, an Ang II type 1 receptor (AT1) antagonist, were acutely administered iv to block the action of endogenous Ang II. MAP after Ang II blockade was maintained at pre-losartan levels by infusion of methoxamine or phenylephrine to avoid baroreflex action on sympathetic activity. Changes in sympathetic nerve activity after Ang II blockade were examined. In the studies of Chapter 2, central venous pressure (CVP) was monitored. The calibration of CVP was frequently checked to avoid possible draft. Other details of methods are described in METHODS of each chapter.

Specific Aims

Chapter 2. LS, CS and HS rats were used in this project. Studies presented in this chapter tested the hypothesis that endogenous Ang II chronically maintains RSNA, LSNA and HR at basal arterial pressure. It was determined whether the acute AT1 receptor blockade with losartan decreased RSNA, LSNA and HR, and whether or not the decrease was greater in lower sodium intake rats, independent

of arterial pressure or baroreceptor input. If endogenous Ang II chronically maintains LSNA, RSNA, and HR through the AT1 receptor, it is expected that the suppression on these variables by losartan should be greater in lower sodium intake rats when MAP is maintained at pre-losartan basal levels. CVP was monitored to determine if changes in the nerve activity was due to activation of cardiopulmonary receptors.

Chapter 3. LS and HS rats were used in this project. Studies in this chapter tested the hypothesis that endogenous Ang II chronically maintains LSNA and HR over the entire baroreflex MAP range. Baroreflex control of LSNA and HR was examined so that LSNA and HR could be compared at any given MAP level. Contribution of endogenous Ang II to the sensitivity of baroreflex function was also evaluated. The effects of AT1 receptor blockade with losartan, and Ang II type II (AT2) receptor blockade with PD 123319, were studied respectively.

Chapter 4. Studies presented in this chapter tested the hypothesis that the area postrema is necessary for endogenous Ang II to chronically maintain LSNA and HR in LS rats. The effects of losartan on suppression of LSNA and HR over the entire baroreflex arterial pressure range were investigated in rats with or without the area postrema. If the hypothesis is true, it would be expected that the suppression of LSNA and HR by losartan should be less in rats without the area postrema compared to rats with the area postrema.

Chapter 2

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Endogenous Angiotensin II Chronically Maintains Renal and Lumbar Sympathetic Nerve Activity in Conscious, Sodium Deprived Rats

Ling Xu and Virginia L. Brooks

ABSTRACT

The hypothesis that chronic elevations in endogenous angiotensin II (Ang II) increase sympathetic outflow in conscious, normotensive rats was tested by determining if acute blockade of Ang II receptors with losartan (10 mg/kg, iv) decreases renal (RSNA) or lumbar sympathetic activity (LSNA) or heart rate (HR) more in rats with higher Ang II levels due to a low sodium diet (LS), compared to rats on either a control sodium (CS) or high sodium (HS) diet. In LS rats, losartan decreased ($p<0.05$) MAP in two phases: an immediate decrease of 23±2 mm Hg and a slower fall of 35±4 mm Hg below control levels in 40 min. RSNA (149±13%), LSNA (143±5%) and HR (109±2%) were increased ($p<0.05$) within 5 min after losartan. Despite further falls in MAP, the elevation in RSNA and HR remained constant and LSNA decreased toward control (119±4%). Following restoration of MAP to basal levels with iv methoxamine or phenylephrine infusion, RSNA (46±8%), LSNA (49±11%) and HR (76±2%) were suppressed ($p<0.05$). In CS rats, losartan initially decreased ($p<0.05$) MAP by 6±2 mm Hg and increased ($p<0.05$)

RSNA to 129±13%. When MAP was returned to control, RSNA was decreased (70±8%; $p<0.05$), but less than in LS rats. In contrast, no changes in MAP, RSNA, LSNA or HR were observed after losartan in HS rats. In conclusion, endogenous Ang II chronically maintains RSNA, LSNA and HR in conscious, normotensive low and normal sodium intake rats.

INTRODUCTION

It is well-accepted that the sympathetic nervous system plays a critical role in arterial pressure maintenance during acute changes in blood volume, largely through the actions of the baroreceptor reflex. However, whether the sympathetic nervous system also participates in long-term regulation of arterial pressure is less clear.

One line of evidence to support an important role for the sympathetic nervous system in day-to-day arterial pressure control is that chronic changes in blood volume are associated with changes in indices of sympathetic activity. For example, sodium deprivation, which decreases blood volume, has been shown to increase circulating catecholamine (60), and total and renal norepinephrine spill-over (56,118,160). Recent studies also indicate that renal and muscle sympathetic nerve activity (3,36) and adrenal and ganglionic mRNA levels for tyrosine hydroxylase, the rate limiting enzyme involved in production of norepinephrine (141), are elevated as well. Conversely, a high sodium diet decreases norepinephrine turnover, total peripheral resistance (93) and tyrosine hydroxylase

mRNA levels (141). A second line of evidence is that an increase in sodium intake produces hypertension in animals in which the sympathetic nervous system is rendered unresponsive by chronic infusion of adrenergic agonist or antagonist (28,124).

Such studies suggest that chronic increases or decreases in blood volume elicit chronic changes in sympathetic activity and that these nervous system activity changes are necessary for long-term maintenance of arterial pressure. The question then becomes, what is the mechanism by which chronic alterations in blood volume lead to changes in sympathetic activity? One idea invokes a key role for angiotensin II (16). More specifically, it is hypothesized that long-term decreases in blood volume produce sustained increases in Ang II, which then chronically increase sympathetic activity. A high sodium diet, by suppressing Ang II, would lead to chronic decreases in sympathetic activity.

The purpose of the present experiments was to test this hypothesis using conscious rats instrumented with either renal or lumbar nerve electrodes to measure sympathetic activity. The rats were placed on a low, high or control sodium diet to chronically alter blood volume and plasma Ang II levels. It was reasoned that if Ang II maintains sympathetic activity in rats on a low salt diet, then blockade of Ang II receptors with losartan should decrease sympathetic activity. In these experiments, because Ang II blockade produced profound hypotension which would reflexly activate the sympathetic nervous system, nerve activity was also measured after arterial pressure was returned to basal levels by infusion of α-

adrenergic agonist.

METHODS

Forty nine male, Sprague-Dawley rats (Simonsen), weighing 185±6 g (range: 109-261g) were used for the nerve recording experiments. At 4 weeks of age, they were randomly placed on one of three diets (Harlan Teklad, Madison, WI) with different sodium contents: low sodium (LS; NaCl < 0.02%, n=23), control sodium (CS; Na=0.5%, n=11) and high sodium (HS; Na=4%, n=15). During the first two days on diet, rats in the LS group received a furosemide injection (1 mg/kg/day, IP; Abbott Labs, North Chicago, IL) to increase sodium excretion, while CS and HS rats received vehicle injection (0.9% saline, IP). Rats were on the diets for 2 to 3 weeks before experiments were performed. All animals were housed in a room maintained on a 12:12 hour light-dark cycle and were allowed to have food and distilled water *ad libitum*. One day before the experiment, the rats were habituated to the wire restrainer which would be used during experiments. The restrainer allowed the rats to move forward and backward, but not to turn around.

Surgical Procedures

Rats were anesthetized initially with Brevital (100 mg/kg IP, Eli Lilly, Indianapolis, IN). After a femoral vein catheter was placed for drug administration, anesthesia was maintained by iv Brevital infusion as needed. Another catheter was advanced into abdominal aorta from a femoral artery for mean arterial pressure

(MAP) measurement. A third one was placed close to the right atrium via the jugular vein for measurements of central venous pressure (CVP).

In all rats, either a renal or lumbar nerve electrode was then surgically placed. For renal nerve electrode implantation, the left kidney was exposed retroperitoneally through a flank incision. After a renal nerve branch was exposed and dissected free from the surrounding tissue, it was placed on a bipolar electrode made of Teflon coated stainless steel wire (A&M Systems, Everett, WA). When optimal nerve activity was confirmed on an oscilloscope (Tektronix, Model 2212), the nerve and electrode were embedded in a small amount of dental gel (President Light Body, Coltene, Hudson, MA).

For the lumbar nerve dissection, an abdominal midline incision was made. After retracting the intestines, the abdominal aorta and vena cava were gently pulled aside to expose the lumbar nerve. The nerve was then dissected free, and using the same method as for the renal nerve, the lumbar nerve electrode was implanted.

After catheters and the electrode lead were tunnelled subcutaneously to the back of the neck and exteriorized, all incisions were closed. Rats returned to consciousness and were allowed 2-5 hours for recovery from surgery in the restrainer before the experiment commenced.

Hemodynamic and Nerve Recording

MAP was monitored via the femoral arterial catheter connected to a Statham

pressure transducer which then feeds a Grass preamplifier (7P1). Heart rate (HR) was measured using a Grass tachograph (7P4) triggered by the amplified arterial pressure pulse. CVP was monitored via the jugular catheter using a similar set-up as for MAP. The raw nerve signal was amplified by a Grass differential preamplifier (P511) with a band pass filter of 100 Hz to 10 kHz. The amplified nerve traffic was observed on a storage oscilloscope (Tektronix 2212 , Beaverton, OR) and was whole wave rectified and integrated using a Grass integrator (7P10) with a reset time of 10 sec. Together with MAP, HR and CVP, the integrated nerve signal was recorded on chart paper using a Grass polygraph (7D). Nerve activity was first quantified by averaging the integrated activity just before reset (Fig. 2.3) over 1-2 min (6-12 peaks). In addition, the noise level was assessed at the end of the experiment by determining the integrator noise output after efferent nerve activity was eliminated using the ganglionic blocker hexamethonium chloride (30 mg/kg; Sigma, St. Louis, MO). For the lumbar nerve, possible preganglionic nerve activity after ganglionic blockade was abolished by infusion of methoxamine to increase MAP above 150 mm Hg. The noise output was then subtracted from all average integrated nerve activity values to yield a measure of net sympathetic nerve activity. For each animal, this net nerve activity was expressed as percent of basal or control nerve activity which was considered to be 100%. Control or basal nerve activity was defined as the average of resting activity recorded for 1-2 min at 3 time points (10, 20 and 30 min) during the control period.

Protocols

Experiments were performed while the rat remained in the restrainer. MAP, HR, CVP and renal sympathetic nerve activity (RSNA) or lumbar sympathetic nerve activity (LSNA), were monitored continuously. After parameters remained stable for at least 30 min, an iv bolus injection of nitroprusside (3-10μg; Elkins-Sinn, Cherry Hill, NJ) was given to verify the reflex activation of nerve activity by arterial pressure reduction. Only rats demonstrating reflex increases in nerve activity were used for experiments. A nitroprusside bolus was also administered at the end of the experiment to establish that viable nerve activity remained. In one case, an inadequate response was observed (maximal hypotension-induced nerve activity was 200% at the beginning of the experiment, and only 130% at the end) and the results of this experiment were not included in the data analysis. All protocols assessed responses of LS and HS rats, with the exception of protocol 4, which compared responses of LS and CS rats.

Protocol 1. The purpose of this protocol was to test the temporal stability of the nerve recordings over the course of the experiment, and to determine the effect of vehicle infusion on nerve and hemodynamic parameters. Experiments were performed on rats from all three diet groups. The protocol consisted of three sequential recording periods during which MAP, HR, CVP and RSNA were recorded continuously. The experiment began with a 30 min control period from which basal values were obtained. Then, a bolus of 100 μl 0.9% saline (vehicle volume) was given intravenously followed by a second recording period which lasted 40 min.

In the final recording period of 30 min, 0.9% saline was infused at a rate similar to that in other protocols (2-5 µl/min).

Protocol 2. This protocol had multiple purposes: 1) to determine if RSNA in LS rats was suppressed by Ang II receptor blockade, and if the suppression was greater than that observed in HS rats; 2) to determine the time course of effects of losartan on MAP and nerve activity; and 3) to determine the effects of Ang II blockade after MAP was restored to the basal level. Following 30 min of basal recordings, the AT1 receptor antagonist, losartan (10 mg/kg, iv), was given. Changes in MAP, CVP, HR and nerve activity were then followed for 40 min. In preliminary experiments this was determined to be the time required for arterial pressure to reach its nadir. Then, methoxamine (Sigma, St. Louis, MO), an α1 adrenergic receptor agonist dissolved in 0.9% saline, was infused intravenously (0-12.5 µg/min) to restore MAP to basal values. Measurements of RSNA, MAP, HR and CVP continued for a final 30 min when MAP was maitained at pre-losartan basal levels. Care was taken to maintain MAP to within 5 mm Hg below the basal value. Only vehicle was infused if MAP was not lowered by losartan. Before giving hexamethonium, an Ang II bolus (100 ng/kg, iv) was injected to test if Ang II blockade was still effective. Before losartan, Ang II injection increased ($p<0.05$) MAP by 14±2 mm Hg in LS rats (n=6), by 30±4 mm Hg in CS rats (n=4) and by 30±10 mm Hg in HS rats (n=3). After losartan, injection of the same dose of Ang II did not produce a detectible change in MAP in any group, indicating significant blockade of Ang II receptors.

Protocol 3. The purpose of this protocol was to test if losartan suppressed LSNA while MAP was maintained at basal levels in LS, but not HS, rats. This protocol was identical to Protocol 2 except that LSNA was measured instead of RSNA. In 2 rats, the experiment was delayed until the day after surgery, because their MAP was abnormally high (>130 mm Hg) on the day of surgery. The results of another LS rat were eliminated from data analysis, also because of high MAP (>130 mm Hg).

Protocol 4. The purpose of this protocol was to determine if the suppression of nerve activity observed after methoxamine was due to a nonspecific action of the drug, by using another adrenergic agonist, phenylephrine. A second purpose was to determine if the response of LS rats differs from CS rats. The experimental procedure was the same as that in Protocol 2 except that phenylephrine (0-5.9 μg/min, Elkins-Sinn, Cherry Hill, NJ) was used instead of methoxamine to restore and maintain MAP at basal values during the final recording period.

Plasma angiotensin II and statistical analyses

At times, viable nerve activity was lost either during surgery or before beginning the experiment. In many of these animals (n=12), an arterial blood sample (1 ml) was instead collected 2-3 hr after completion of surgery into ice-chilled tubes containing EDTA, for measurement of plasma Ang II concentration. Blood was also collected 2-3 hr after recovery from surgery and anesthesia from an additional 6 sham animals that were prepared with catheters and a flank incision,

but not nerve electrodes. The blood was centrifuged at 4 °C, and stored at -20 °C until assayed. Ang II concentration was measured from plasma extracted using 100 mg C18 Octadecyl columns (Varian, Sunnyvale, CA) by radioimmunoassay and a previously published procedure (14).

One min averages were collected at each time point and these values are presented as mean±SE. Results were analyzed by two way ANOVA, with repeated measures across time, and the post hoc Newman-Keuls test, using the statistical program GB STAT (Dynamic Microsystems, Inc.; Silver Spring, MD) (161). A significance level of $p<0.05$ was accepted. In some experiments, the between group variance was nonhomogeneous (Levene's F test), so the data was subjected to logarithmic transformation before statistical analysis.

RESULTS

On the day of the experiment, rats maintained for 2-3 weeks on a low sodium diet (155±7 g, n=21) weighed less ($p<0.05$) than rats on either the high sodium (203±7 g, n=15) or normal sodium (225±8 g, n=11) diets. Plasma Ang II levels were elevated ($p<0.0001$) in LS rats (202±44 pg/ml, n=7) compared to either HS (7.3±3.1 pg/ml, n=7) or CS (12.5±3.3 pg/ml, n=4) rats. In addition, there was a tendency for Ang II to be higher in CS compared to HS rats, but this difference did not reach statistical significance because one HS animal had high Ang II levels (20.4 pg/ml). When this outlying value was eliminated, Ang II was lower ($p<0.05$) in HS (5.1±2.6 pg/ml) compared to CS rats.

Time controls

MAP, HR, and RSNA did not change with time in any of the treatment groups (Fig. 2.1), indicating that the experimental model was stable. There was also no difference in MAP or HR between groups at any time.

Losartan and methoxamine

Basal values of MAP were not different between the two groups of rats instrumented with renal nerve electrodes (Fig. 2.2). In the LS group (n=6), losartan decreased MAP, and the hypotensive effect appeared to consist of 2 phases (Fig. 2.2). The first phase was an immediate fall in MAP of 23±2 mm Hg by 5 min after losartan. However, pressure continued to fall ($p<0.01$) to reach a nadir of 81±6 mm Hg after 40 min (Fig. 2.2 and 2.3). RSNA increased to 149±13% after 5 min and was 163±13 % after 40 min. Thirty min after MAP was restored and maintained close to the basal value (115±3 vs. 111±3 mm Hg) by methoxamine infusion, RSNA decreased to 46±8 % of basal. In contrast, MAP and RSNA did not significantly change following losartan injection or saline infusion during the third recording period in HS rats (n=4). As a result, the decrease in RSNA in the LS group was greater ($p<0.05$) than that in the HS group when MAP was controlled.

The changes in MAP and nerve activity of the rats with lumbar nerve electrodes were generally similar to the RSNA experiments (Fig. 2.4). In the LS group (n=6), losartan decreased MAP by 22±5 mm Hg, and increased LSNA to 143±5% after 5 min. Unlike RSNA, LSNA then decreased toward the basal level,

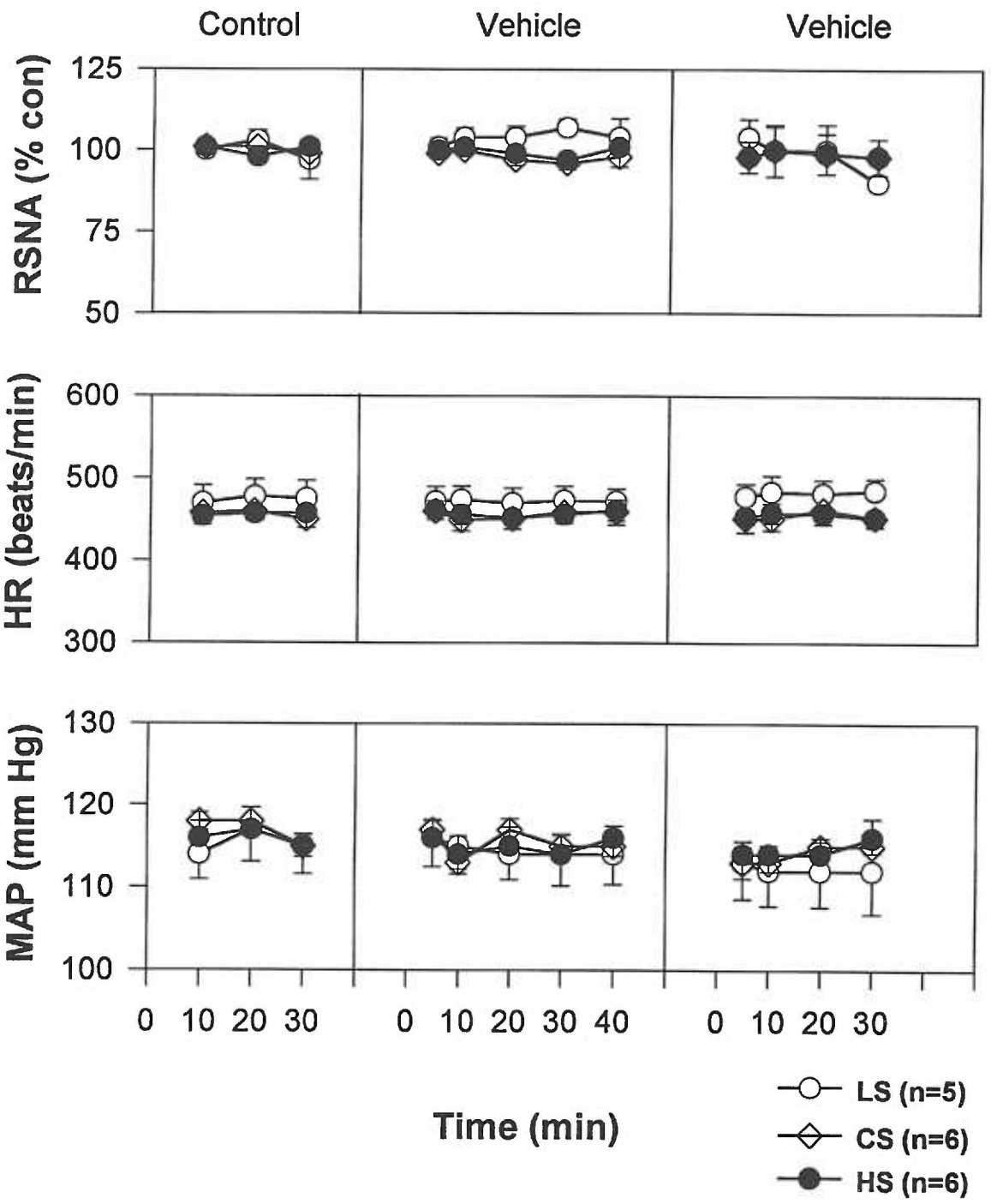

Figure 2.1 Mean arterial pressure (MAP, bottom panel), heart rate (HR, middle panel) and renal sympathetic nerve activity (RSNA, top panel) during the periods before (Control) and after (Vehicle) iv infusion of vehicle (0.9 % saline) in low sodium (LS), control sodium (CS) and high sodium (HS) diet rats.

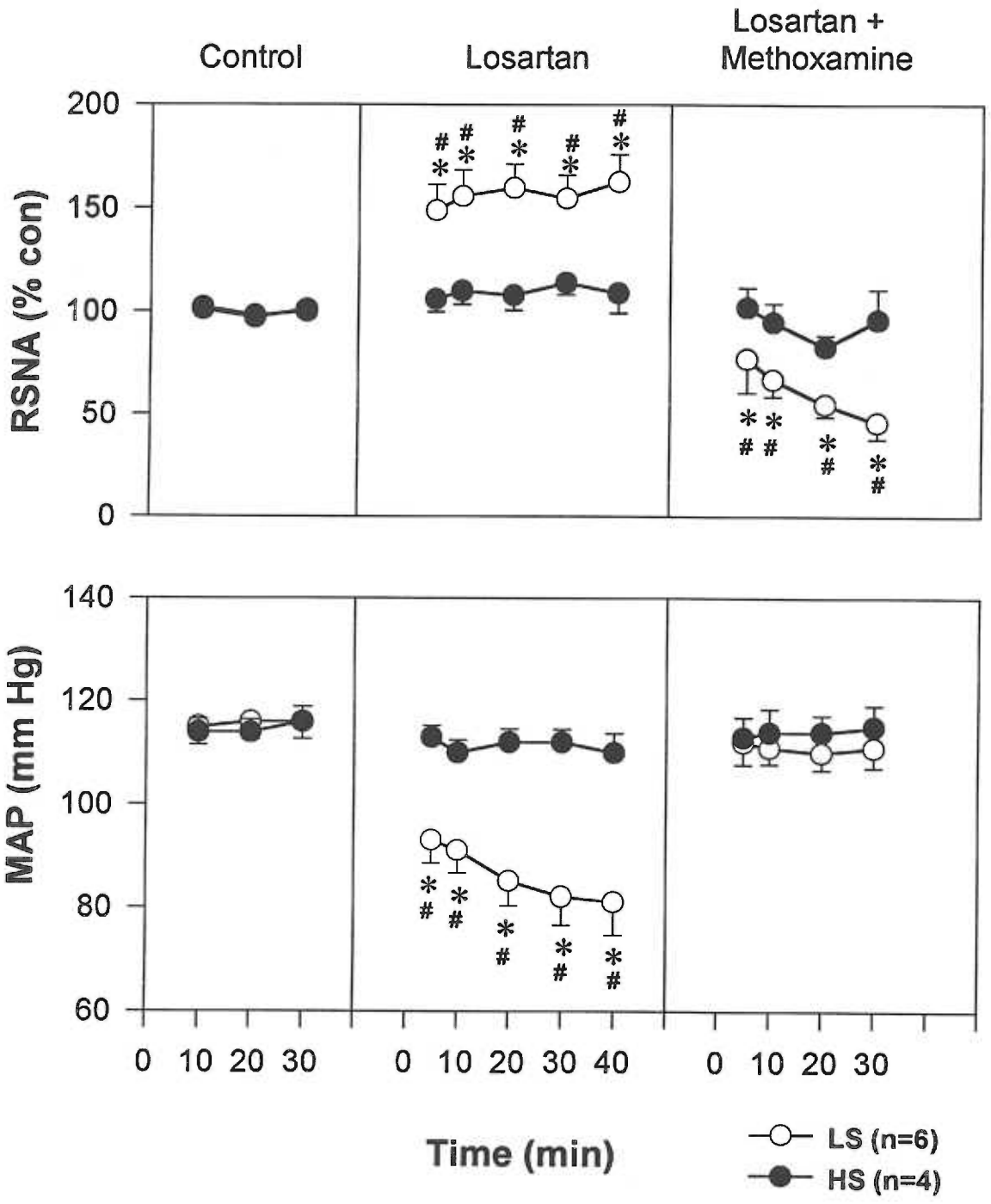

Figure 2.2 Mean arterial blood pressure (MAP, bottom panel) and renal sympathetic nerve activity (RSNA, top panel) during the periods before losartan injection (Control), after iv injection of losartan (Losartan), and after MAP was restored to the pre-losartan basal values with iv infusion of methoxamine (Losartan+Methoxamine) in low sodium diet rats (LS) or with iv infusion of vehicle in high sodium diet rats (HS). * indicates significance compared with the pre-losartan basal values in the same diet group; # indicates significant difference between the two diet groups.

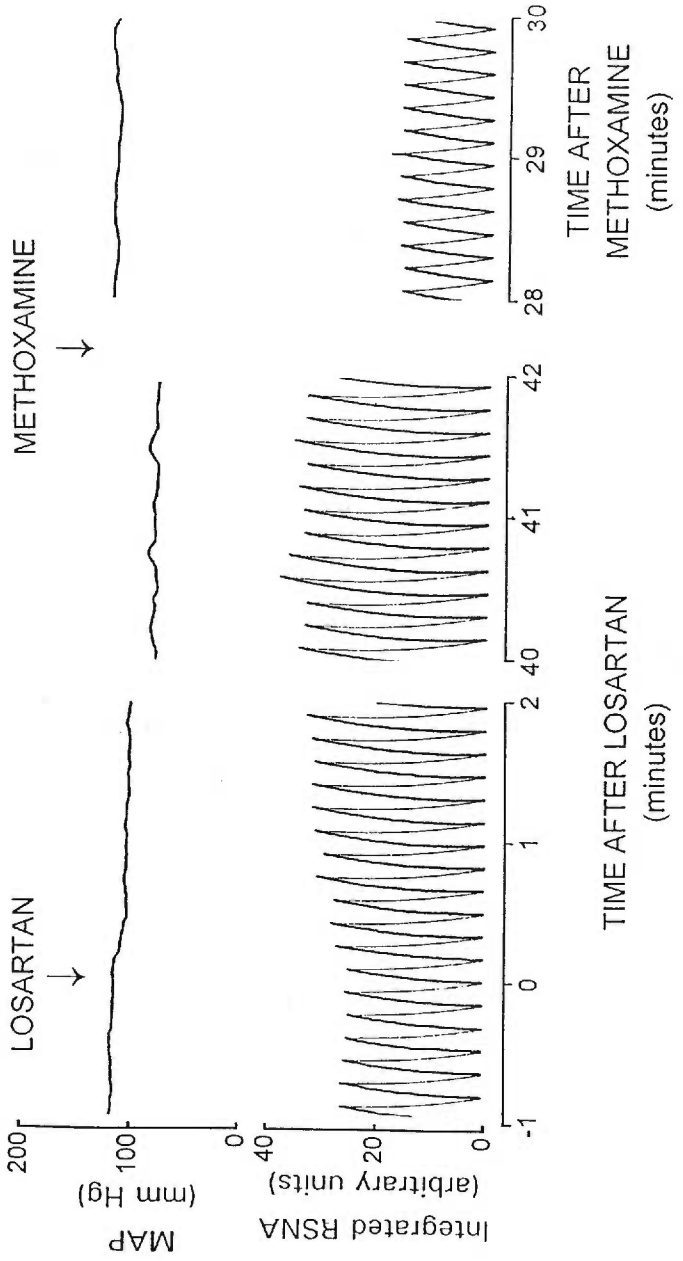

Figure 2.3 Effect of losartan on mean arterial pressure (MAP) and integrated renal sympathetic nerve activity (RSNA) in one low sodium diet rat. Results are shown before (left 2 panels) and after (right panel) MAP was restored to pre-losartan basal values by iv infusion of methoxamine. Note that the time scale indicates the time after losartan injection as well as the time after pressure was restored with methoxamine.

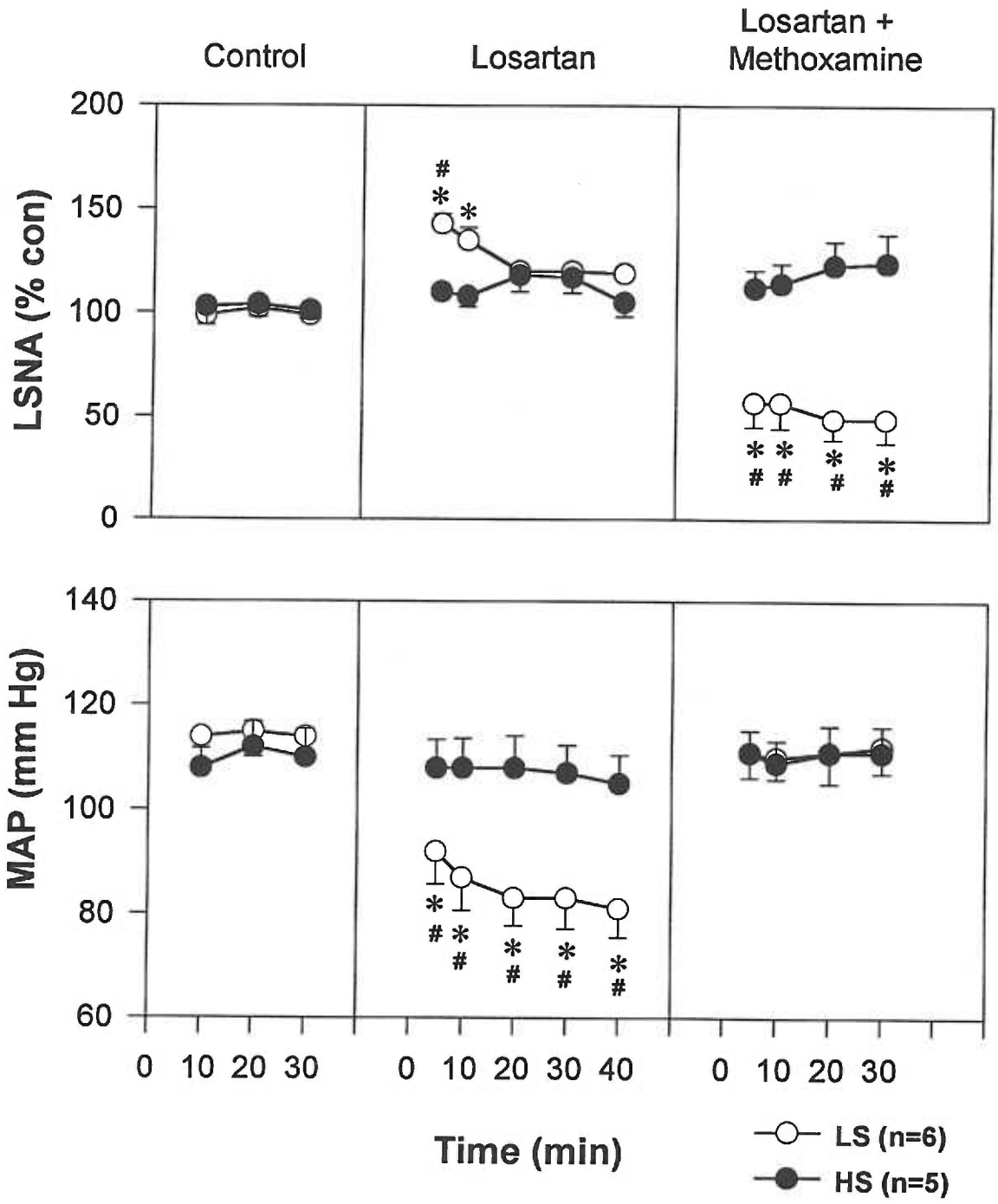

Figure 2.4 Mean arterial blood pressure (MAP, bottom panel) and lumbar sympathetic nerve activity (LSNA, top panel) during the periods before losartan injection (Control), after iv injection of losartan (Losartan), and after MAP was restored to the pre-losartan basal values with iv infusion of methoxamine (Losartan+Methoxamine) in low sodium diet rats (LS) or with iv infusion of vehicle in high sodium diet rats (HS). * indicates significance compared with the pre-losartan basal values in the same diet group; # indicates significant difference between the two diet groups.

reaching 119±4% of control at 40 min after losartan despite the low MAP at the same time (81±6 mm Hg). Thirty min after MAP was returned to the basal value by methoxamine infusion, LSNA was decreased to 49±11% of control. In contrast, losartan did not change MAP or LSNA in HS group (n=5). Thus, Ang II blockade decreased LSNA more ($p<0.01$) in LS compared to HS rats, when MAP was maintained near control levels.

The HR data from the RSNA and LSNA studies were combined and are summarized in Fig. 2.5. There was no significant difference in the basal values between LS and HS rats. In the LS group (n=12), losartan increased HR from 476±11 to 519±12 beats/min after 5 min and to 515±13 beats/min after 40 min. Thirty min after MAP was restored with methoxamine infusion, HR was suppressed by 76±16 beats/min below the pre-losartan values and by 67±28 beats/min below the HS values (456±10 beats/min) at the same time. In the HS group (n=9), losartan did not change HR.

Losartan and phenylephrine

As shown in Fig. 2.6, basal MAP in the LS group (n=4) was lower than that in the CS group (n=5). Forty min after losartan injection in LS rats, MAP decreased by 31±2 mm Hg, and RSNA increased to 180±17%. MAP also decreased by 6±2 mm Hg in the CS group, and RSNA increased to 129±13% of control. Twenty min after MAP was restored to and maintained at basal values with phenylephrine infusion, RSNA was decreased to 45±15% in the LS group and to 70±8% in the CS

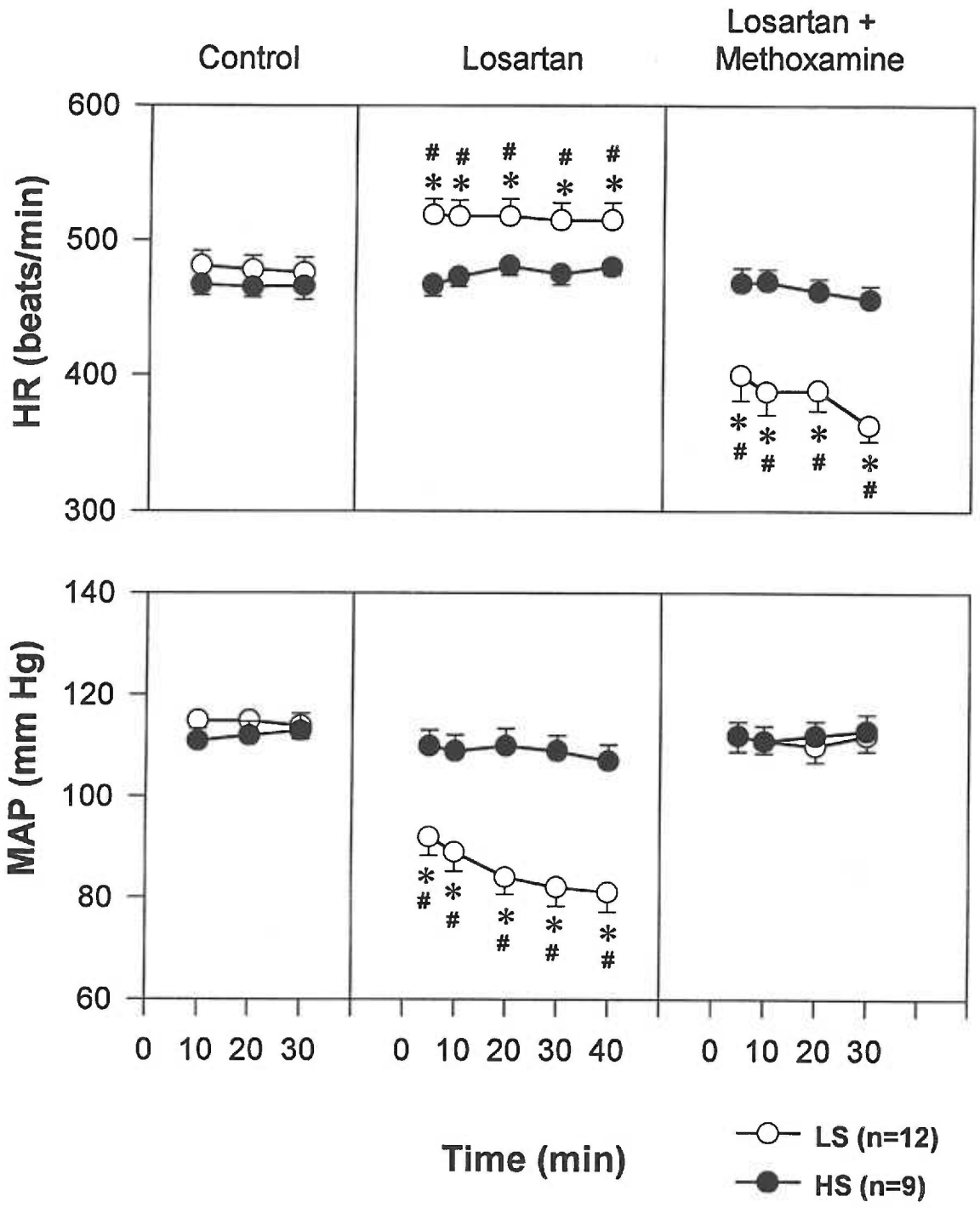

Figure 2.5 Mean arterial blood pressure (MAP, bottom panel) and heart rate (HR, top panel) during the periods before losartan injection (Control), after iv injection of losartan (Losartan), and after MAP was restored to the pre-losartan basal values with iv infusion of methoxamine (Losartan+Methoxamine) in low sodium diet rats (LS) or with iv infusion of vehicle in high sodium diet rats (HS). ✻ indicates significance compared with the pre-losartan basal values in the same diet group; # indicates significant difference between the two diet groups.

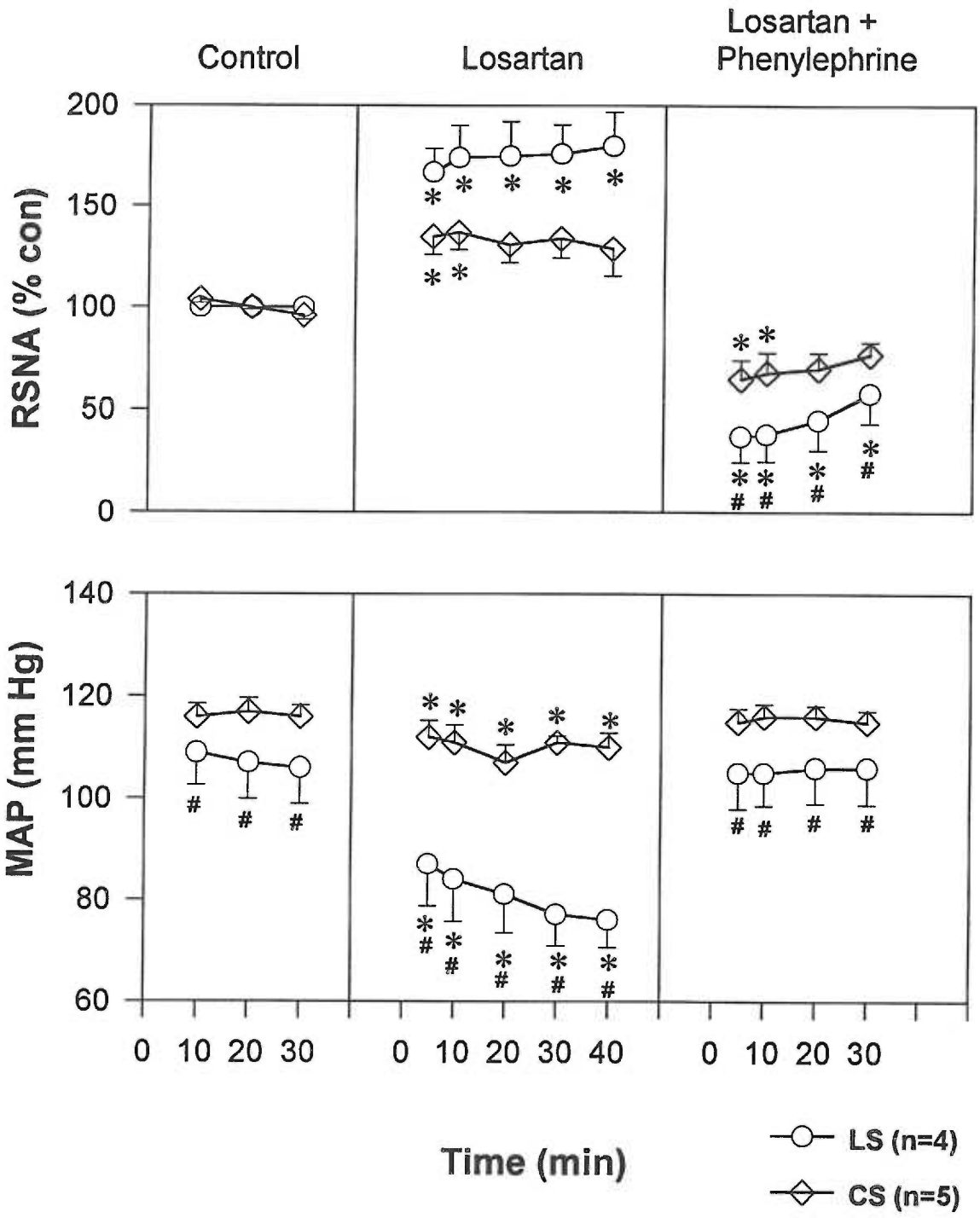

Figure 2.6 Mean arterial blood pressure (MAP, bottom panel) and renal sympathetic nerve activity (RSNA, top panel) during the periods before losartan injection (Control), after iv injection of losartan (Losartan), and after MAP was restored to the pre-losartan basal values with iv infusion of phenylephrine (Losartan + Phenylephrine) in low sodium (LS) and control sodium (CS) diet rats. * indicates significance compared with the pre-losartan basal values in the same diet group; # indicates significant difference between the two diet groups.

group. These decreases were significantly different. In both groups, HR tended to increase after losartan (LS: 465±14 to 481±24 beats/min; CS: 446±13 to 472±10 beats/min) and to be suppressed when MAP was returned to control with phenylephrine (LS: 438±19 beats/min; CS: 418±10 beats/min), but these changes did not reach statistical significance.

Central Venous Pressure

CVP averaged 0.1±0.7 cm H_2O in LS rats (n=7) and 1.3 ±1.7 cm H_2O in HS rats (n=7) at the end of the control period, and did not change in either group following losartan injection, either before (-0.1±0.6 cm H_2O, LS; 2.3±2.2 cm H_2O, HS) or after MAP restoration (0±0.5 cm H_2O, LS; 2.1±1.8 cm H_2O, HS).

DISCUSSION

The important new finding in the present study is that acute blockade of Ang II receptors with losartan suppressed RSNA, LSNA and HR to below basal values when MAP was maintained at pre-losartan levels. This suppression was observed in both LS and CS but not in HS rats. The results suggest that Ang II chronically maintains RSNA, LSNA and HR in sodium deprived and normal rats, and that this action is only revealed when MAP is maintained at basal values.

Supportive data indicates that the suppression of nerve activity after losartan is due to blockade of an action of Ang II rather than to other nonspecific effects. For example, the decrease in nerve activity was not due to deterioration of the

nerve recording signal since time control experiments revealed that the preparation used in this study was stable. The lack of suppression of RSNA, LSNA or HR after losartan in HS rats is also consistent with this conclusion. Another potential explanation for the suppression of nerve activity is that the combination of losartan and methoxamine increased cardiac pressures and activated cardiopulmonary stretch receptors to reflexly inhibit sympathetic outflow. This is also unlikely since central venous pressure did not change after losartan or after MAP restoration. The decrease in sympathetic nerve activity and heart rate is also probably not due to a nonspecific effect of drugs used for restoration of MAP, since the suppression was observed in rats infused with either methoxamine or phenylephrine. Finally, it could be argued that acute pressure-dependent resetting (23,88) caused the baroreflexes to shift to a lower pressure level during the hypotension induced by losartan administration and that the return of MAP to control simply caused a reflex decrease in nerve activity. However, there is abundant evidence that acute resetting is complete within 15 min (23,88). In the present experiment, arterial pressure was returned and held near control for 30 min, which would be sufficient time for the reflex to shift back to the control position, but nerve activity and heart rate continued to be suppressed. Moreover, we have found that losartan decreases LSNA even when arterial pressure is not allowed to fall. Thus, acute resetting cannot account for the suppression of nerve activity observed.

It is noteworthy that both lumbar and renal sympathetic activity, as well as heart rate, were suppressed by losartan in the present study. These results are

consistent with previous studies investigating the role of Ang II in regulation of other baroreflex efferent pathways during changes in sodium intake. In these studies, baroreflex curves relating arterial pressure and heart rate or plasma concentrations of vasopressin, glucocorticoids or ACTH were shifted to lower arterial pressure levels, or to the left, after blockade of the renin-angiotensin system in sodium deprived subjects (15,17,40,41,69). As a result, heart rate or hormone concentrations were decreased at any given level of arterial pressure. In contrast, Ang II blockade did not shift curves in sodium replete individuals (15,17). Collectively, the prior and present data suggest that chronic elevations in endogenous Ang II may exert a global effect on baroreflex efferent pathways.

The focus of the present study was the role of Ang II in regulation of sympathetic outflow during chronic changes in blood volume in normal animals. The results are in agreement with prior studies of other pathophysiological states associated with elevated Ang II levels. In another model of chronic fluid depletion, water deprivation, the Ang II competitive antagonist saralasin shifted baroreflex regulation of heart rate, vasopressin and ACTH/glucorticoid levels to a lower pressure level (10,13). Elimination of Ang II in a number of hypertensive models shifts baroreflex curves of sympathetic nerve activity or heart rate to the left, effectively decreasing sympathetic activity at any given level of arterial pressure (11,71,95,96,151). Two studies suggest that these shifts are not due to the concomitant large decreases in arterial pressure causing pressure-dependent baroreflex resetting. We demonstrated that leftward shift of baroreflex control of

heart rate, norepinephrine or corticosterone concentrations occurred upon termination of a chronic Ang II infusion, even when the hypertension was maintained by infusion of other vasoconstrictors (11, Brooks and Hatton: unpublished results). Heesch et al (71) reported that converting enzyme inhibition shifted reflex regulation of renal sympathetic nerve activity to a lower pressure in renal hypertensive rats, and this shift was sustained when pressure was returned to initial hypertensive values by infusion of phenylephrine. Similar shifts in reflex control of renal sympathetic activity have been reported following blockade of the renin-angiotensin system in newborn sheep (138) and in rats with chronic congestive heart failure (34). Again, a key feature of these latter studies was that pressure was not allowed to fall after Ang II blockade because of infusion of adrenergic agonists. Thus, there is considerable evidence that Ang II may maintain sympathetic outflow and hormonal release in a number of pathophysiological states.

The present study also demonstrated that, at basal MAP, losartan produces a small but significant decrease in renal sympathetic nerve activity in rats on a control salt diet. Thus, Ang II may tonically maintain nerve activity in animals with relatively low Ang II levels. This result is in conflict with a prior report that losartan does not alter baroreflex control of renal sympathetic nerve activity in conscious rabbits (97). The reason for the conflict is not clear, but may be explained by species differences, since other studies indicate a lack of effect of Ang II on the rabbit renal nerve (97,110), or by a different degree of activation of the renin-angiotensin system.

The site of action of losartan was not investigated in the present study, but the brain is a likely candidate for several reasons. First, in the present study, losartan decreased activity of the lumbar nerve, which can contain a population of preganglionic neurons. Second, the fact that multiple baroreflex efferents (including 2 sympathetic nerves) are altered by blockade of the renin-angiotensin system suggests a common site of action, most likely the central nervous system. Third, DiBona et al reported that intracerebroventricular injection of low doses of losartan decreased renal sympathetic nerve activity without affecting arterial pressure in rats with congestive heart failure (34).

Because losartan in plasma appears to be able to cross the blood-brain barrier (103), it may act at a circumventricular organ which lacks this barrier, such as the area postrema, or at a medullary site behind the barrier such as nucleus tractus solitarius or caudal or rostral regions of the ventral lateral medulla. All these regions have AT1 receptors and are known to influence arterial pressure regulation (49). It is interesting that the neural and hemodynamic responses to losartan appear to include two phases in the present study. In the initial immediate phase, lumbar and renal nerve activity increased as arterial pressure rapidly fell. In the next phase, pressure continued to fall more slowly and lumbar nerve activity actually decreased back toward control. It is possible that with time, delayed permeation of losartan beyond areas lacking a patent blood-brain barrier allowed recruitment of larger areas of brain receptive sites.

There is substantial evidence that basal sympathetic activity increases

during chronic effective arterial blood volume depletion due to sodium or water deprivation or congestive heart failure (for review, see reference (16)). Results from the present and prior studies suggest that Ang II may contribute to the increased activity. Thus, we propose that Ang II plays a critical role in long-term control of arterial pressure at least in part by maintaining sympathetic outflow.

In summary, injection of the Ang II type 1 receptor antagonist, losartan, decreased arterial pressure and increased RSNA, LSNA and HR in conscious control sodium intake, sodium deprived, but not sodium loaded, rats. When arterial pressure was returned to control values by infusion of either methoxamine or phenylephrine, nerve activity and heart rate were suppressed to below basal levels. These data indicate that Ang II tonically maintains sympathetic outflow. We suggest that this sympathoexcitatory action of Ang II is necessary for long-term regulation of arterial pressure.

Chapter 3

Based on *Hypertension* 29 [part 2]: 450-457, 1997

Sodium Intake, Angiotensin II Receptor Blockade and Baroreflex Function in Conscious Rats

Ling Xu and Virginia L. Brooks

ABSTRACT

To investigate if endogenous angiotensin II (Ang II) maintains baroreflex function during normotension, the effect of blockade of either Ang II AT1 receptors with losartan, or AT2 receptors with PD123319, on baroreflex control of lumbar sympathetic nerve activity (LSNA) and heart rate (HR) was examined in conscious rats. Rats were fed either a sodium deficient diet (LS) to increase circulating Ang II, or a high sodium diet (HS), for 2 to 3 weeks. One to two days after surgery to implant catheters and nerve electrodes, baroreflex curves were produced before and 40 min after iv administration of losartan (10 mg/kg) or PD123319 (500 μg/kg+50 μg/kg/min). MAP post-losartan was maintained at basal levels with methoxamine. Forty min after losartan in LS rats, LSNA (46±5 to 22±1 % max) and HR (414±7 to 387±8 beats/min) were decreased ($p<0.05$). Losartan decreased reflex control of LSNA more in LS than HS rats ($p<0.05$), as indicated by decreases in maximal LSNA (98±2 to 78±3 % max) and minimal LSNA (42±5 to 21±5 % max). Losartan also shifted reflex control of LSNA to a lower MAP level, or more to the left, in LS than in HS rats (-21±3 vs -9±2 mm Hg at basal LSNA; $p<0.05$), although

maximal gain was unaltered in either group. Similarly, losartan reduced maximal HR (534±6 to 495±9 beats/min) and shifted the HR curve to the left (114±5 to 105±4 mm Hg) in LS but not HS rats. In general, no changes were observed in MAP or baroreflex control of LSNA and HR after PD123319 in LS rats. These results suggest that endogenous Ang II maintains baroreflex control of LSNA and HR in conscious, normotensive LS rats through AT1 but not AT2 receptors.

INTRODUCTION

Arterial baroreceptor reflex function is impaired in established human hypertension and in experimental hypertension, as exemplified by a resetting of the baroreflex to a higher pressure level and a decrease in baroreflex gain (88,92). In some types of hypertension, elevated plasma angiotensin II (Ang II) co-exists with elevated indices of sympathetic activity, such as plasma norepinephrine levels (119). Blockade of the renin-angiotensin system lowers arterial pressure and shifts the baroreflex control of heart rate (HR) and sympathetic activity towards the normotensive range (12,71,95). Moreover, Ang II blockade maintains baroreflex function in hypertensive subjects by increasing reflex gain (8,82,95,137). These results are consistent with a role for Ang II in the alteration of the baroreflex during hypertension, but whether Ang II contributes to baroreflex function in the normotensive state is not clear.

It is well established that chronic and acute blockade of Ang II lowers arterial pressure in normotensive animals with elevated Ang II levels due to low sodium

intake (67,79). Moreover, renal (RSNA) and lumbar (LSNA) sympathetic activity are reduced in sodium deprived rats following acute blockade of Ang II type 1 (AT1) receptors, when the hypotensive effect of Ang II blockade is reversed by infusion of α-adrenergic agonists (162). Blockade of the renin-angiotensin system shifts reflex control of HR and plasma vasopressin or ACTH levels to a lower pressure level (15,17,69). These findings indirectly suggest that the profound hypotension following Ang II blockade is due in part to an attenuation of reflex increases in sympathetic activity. However, whether Ang II blockade alters reflex control of sympathetic activity during sodium depletion has not been investigated. Therefore, the present experiments tested the hypothesis that acute blockade of Ang II receptors shifts baroreflex control of LSNA to a lower arterial pressure level, in low sodium intake rats.

It has become increasingly apparent that Ang II can bind to at least 2 types of binding sites (145). The AT1 receptor mediates most of the cardiovascular actions of Ang II (145). Blockade of AT1 receptors with losartan decreases sympathetic activity relative to arterial pressure (34,95,162), suggesting that losartan would shift baroreflex control of sympathetic activity to a lower pressure level in sodium deprived animals. This hypothesis was tested in the present study by comparing the effects of losartan in conscious rats on either a high sodium or low sodium diet. On the other hand, there is little evidence that circulating Ang II significantly alters cardiovascular function via AT2 receptors (145). Indeed, Ang II receptors in circumventricular organs, presumed major sites of action of chronic

increases in Ang II, do not exhibit AT2 binding (130,145,153). Nevertheless, a recent study suggests that prolonged elevation of plasma Ang II may increase effects of Ang II mediated by the AT2 receptor (114). Because sodium deprivation is a state of chronically elevated plasma Ang II levels, it was also determined if blockade of AT2 receptors alters arterial pressure and baroreflex curves in low sodium intake rats.

METHODS

Eighteen male, Sprague-Dawley rats (Simonsen Lab, Gilroy, CA) were used in this study. At 8 weeks of age (240-292 g), rats were placed on one of two diets (Harlan Teklad, Madison, WI): sodium deficient (LS; Na<0.02%) or high sodium (HS, Na=4%) rat chow. On the first two days on diet, rats in the LS group received a furosemide injection (1 mg/kg/day, IP; Abbott Labs, North Chicago, IL), while rats in the HS group received the 5% dextrose (D5W) vehicle (1 ml/kg, IP). Rats were maintained on diet for 2 to 3 weeks before surgery for catheterization and nerve electrode implantation. All rats were housed in a room maintained on a 12 hr-12 hr light-dark cycle and were allowed to have food and distilled water *ad libitum*.

Surgical Procedures

Rats (272-352 g) were anesthetized initially with Brevital Sodium (100 mg/kg in D5W in two IP injections over 5 min; Eli Lilly, Indianapolis, IN). After a venous catheter was inserted, anesthesia was maintained by Brevital infusion as needed

(2.7-4 µl/min, 10 mg/ml D5W, iv). Two tygon catheters (Norton Performance Plastics, Akron, OH) were inserted into the right jugular vein and two into the left femoral vein for drug delivery. Finally, a catheter was advanced into the abdominal aorta via a femoral artery for the measurement of MAP and HR.

For the lumbar nerve electrode implantation, a midline abdominal incision was made. After retracting the intestines, the abdominal aorta and vena cava were gently pulled aside to expose the lumbar nerve. The nerve was then dissected free and placed on a bipolar electrode made of Teflon coated stainless steel wire (A&M Systems, Everett, WA). When optimal nerve traffic was confirmed on an oscilloscope (Model 2212, Tektronix, Beaverton, OR), the nerve and electrode were embedded in dental gel (President Light Body, Coltene, Hudson, MA).

Catheters and the electrode lead were tunneled subcutaneously to the back of the neck and exteriorized, and all incisions were closed with silk suture. The rats were returned to their home cage and allowed 20-40 hr for recovery. Experiments were performed while rats remained in their home cage.

Hemodynamic and Nerve Activity Recordings

MAP was monitored via the femoral arterial catheter connected to a Statham pressure transducer and a Grass preamplifier (7P1). HR was measured using a Grass tachograph (7P4) triggered by the amplified arterial pressure pulse. The raw lumbar nerve activity was amplified using a Grass differential preamplifier (P511) with a band pass filter of 30 Hz to 10 kHz. The gain (25,000-70,000X) of the

preamplifier was adjusted so that the output of maximal nerve activity amplitude did not exceed the linear input range (±1.5 v peak-peak) of the Grass integrator (7P10), which was used for integrating raw nerve activity. The amplified nerve traffic was observed on the storage oscilloscope and was whole wave rectified and integrated with a reset time of 1 sec. Together with MAP and HR, integrated LSNA was recorded on chart paper using a Grass polygraph (7D). Nerve activity was first quantified by averaging the integrated activity just before reset over 12 sec (12 peaks) during stable and quasi-stable periods (slow or no change in measured parameters), or 3-4 sec (3-4 peaks) during transient periods (e.g. baroreflex curve). In addition, the noise level was quantified at the end of the experiment by averaging the integrated output over 12 sec after efferent nerve activity was eliminated by an iv bolus of hexamethonium chloride (30 mg/kg in D5W; Sigma, St. Louis, MO), a ganglionic blocker, followed by iv infusion of methoxamine to increase MAP above 150 mm Hg. This combined use of ganglionic blockade and increasing MAP eliminated both pre- and post- ganglionic nerve activities. The noise output was then subtracted from average integrated nerve activity to yield a measure of net LSNA. For each animal, LSNA was normalized using two methods. First, LSNA was normalized to basal nerve activity in the control period and was expressed as percent of control (% con). Basal nerve activity was defined as the average of resting activity at 2 time points 10 min before the first baroreflex curve was generated. Second, LSNA was normalized to the maximal nerve activity during the control period and was expressed as percent of maximal (% max). Maximal LSNA

was the peak LSNA in the baroreflex curves induced by nitroprusside (NP) infusion during the control period.

Baroreceptor Reflex Curves

Arterial pressure was varied by a slow infusion of either phenylephrine (PE) or NP (both from Elkins-Sinn, Cherry Hill, NJ). Increasing doses of PE were infused (0.68-27 µl/min, 1 mg/ml D5W, iv) to increase MAP up to 175-180 mm Hg, while increasing doses of NP were infused (1.35-68 µl/min, 1 mg/ml D5W, iv) to decrease MAP to about 50 mm Hg (Fig. 3.1). The ramp increase or decrease of MAP was completed in about 2 min. Infusions of PE or NP were performed randomly. MAP, LSNA and HR were allowed to returned to baseline (about 30 min) before a subsequent ramp of MAP was made.

Protocols

Protocol 1. This study tested the hypothesis that acute systemic blockade of the AT1 receptor with losartan shifts baroreflex control of LSNA and HR to a lower arterial pressure level and that the shift is greater in LS rats than in HS rats. After basal parameters were obtained and baroreflex control of HR and LSNA was studied during the control period, losartan was injected (10 mg/kg, in 100 µl D5W followed by 100 µl flush, iv) in both LS (n=7) and HS (n=5) rats. Immediately after losartan, iv infusion of methoxamine (5-33 µg/min, in D5W) was begun to prevent MAP from dropping. MAP, HR and LSNA were monitored for at least 40 min

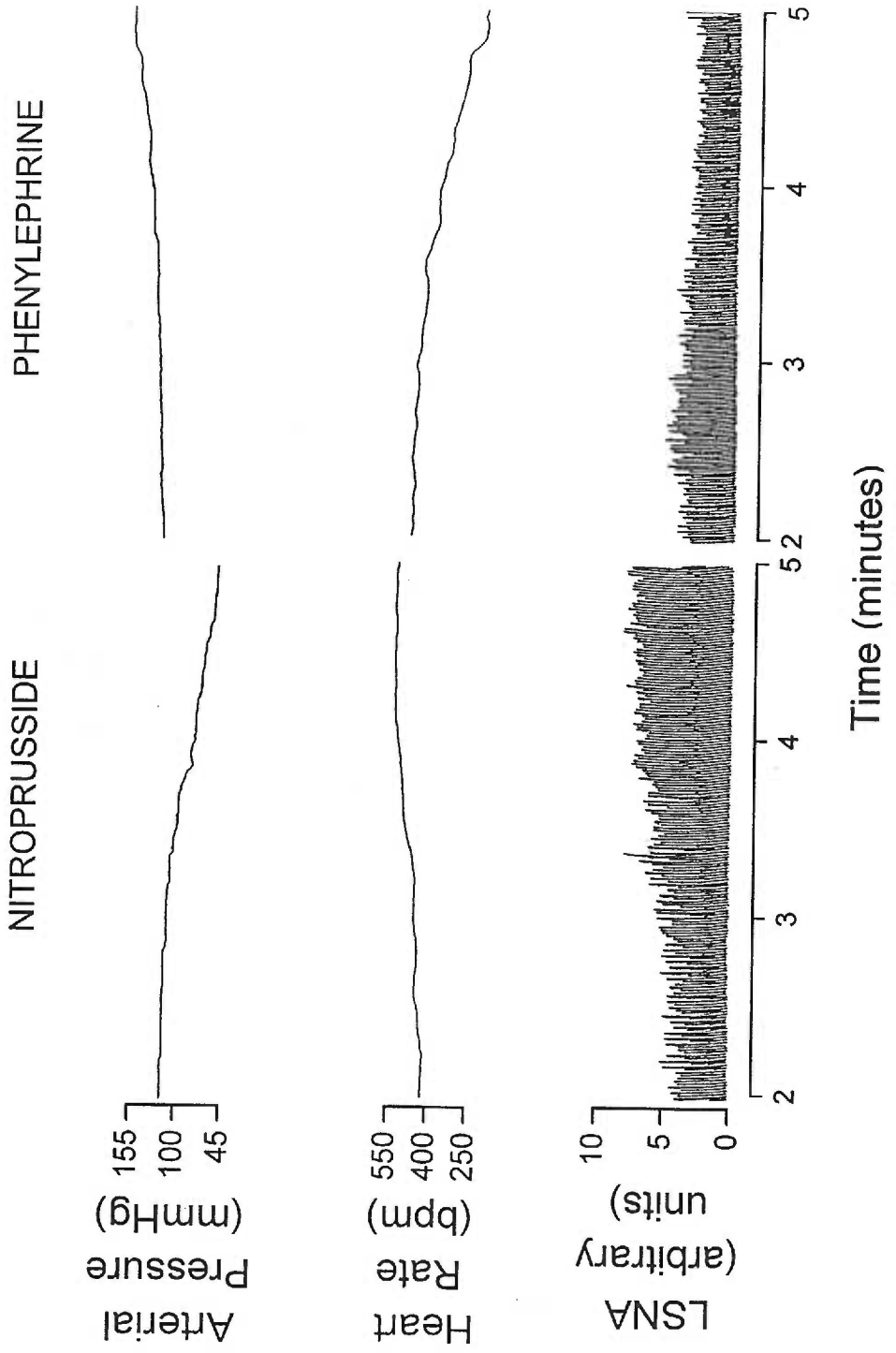

Figure 3.1 Effects of iv infusion of increasing doses of nitroprusside (NP, left) or phenylephrine (PE, right) on mean arterial pressure, heart rate and lumbar sympathetic nerve activity in a representative experiment. Time is minutes after beginning the NP or PE infusion.

following losartan administration, because previous studies (162) indicate that it takes at least 40 min for the depressor effect of losartan to stabilize. After this, the post-losartan baroreflex control of HR and LSNA was determined. The pressor response to an Ang II bolus (100 ng/kg, iv) was tested after losartan at the end of each experiment and was always completely prevented, indicating complete blockade of AT1 receptors. In one HS rat, the HR tracing was not adequate because of the small arterial pressure pulse which failed to trigger the tachograph. Thus, the HR results are presented for only 4 out of 5 HS rats.

Protocol 2. This experiment determined if acute systemic blockade of the AT2 receptor alters MAP or baroreflex control of LSNA and HR. Experiments were only conducted in LS rats with chronically elevated plasma Ang II levels (n=6). Procedures were the same as in Protocol 1, except that the AT2 receptor antagonist, PD123319, was given instead of losartan. A dose (500 μg/kg prime + 50 μg/kg/min, 10 μl/min, iv) was chosen that is within a range which has proved effective in previous studies (84,106,114). No manipulation was applied to maintain MAP, since PD123319 did not affect MAP. The pressor responses to Ang II before (29±5 mm Hg) and after (31±6 mm Hg) PD 123319 administration were not different. LSNA was lost before the experiment in 2 of the 6 LS rats. The HR tracing of 3 rats was not acceptable during baroreflex curve generation at high MAP.

Data Analysis and Statistics

A logistic relation, slightly modified from Kent (85), was used to analyze baroreflex curves: $Y=d+(a-d)/(1+\exp[b(X-c)])$, where X, MAP; Y, LSNA or HR; a, maximal LSNA or (HR); b, slope coefficient; c, MAP at the midpoint of the range of LSNA or HR; and d, minimal LSNA (or HR). In each animal, raw data of MAP and LSNA (or HR) were fit to the logistic function to generate parameters a, b, c and d, using graphics software (Sigmaplot, Jandel Scientific, Corte Madera, CA). Constraints of maximum and minimum of LSNA (or HR) were set for the fitting process and were determined in each experiment when the high (or low) plateau of LSNA and HR was reached while MAP was still decreasing by NP infusion (or increasing by PE infusion). The range of the baroreflex curve, e, was defined as (a-d), and the maximal gain of the baroreflex curve as -be/4 (85). Means ± SEM of individual curve fit parameters were calculated, and statistical analysis was performed to determine within and between group differences in parameters (Table 3.1 and 3.3). The averaged a, b, c and d were then used to generate averaged baroreflex curves (Fig. 3.4 and 3.6).

In addition, the MAP, LSNA (% max) and HR data of all rats in each diet group were pooled by calculating the mean and SEM of all data points collected within 5 mm Hg increments of MAP. Multiple points within the same MAP range in each animal were averaged before pooling. The means of pooled data were plotted with SEM of LSNA (or HR) and then fit to the logistic function (Fig. 3.3, 3.5 and 3.8)

All data are presented as means±SEM. Data were analyzed using two or

one way ANOVA repeated one way (time or drug administration) and the post hoc Newman-Keuls test (161). When two groups of data were compared, paired or unpaired Student *t*-tests were employed (161). All analyses were performed using GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD). A significance level of $p<0.05$ was accepted.

RESULTS

AT1 receptor blockade

Time course of changes in LSNA and HR. As shown in Fig. 3.2, basal HR and LSNA were not different between LS and HS groups, although basal MAP was lower in LS rats than in HS rats ($p<0.05$). MAP during the first 10 min after losartan decreased slightly, due to the time needed to adjust the infusion speed of methoxamine, but was maintained at levels not different from basal levels thereafter in each group. Losartan reduced both LSNA and HR in LS rats ($p<0.05$), but this effect took time to be developed. LSNA decreased beginning 20 min after losartan, reaching 22±1 % max at 40 min. The reduction in HR was significant only at 40 min after losartan. In contrast, no significant changes in LSNA or HR were observed in HS rats after losartan.

Baroreflex control of LSNA. Losartan reduced LSNA at any given MAP more in LS than HS rats (Fig. 3.3 and 3.4; Table 3.1), as indicated by significant differences in several logistic parameters (Table 3.1). First, losartan decreased the maximal LSNA in LS rats, but not in HS rats. This difference was observed whether

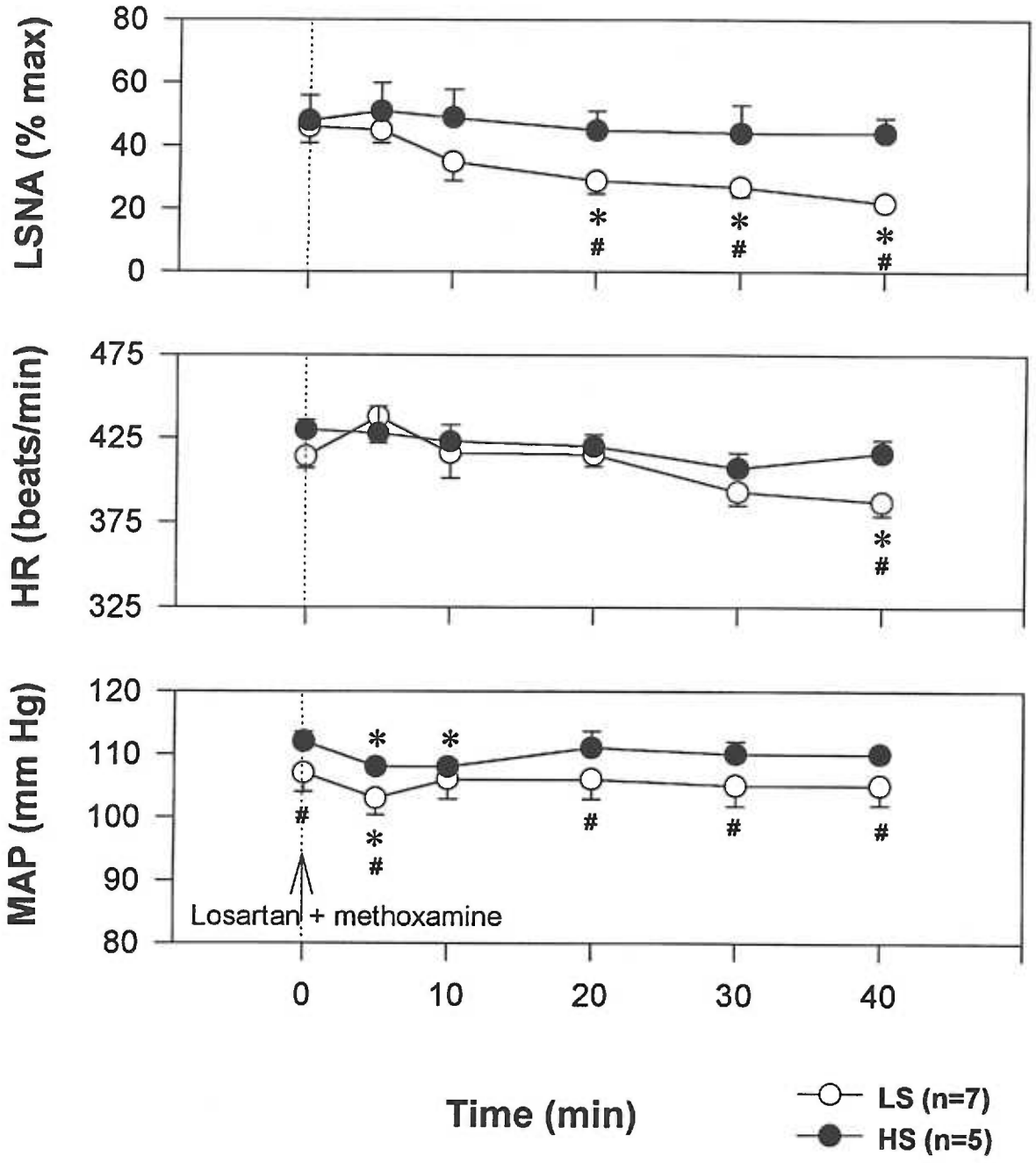

Figure 3.2 Time course of changes in lumbar sympathetic nerve activity (LSNA, top panel) and heart rate (HR, middle panel) after iv injection of losartan, while mean arterial pressure (MAP, bottom panel) was maintained at the pre-losartan basal levels with iv infusion of methoxamine in low sodium (LS) and high sodium (HS) diet rats. * indicates significant difference compared with basal values in either diet group ($p<0.05$); # indicates significant difference at the same time points between LS and HS rats ($p<0.05$).

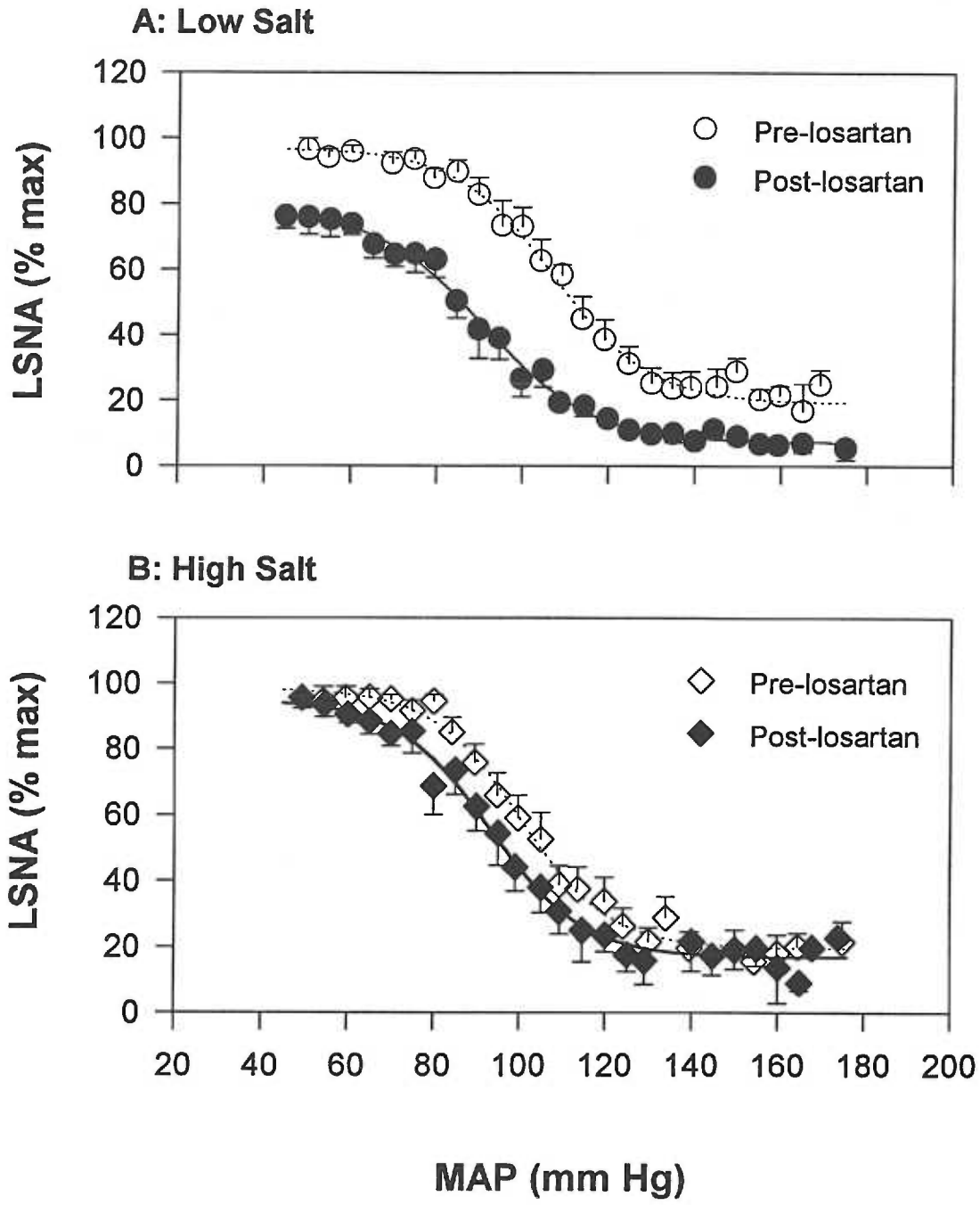

Figure 3.3 Effect of losartan on baroreflex control of lumbar sympathetic nerve activity (LSNA; % baroreflex maximum) in low sodium (n=7, Panel A) and high sodium (n=5, Panel B) diet rats. Data were pooled as described in the text, and plotted as means of mean arterial pressure (MAP) and LSNA, with SEM of LSNA.

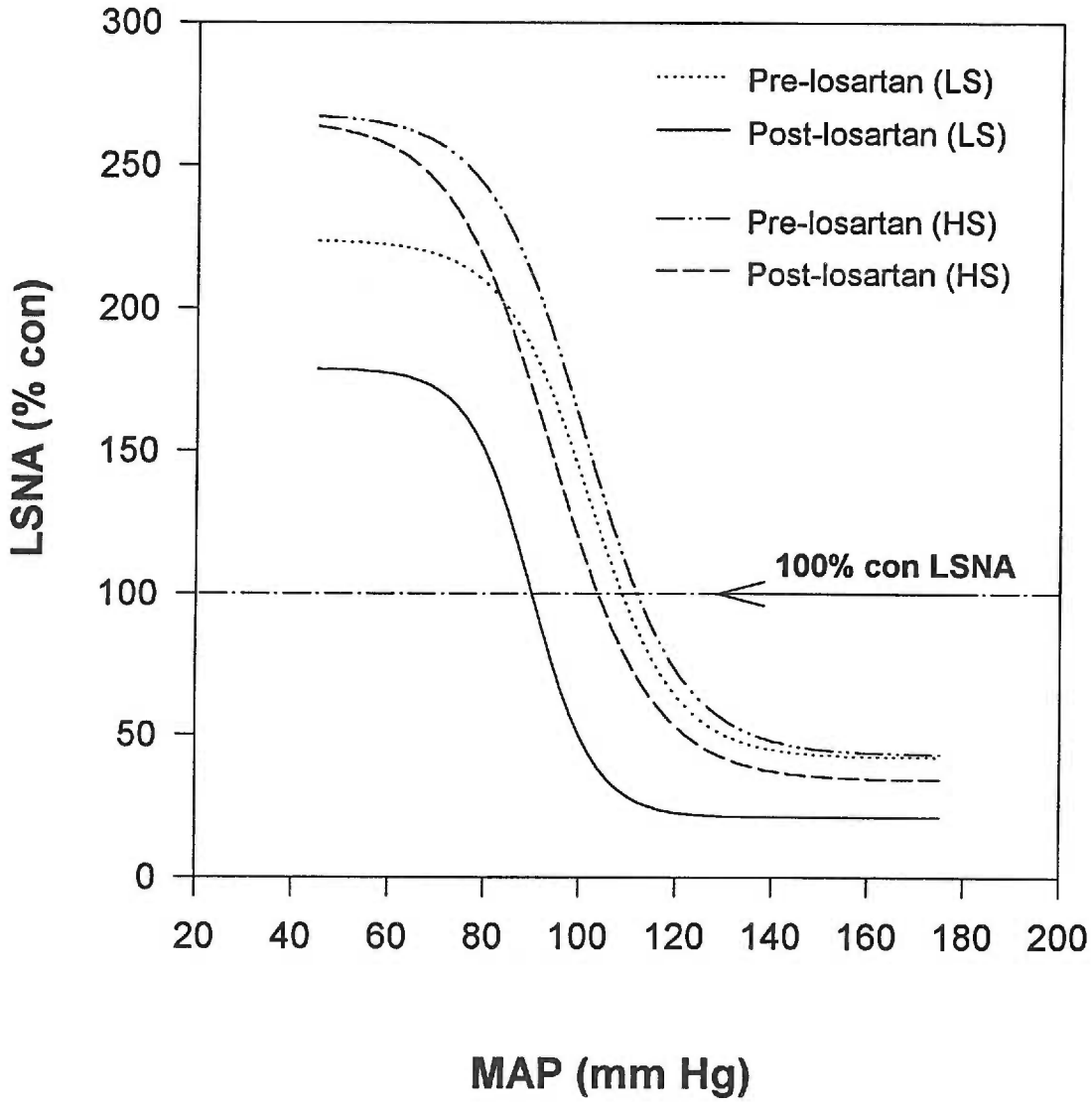

Figure 3.4 Effects of sodium intake and losartan on baroreflex control of lumbar sympathetic nerve activity (LSNA; % control) in low sodium (LS, n=7) and high sodium (HS, n=5) diet rats. Sigmoidal curves were generated from averaged parameters as described in the text. MAP, mean arterial pressure; 100% con LSNA, basal LSNA before losartan injection.

Table 3.1 Effects of Losartan on Baroreflex Control of Lumbar Sympathetic Nerve Activity (LSNA) in Low Sodium and High Sodium Diet Rats

	Low Sodium (n=7)		High Sodium (n=5)	
	Pre-losartan	Post-losartan	Pre-losartan	Post-losartan
LSNA (% con)				
Maximum	224±21 †	179±20 *‡	268±36	265±39
Slope Coefficient	0.110±0.008	0.151±0.045	0.098±0.010	0.093±0.010
MAP50	102±3	90±3 *	101±3	94±3 *
Minimum	42±5	21±5 *	43±7	34±7
Range	182±20 †	156±17 ‡	225±34	232±45
Maximal Gain	-5.05±0.78	-5.55±1.29	-5.62±1.20	-5.55±1.23
LSNA (% max)				
Maximum	98±2	78±3 *‡	100±0	98±3
Slope Coefficient	0.110±0.009	0.150±0.045	0.098±0.010	0.093±0.010
MAP50	102±3	90±3 *	101±3	94±3 *
Minimum	19±3	9±2	17±3	15±4
Range	79±3	70±4 ‡	83±4	84±7
Maximal Gain	-2.18±0.22	-2.50±0.67	-2.04±026	-1.99±0.31

Values are means ± SEM of baroreflex logistic curve parameters. Data were analyzed with ANOVA and Newman-Keuls test.

*$p<0.05$ post- vs pre-losartan in the same diet group

†$p<0.05$ low vs high sodium during pre-losartan period

‡$p<0.05$ low vs high sodium during post-losartan period.

LSNA was normalized to the maximal (Fig. 3.3, Table 3.1) or basal LSNA (Fig. 3.4, Table 3.1). Second, losartan reduced minimal LSNA in LS rats but not in HS rats (Fig. 3.4, Table 3.1). Finally, losartan decreased ($p<0.05$) the extrapolated LSNA at basal MAP (LS: 109±2 mm Hg; HS: 111±1 mm Hg) in LS (46±5 to 18±3 % max) but not HS rats (40±3 to 30±5 % max).

Baroreflex control of LSNA was shifted to the left by losartan in both groups, but the shift was greater in LS rats (Fig. 3.3 and 3.4, Table 3.1). MAP at the midrange of the curve (MAP50) was reduced in both groups (Table 3.1), and the shift in MAP50 was not significantly different ($p=0.06$) between LS (-12±1.9 mm Hg) and HS (-7±1.3 mm Hg). However, because the logistic parameter MAP50 may be altered by the decreased maximal of LSNA in LS rats after losartan (Table 3.1), comparing shift in MAP50 between LS and HS groups may not reflect a true difference in the shift of baroreflex curve caused by losartan. Therefore, values of MAP at 100 % con LSNA (MAP100), extrapolated from the fitted baroreflex curves (Fig. 3.4) were compared. In the control period, MAP100 was not different between groups (LS: 109±3 mm Hg vs HS: 111±2 mm Hg, Fig. 3.4). After losartan, MAP100 was reduced in both groups, but the reduction was greater in LS rats (-21±3 mm Hg) than in HS rats (-9±2 mm Hg) (all $p<0.05$), indicating baroreflex control of LSNA was shifted to the left more in LS rats than in HS rats.

The slope coefficient or maximal gain of the baroreflex curves were not changed significantly by diet or losartan as shown in Table 3.1.

The data were also compared, when they were normalized as % control, to

determine if there were between group differences in maximal LSNA during the control period (Fig. 3.4). Maximal LSNA before losartan was lower in LS rats compared to HS rats (Fig. 3.4, Table 3.1). Again, losartan decreased maximal LSNA in LS rats but not HS rats (Table 3.1).

Baroreflex control of heart rate. Losartan altered baroreflex control of HR in LS rats but not in HS rats (Fig. 3.5 and 3.6). After losartan administration in LS rats (n=7), maximal HR, range, MAP50 as well as maximal gain were all reduced, although the slope coefficient and minimal HR of the baroreflex curve did not change (Table 3.2). In contrast, losartan did not significantly alter any of the parameters in HS rats (Table 3.2).

Maximal HR during the control period was higher in LS rats compared to that in HS rats (Table 3.2, Fig. 3.6). After losartan, maximal HR in LS rats was reduced to a level not significantly different from maximal HR in HS rats before or after losartan (Table 3.2, Fig. 3.6).

AT2 receptor blockade

Time course of changes in LSNA and HR. No changes in MAP (n=6), HR (n=6) or LSNA (n=4) were observed after PD123319 in LS rats (Fig. 3.7).

Baroreflex control of LSNA and HR. In LS rats, PD123319 had little effect on baroreflex control of LSNA (Fig. 3.8). No logistic parameters were altered, except the range of the baroreflex curve (Table 3.3). Similarly, PD123319 did not affect baroreflex control of HR (Table 3.3).

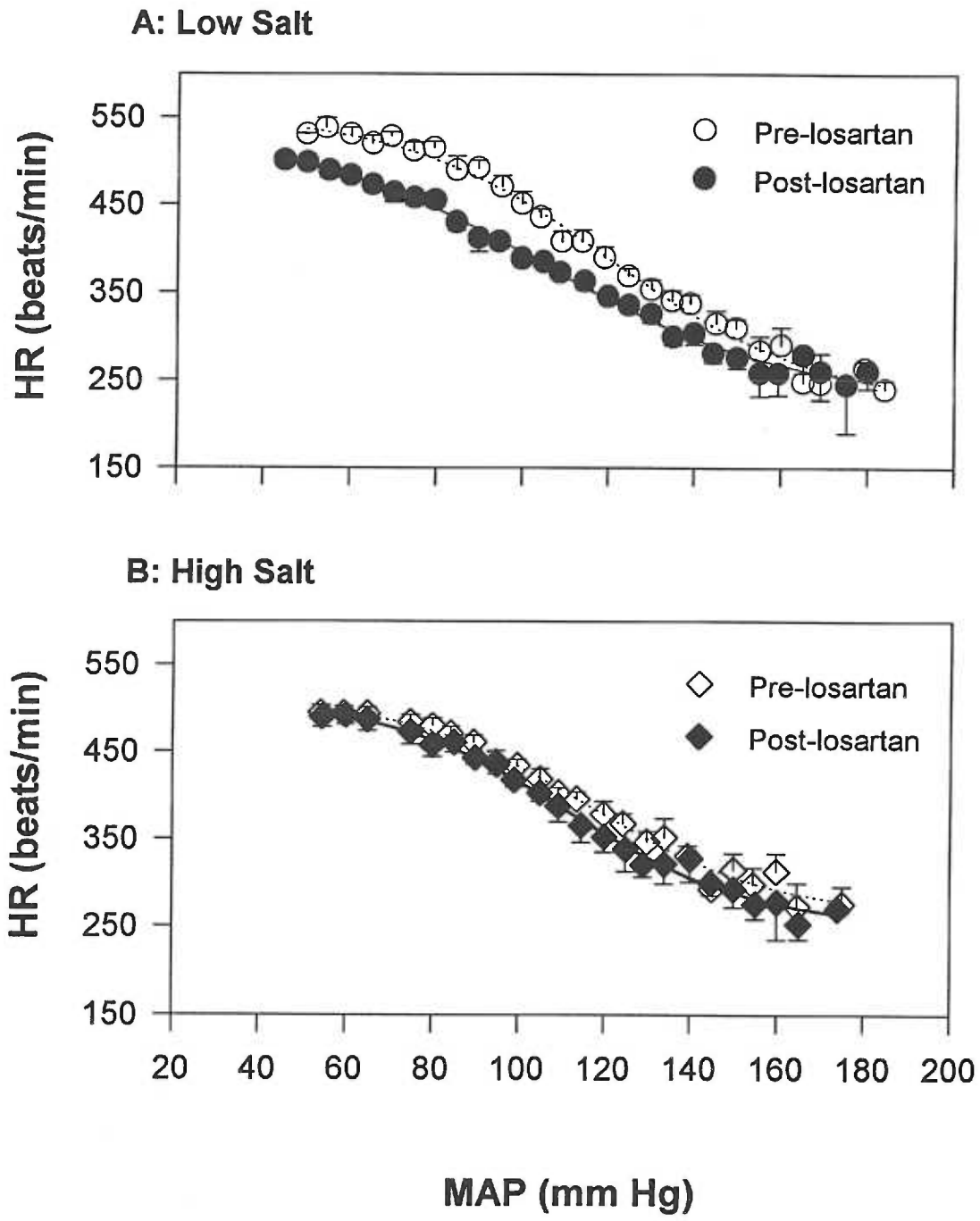

Figure 3.5 Effect of losartan on baroreflex control of heart rate (HR) in low sodium (n=7, Panel A) and high sodium (n=4, Panel B) diet rats. Data were pooled as described in the text, and plotted as means of mean arterial pressure (MAP) and HR, with SEM of HR.

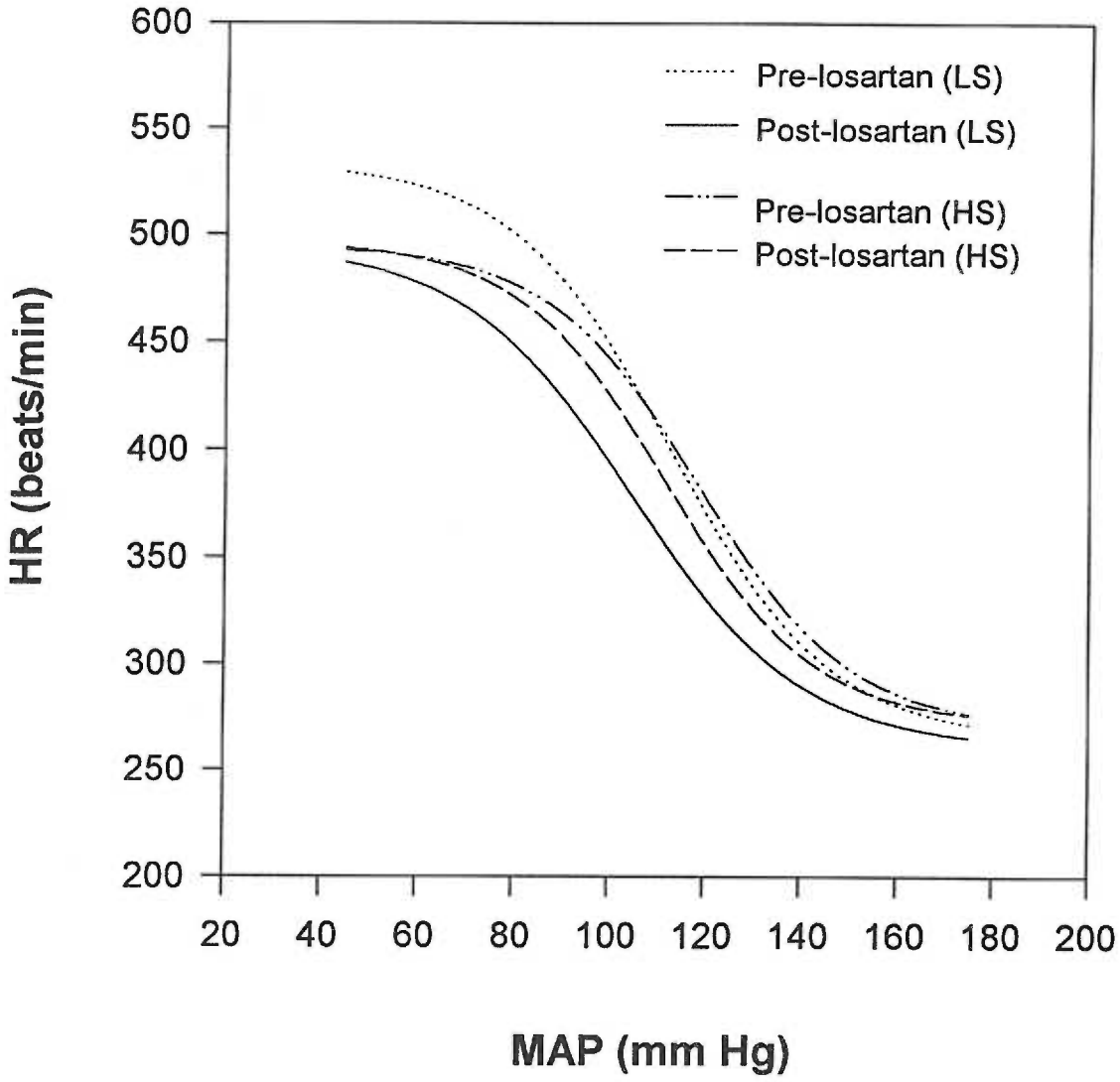

Figure 3.6 Effects of sodium intake and losartan on baroreflex control of heart rate (HR) in low sodium (LS, n=7) and high sodium (HS, n=4) diet rats. Sigmoidal curves were generated from averaged parameters as described in the text. MAP, mean arterial pressure.

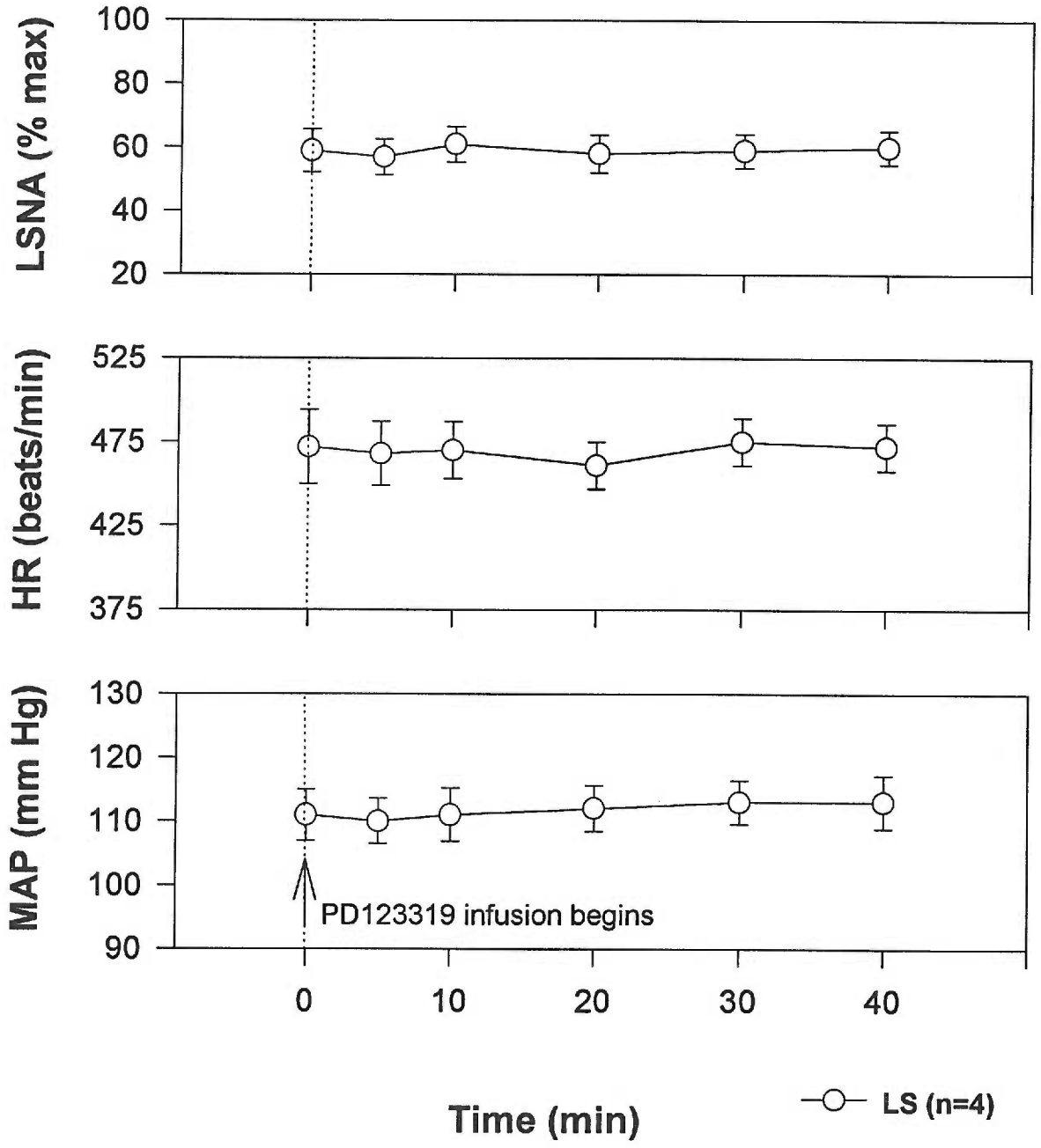

Figure 3.7 Lack of changes in mean arterial pressure (MAP), heart rate (HR) and lumbar sympathetic nerve activity (LSNA, % baroreflex maximum LSNA) during 40 min of iv infusion of PD123319 in low sodium diet rats (LS, n=4).

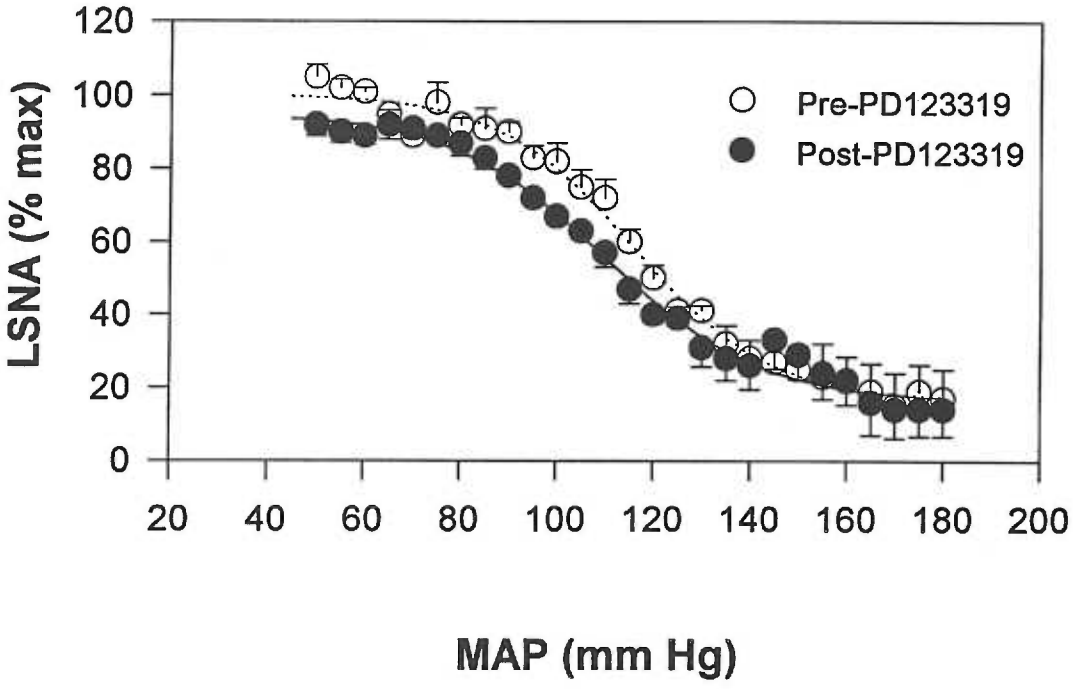

Figure 3.8 Effect of iv infusion of PD123319 on baroreflex control of lumbar sympathetic nerve activity (LSNA) in low sodium (LS, n=4) diet rats. Data were pooled as described in the text, and plotted as means of mean arterial pressure (MAP) and LSNA, with SEM of

Table 3.2 Effects of Losartan on Baroreflex Control of Heart Rate in Low Sodium and High Sodium Diet Rats.

	Low Sodium (n=7)		High Sodium (n=4)	
	Pre-losartan	Post-losartan	Pre-losartan	Post-losartan
Maximum	534±6 †	495±9 *	495±9	497±11
Slope Coefficient	0.059±0.003	0.056±0.003	0.062±0.007	0.065±0.012
MAP50	114±5	105±4 *	119±2	112±4
Minimum	265±19	261±18	271±19	272±12
Range	270±22 †	234±21 *	225±23	225±19
Maximum Gain	-3.94±0.29	-3.20±0.23 *	-3.49±0.51	-3.65±0.75

Values are means ± SEM of baroreflex logistic curve parameters. Data were analyzed with ANOVA and Newman-Keuls test.

*$p<0.05$ pre- vs post-losartan in the same diet group.

† $p<0.05$ low vs high sodium diet during pre-losartan period.

Table 3.3 Effects of PD123319 (PD) on Baroreflex Control of Lumbar Sympathetic Nerve Activity (LSNA) and Heart Rate (HR) in Low Sodium Diet Rats

	LSNA (% con)		LSNA (% max)		HR	
	Pre-PD	Post-PD	Pre-PD	Post-PD	Pre-PD	Post-PD
Maximum	177±16	162±15	100±1	93±3	554±42	549±35
Slope Coefficient	0.078±0.005	0.075±0.008	0.078±0.005	0.072±0.009	0.131±0.062	0.107±0.036
MAP50	116±4	106±2	116±4	105±3	123±9	125±9
Minimum	23±9	30±9	15±6	18±7	304±13	282±22
Range	154±24	133±2 *	85±6	75±7 *	250±54	268±42
Maximal Gain	-2.97±0.46	-2.41±0.30	-1.65±0.13	-1.3±0.10	-6.56±1.43	-6.70±1.53

Values are means ± SEM of baroreflex logistic curve parameters. Data were analyzed with ANOVA and Newman-Keuls test.

* $p<0.05$ pre- vs post-PD 123319.

DISCUSSION

Salt intake can change on almost a daily basis in animals. Subsequent salt imbalance could lead to potentially serious consequences, including threats to the constancy of the extracellular fluid volume and therefore arterial pressure. However, powerful homeostatic mechanisms exist which act to maintain day-to-day levels of arterial pressure in the face of a constantly changing sodium balance. One such mechanism involves the sympathetic nervous system. It has been proposed (16) that chronic decreases in sodium balance and/or extracellular fluid volume increase circulating levels of Ang II, which in turn maintain elevated levels of sympathetic activity. The increases in sympathetic activity, in concert with other homeostatic mechanisms, act to maintain arterial pressure despite decreases in volume.

Consistent with this idea, a previous study showed that losartan decreases renal and lumbar sympathetic activity in sodium deprived rats, but only if the hypotensive effect of Ang II blockade is reversed (162). Importantly, losartan had no effect in rats on a high salt diet, and produced only a small decrease in nerve activity in rats on a normal salt diet, suggesting a role for a sympathetic nervous system-angiotensin interaction in sodium balance homeostasis (162). The present study sought to extend these results by determining if the suppression of nerve activity could be observed over the entire baroreflex range of arterial pressure. The important new findings are 1) losartan suppresses LSNA and HR in LS rats when MAP is not allowed to decrease significantly, and the suppression takes a slow time

course; 2) losartan shifts reflex control of LSNA to the left more in LS rats than in HS rats without affecting maximal gain, effectively decreasing LSNA at any given levels of MAP; 3) losartan shifts baroreflex control of HR to a lower MAP level and decreases maximal HR in LS rats only; and 4) PD123319 is generally without effect on resting parameters or baroreflex control of LSNA and HR in LS rats. These findings support the conclusion that endogenous Ang II increases LSNA and HR and maintains baroreflex function in LS rats through AT1 but not AT2 receptors.

It is likely that the suppression of LSNA and HR after losartan in LS rats is due to blockade of AT1 receptors rather than other nonspecific effects. One potential problem is that Ang II blockade often decreases arterial pressure which could produce acute pressure-dependent baroreflex resetting (4). However, a key feature of the present study is that MAP was maintained at basal levels after losartan with methoxamine infusion. Second, a direct effect of methoxamine to decrease LSNA and HR, independent of effects on pressure, is unlikely, since in our previous study (162), nerve activity and HR were suppressed after losartan regardless of whether MAP was maintained at basal levels with either methoxamine or PE. Moreover, adrenergic agonists decrease sympathetic activity by increasing pressure to activate baroreceptor afferents (38,88,154). In the present study, methoxamine was used to maintain pressure, not increase it. Third, the sympathoinhibition was not due to activation of cardiopulmonary baroreceptors, since neither losartan nor losartan plus methoxamine significantly affects central venous pressure (162)

In LS rats, losartan decreased sympathetic activity over the entire pressure range of the baroreflex, suggesting that Ang II is critical for maintenance of sympathetic activity and its baroreflex regulation during decreases in salt intake. Blockade of the renin-angiotensin system also shifts baroreflex control of sympathetic activity in other pathophysiological states such as hypertension, congestive heart failure and birth (34,71,138). Thus, it is becoming increasingly apparent that Ang II is a major participant in long-term control of the sympathetic nervous system in both hypertensive and normotensive states (16).

While the present study clearly demonstrates that losartan decreases sympathetic activity in LS rats, it is not clear if it is blocking an effect of Ang II to maintain normal nerve activity or to increase nerve activity above normal. The uncertainty lies in the difficulty in quantifying long-term changes in sympathetic activity. Nevertheless, present and previous results suggest that Ang II may increase sympathetic activity above normal during sodium deprivation. In the present study, baroreflex-induced maximal LSNA before losartan was lower in LS than in HS rats, when nerve activity was expressed as % of control, in agreement with a previous report (74). This result could be explained by an effect of sodium deprivation to decrease maximal reflex activity or to increase basal activity. The latter possibility is supported by several lines of evidence. First, in the present study, baroreflex-induced maximal HR, for which absolute values can be obtained, were higher in LS rats. Second, a number of studies indirectly assessing the degree of activation of the sympathetic nervous system, through measurements of

circulating catecholamines, norepinephrine turnover, absolute nerve activity or levels of tyrosine hydroxylase, the rate limiting enzyme involved in catecholamine production, conclude that sympathetic activity is increased during sodium deprivation (16,60,141). Finally, other pathophysiological states associated with decreases in effective arterial blood volume, such as congestive heart failure, also appear to exhibit increased sympathetic activity (16).

Collectively, these data suggest that sodium deprivation, presumably by decreasing extracellular fluid volume, increases renin and Ang II levels. The increased Ang II then chronically maintains elevated sympathetic outflow and the baroreflex regulation, which will tend to help maintain arterial pressure at normal levels by increasing peripheral resistance and promoting fluid retention, despite volume depletion.

Ang II could also participate in the regulation of baroreflex function during decreases in salt intake by decreasing reflex gain. However, neither changes in salt intake or losartan administration altered LSNA baroreflex gain. The lack of effect of salt intake on gain has been observed by others (17,69,74), but the lack of effect of losartan on gain is in conflict with reports that Ang II blockade increases baroreflex sensitivity in animals with hypertension or congestive heart failure (8,34,82,95,96,115,137). While the explanation for this difference is not known, it is not surprising from a physiological point of view that baroreflex sensitivity is regulated differently in normotensive versus diseased rats.

Baroreflex control of HR was also decreased by losartan in LS rats. This

result is in agreement with previous work (17,69), and suggests that Ang II increases activity of a number of baroreceptor reflex efferents. Interestingly, there were subtle differences in the effect of losartan on HR and LSNA. In particular, losartan did not alter minimal HR, as it suppressed minimal LSNA, which led to a decrease in the slope of the HR reflex curve. Thus, Ang II may act through somewhat different mechanisms to influence reflex control of LSNA and HR. This is not surprising, since various sympathetic nerves can be regulated differently, and changes in HR are effected via changes in both the sympathetic and parasympathetic nervous systems.

The site of action of losartan was not investigated, but the brain is the most plausible candidate given the wide range of baroreceptor efferents affected by Ang II blockade in sodium deprived animals. Since losartan can penetrate the blood brain barrier (103,167), it is possible that losartan suppresses sympathetic nerve activity by blockade of AT1 receptors beyond the blood brain barrier in the brain, or by blockade of receptors in circumventricular organs lacking this barrier, such as the area postrema or subfornical organ. Because losartan takes a slow time course to decrease MAP (162), LSNA and HR, it is tempting to speculate that Ang II acts in part at a site behind the blood brain barrier. However, losartan also slowly reverses the hypertension produced by iv Ang II infusion (62). This finding suggests that at least a component of the effect of losartan is via blockade of circulating Ang II binding in circumventricular organs.

Baroreflex control of LSNA in HS rats was also shifted slightly after losartan

to a lower MAP level, although this shift was smaller than that in LS rats. However, experiments were performed 1-2 days after surgery, which attenuated eating and drinking behavior (100). The subsequent volume depletion may offset the effect of chronic HS diet on the suppression of the renin-angiotensin system. In previous experiments, which were performed 2-5 hr after surgery, losartan did not affect MAP, LSNA or HR in HS rats (162).

While abundant research documented that neither acute nor chronic AT2 receptor blockade significantly alters arterial pressure, a few reports suggest that under some circumstances AT2 receptor effects may be revealed. For example, increases in exogenous Ang II in combination with AT2 receptor blockade increases arterial pressure and vessel density (114). AT2 effects on the cerebral vasculature have also been reported (116). However, the specific AT2 antagonist, PD 123319, failed to alter arterial pressure (106) or baroreflex function during sodium depletion in sharp contrast to the effects of AT1 blockade. This finding reaffirms the dominance of AT1 over AT2 cardiovascular effects. The results are also consistent with a previous study that AT2 blockade does not alter arterial pressure or reflex control of sympathetic activity in spontaneously hypertensive rats (95).

In conclusion, endogenous Ang II chronically maintains LSNA and HR through AT1 but not AT2 receptors in conscious normotensive LS rats. During sodium deprivation, baroreflex function, which regulates arterial pressure over a moment to moment basis, depends in part on the chronically elevated endogenous Ang II levels.

Chapter 4

The Area Postrema Is Not Required for Circulating Angiotensin II to Maintain Sympathetic Activity in Conscious, Sodium Deprived Rats

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ABSTRACT

This study tests the hypothesis that the area postrema (AP) is necessary for endogenous Angiotensin II (Ang II) to chronically maintain lumbar sympathetic nerve activity (LSNA) and heart rate (HR) in conscious sodium deprived rats. The effect of the AT1 antagonist, losartan, on LSNA and HR was determined in AP lesioned (APx), and sham lesioned, with (SFR) or without food restriction (SAL), rats. Before losartan, basal mean arterial pressure (MAP), HR, and baroreflex control of LSNA and HR were similar between groups, with the exception of lower maximal LSNA and higher maximal gain of the HR/MAP curve in APx rats. In all groups, losartan similarly shifted ($p<0.01$) the LSNA/MAP curve to the left without altering maximal gain. Losartan also decreased ($p<0.05$) minimal LSNA in all groups, and suppressed ($p<0.01$) maximal LSNA (% control) in SFR (240±13 to 205±15) and SAL (231±21 to 197±26) but not APx rats (193±10 to 185±8). In general, losartan similarly shifted the HR/MAP curve to the left in all groups. The

results suggest that the AP is not necessary for endogenous Ang II to chronically maintain LSNA and HR at most MAP levels in sodium deprived rats, but is required for Ang II to maintain maximal reflex LSNA.

INTRODUCTION

Recent studies suggest that chronic elevation of endogenous angiotensin II (Ang II) maintains sympathetic outflow in rats. For example, blockade of the renin-angiotensin system with antagonists or angiotensin converting enzyme (ACE) inhibitors decreases sympathetic nerve activity in rats with chronic heart failure (34), renal hypertension (71), spontaneous hypertension (95), and sodium deprivation (35,162,163). A key feature of most of these studies is that the hypotension due to blockade of the renin-angiotensin system was reversed by iv infusion of α1 adrenergic agonists to avoid baroreflexly increasing sympathetic outflow. In these studies, Ang II blockade also shifted baroreflex control of sympathetic nerve activity or heart rate (HR) to a lower mean arterial pressure (MAP) level (34,35,71,95,163). The net result is that sympathetic nerve activity and HR after Ang II blockade were lower at any given levels of MAP over a wide range.

The brain has been suggested as a site at which endogenous Ang II acts to influence the autonomic nervous system (for review, see reference (128)), but it remains unclear where in the brain the action of Ang II takes place. There are several lines of indirect evidence that the area postrema (AP), a circumventricular organ (CVO) located at the floor of the fourth ventricle on the dorsal surface of the

medulla of the brain, may be a site of action of Ang II in rats (44). First, the AP, like other CVOs, lacks a blood brain barrier (65), and therefore, circulating substances such as Ang II can gain access to influence sympathetic nervous system. Second, *in vitro* autoradiographic studies reveal rich ANG II binding sites in the rat AP (58,147), and iv injection of losartan, a nonpeptide Ang II type 1 (AT1) receptor antagonist, dose-dependently competes with the binding sites (167). Third, anterograde and retrograde anatomic tracing studies demonstrate that the rat AP is a relay station for afferents from, and efferents to, other important brain regions integrally involved in cardiovascular regulation, including the nucleus tractus solitarius (NTS) and the parabrachial nucleus (PBN) (29,94,140,156). Fourth, physiological studies show that iv injected Ang II activates some rat AP neurons, independent of changes in arterial pressure (127). Fifth, microinjection of Ang II into the rat AP causes dose-dependent increases in arterial pressure and this increase of arterial pressure can be attenuated by iv injected losartan (104). Finally, the rat AP may be involved in the development of renal and chronic Ang II induced hypertension (52,53).

The present study was conducted to directly test the hypothesis that circulating endogenous Ang II acts at the AP to maintain sympathetic nerve activity under physiological conditions. This hypothesis was tested by determining whether the AP is necessary for endogenous Ang II to maintain lumbar sympathetic nerve activity (LSNA) and HR in conscious sodium deprived rats with elevated levels of circulating Ang II. More specifically, we examined the effects of AP lesion on

baroreflex control of LSNA and HR during sodium deprivation in conscious rats. Then, we determined if lesions of the AP abolished the effect of losartan to suppress LSNA and HR over the entire baroreflex range of arterial pressure.

METHODS

Male, Sprague-Dawley rats (225-333 g, Harlan Sprague-Dawley Inc, Indianapolis, IN), at 8 weeks of age, were subjected to either AP lesion or sham operation and received post surgery care at University of Minnesota, as previously described (24). Briefly, rats were pre-anesthetized with pentobarbital (32.5 mg/kg, IP). Surgical anesthesia was achieved with a second intramuscular injection of an anesthetic cocktail (acetylpromazine 0.2 mg/kg, butorphanol tartrate 0.2 mg/kg, ketamine 25 mg/kg). Rats were then placed in a stereotaxic apparatus, the AP on the dorsal surface of the medulla at the caudal extent of the fourth ventricle was exposed and removed by suction with a 26-gauge needle attached to a vacuum line. Sham lesion operations were identical except that the vacuum line was not attached. Since AP lesioned (APx) rats exhibit decreases in food intake for a few weeks after the lesion (24,75,76), a recovery period of 8 weeks was allowed to restore normal growth rate. There were two groups of sham rats in this study. One group received food and water *ad libitum* (SAL) during the recovery period. The second group was food restricted (SFR) for the first 3 weeks after sham operation to match the spontaneous food intake reduction of AP lesioned rats. Food intake for SFR rats was limited to 50%, 60% and 80% of normal food intake of SAL rats

during the first, second and third week post-sham lesion operation, respectively.

Eight weeks after surgery for the AP lesion or sham lesion, rats were shipped to the Oregon Health Sciences University for nerve activity studies on a blind sample basis. All rats were coded with ear notches by the operator of AP lesion and sham lesion. The coding information was not released to the experimenter until after experiments. All rats, at 16 weeks of age, were then placed on sodium deficient food (Na<0.02%, Harlan Teklad, Madison, WI) for 2 to 3 weeks to increase endogenous Ang II levels before surgery for catheter and nerve electrode implantation. During the first two days on sodium deficient diet, rats received a furosemide injection (1mg/kg/day, IP; Abbott Labs, North Chicago, IL) to increase sodium excretion. All rats were housed in a room maintained on a 12 hr-12 hr light-dark cycle and were allowed to have food and distilled water *ad libitum*. Experiments were conducted in the same room while rats remained in their home cage.

Chemicals

All drugs were dissolved in water of 5% dextrose. Brevital (Eli Lilly, Indianapolis, IN) was used for anesthesia during surgery for nerve electrode and catheter implantation. Phenylephrine (PE) and nitroprusside (NP) (both from Elkins-Sinn, Cherry Hill, NJ) were infused to manipulate MAP in the baroreflex studies. Losartan (gift of Dr. Ronald Smith, Dupont Merck Pharmaceutical, Wilmington, DE) was injected iv for blockade of AT1 receptors. Methoxamine was

infused iv to maintain MAP after losartan injection, and hexamethonium chloride was injected iv for ganglionic blockade at the end of the experiment to block post ganglionic nerve activity (both drugs from Sigma, St. Louis, MO). All injections were given in 100 µl volume, followed by a 100 µl flush of the dead space of catheters.

Catheter and nerve electrode implantation

Rats (298-496 g) were anesthetized with an initial injection of Brevital (50 mg/kg, IP) followed by a second at the same dose about 5 min later. After a venous catheter was inserted into the right jugular vein, anesthesia was maintained by iv Brevital infusion as needed (2.7-4 µl/min, 10 mg/ml). A total of four tygon catheters (Norton Performance Plastics, Akron, OH) were implanted for drug delivery, two of which were inserted into the right jugular vein and two were placed via the left femoral vein. An additional catheter was advanced into the abdominal aorta via the left femoral artery for the measurement of MAP.

For the lumbar nerve electrode implantation, a midline abdominal incision was made. After retracting the intestines, the abdominal aorta and vena cava were gently pulled aside to expose the lumbar nerve. The nerve was then dissected free and placed on a bipolar electrode. The electrode was constructed with two Teflon coated, 3-strained stainless steel wires (#7934, A&M Systems, Everett, WA) and was encased within silicon tubing (0.02" x 0.037"; Specialty Mfg, Saginaw, MI). When optimal nerve traffic was confirmed on an oscilloscope (Model 2212,

Tektronix, Beaverton, OR) by observing the rhythmic bursts of nerve traffic, the nerve and electrode were embedded in a small amount of dental gel (President Light Body, Coltene, Hudson, MA).

Catheters and the electrode lead were tunneled subcutaneously to the back of the neck and exteriorized. All incisions were closed with silk suture. The rats were returned to their home cage and allowed 20-40 hr for recovery.

Hemodynamic and Nerve Activity Recordings

MAP was monitored via the femoral arterial catheter connected to a Statham pressure transducer and a Grass preamplifier (7P1). HR was measured using a Grass tachograph (7P4) triggered by the amplified arterial pressure pulse. The raw lumbar nerve activity was amplified using a Grass differential preamplifier (P511) with a band pass filter of 30 Hz to 10 kHz. The gain (25,000-70,000X) of the preamplifier was adjusted so that the amplitude of maximal nerve activity output did not exceed the linear input range (±1.5 V peak-peak) of the Grass integrator (7P10), which was used for integrating raw nerve activity. The amplified nerve activity traffic was observed on the storage oscilloscope and was whole wave rectified and integrated with a reset time of 1 sec. Together with MAP and HR, integrated LSNA was recorded on chart paper using a Grass polygraph (7D). Nerve activity was first quantified by averaging the integrated activity just before reset over 12 sec (12 peaks) during stable and quasi-stable periods (slow or no change in measured parameters), or 3-4 sec (3-4 peaks) during transient periods

(e.g. baroreceptor reflex curve) (163). In addition, the noise level was quantified at the end of the experiment by averaging the integrated output over 12 sec (12 peaks) after efferent nerve activity was eliminated by the combined use of a bolus injection of hexamethonium and infusion of methoxamine. The noise output was then subtracted from the average integrated nerve activity to provide a measure of net LSNA. For each animal, the net LSNA was normalized using two methods. First, LSNA was normalized to basal nerve activity in the control period and was expressed as percent of control (% con). Basal nerve activity was defined as the average of resting activity at 2 time points within 10 min before the first baroreflex curve was generated. Second, LSNA was normalized to the maximal nerve activity during the control period and was expressed as percent of maximum (% max). Maximal LSNA was the peak LSNA in the baroreflex curves induced by NP infusion during the control period.

Baroreceptor Reflex Curves

MAP was varied by iv infusion of increasing doses of either PE (0.68-27 µl/min, 1 mg/ml) to increase MAP up to ~ 170 mm Hg, or NP (1.35-68 µl/min, 1 mg/ml, iv) to decrease MAP to ~ 50 mm Hg. The corresponding response of LSNA and HR, together with MAP, were recorded. The ramp increase or decrease of MAP was completed in ~ 2 min. Infusions of PE or NP were performed randomly. MAP, LSNA and HR were allowed to returned to baseline (about 30 min) before a subsequent ramp of MAP was made.

Protocol

On the morning of the experiment, NP and PE for the baroreflex study were loaded into separate catheters to fill the dead space. After MAP, HR and LSNA were stable (in ~ 30 min), these variables were recorded for 40-60 min. Starting around noon, time course and baroreflex function studies were begun. After basal parameters were obtained and pre-losartan baroreflex control of HR and LSNA was determined, losartan was injected (10 mg/kg, iv), and immediately after losartan, iv infusion of methoxamine (5-33 μg/min) was started to prevent post losartan hypotension. MAP, HR and LSNA were recorded for at least 40 min after losartan administration, because in our previous study (162), it took at least 40 min for the depressor effect of losartan to stabilize. Then, the post-losartan baroreflex control of HR and LSNA was determined. At the beginning and the end of each experiment, the pressor response to an Ang II bolus (100 ng/kg, iv) was tested by measuring the peak change in MAP.

Verification of the AP lesions

After the experiments, rats were anesthetized with Brevital and then perfused via the left ventricle of the heart with saline followed by 4% paraformaldehyde. The brain was then removed and soaked in 4% paraformaldehyde at 4° C. The following morning, the brain was rinsed with phosphate buffered saline and then transferred to a bottle containing 30% sucrose solution. The brain was then stored in the bottle and shipped back to University of Minnesota for histological study to verify the AP

lesion in APx rats, using methods described previously (24).

To determine if the AP lesion damaged the neighboring NTS, MAP lability was examined and compared between APx and sham rats. MAP was recorded for at least 40 min before baroreflex function studies in individual rats and sampled every 30 sec to generate at least 80 readings. The standard deviation (SD) of MAP was calculated in individual rats. Then, SD of APx rats and sham rats were subjected to statistics to examine if there is a significant difference in MAP lability among groups. SD analysis has been typically used to determine if the NTS is damaged (98,122).

Histological verification of AP lesion was confirmed in all APx rats. Typical histological sections from sham rats and APx rats are shown in Fig 4.1a and 4.1b, respectively. The AP was completely lesioned and the ablation of AP caused little damage to the adjacent NTS in all APx rats. The SD of MAP was not significantly different ($p=0.86$) among groups [APx ($n=4$): 4.66±1.06; SFR ($n=6$): 5.02±0.38; SAL ($n=6$): 5.16±0.55; mm Hg], further indicating that the AP ablation did not damage the NTS.

Data Analysis and Statistics

A logistic relation, slightly modified from Kent (85), was used to analyze baroreflex curves: $Y=d+(a-d)/(1+\exp[b(X-c)])$, where X, MAP; Y, LSNA or HR; a, maximum of LSNA or HR; b, slope coefficient; c, MAP at the midpoint of the range of LSNA or HR; and d, minimum of LSNA or HR. In each animal, raw MAP and

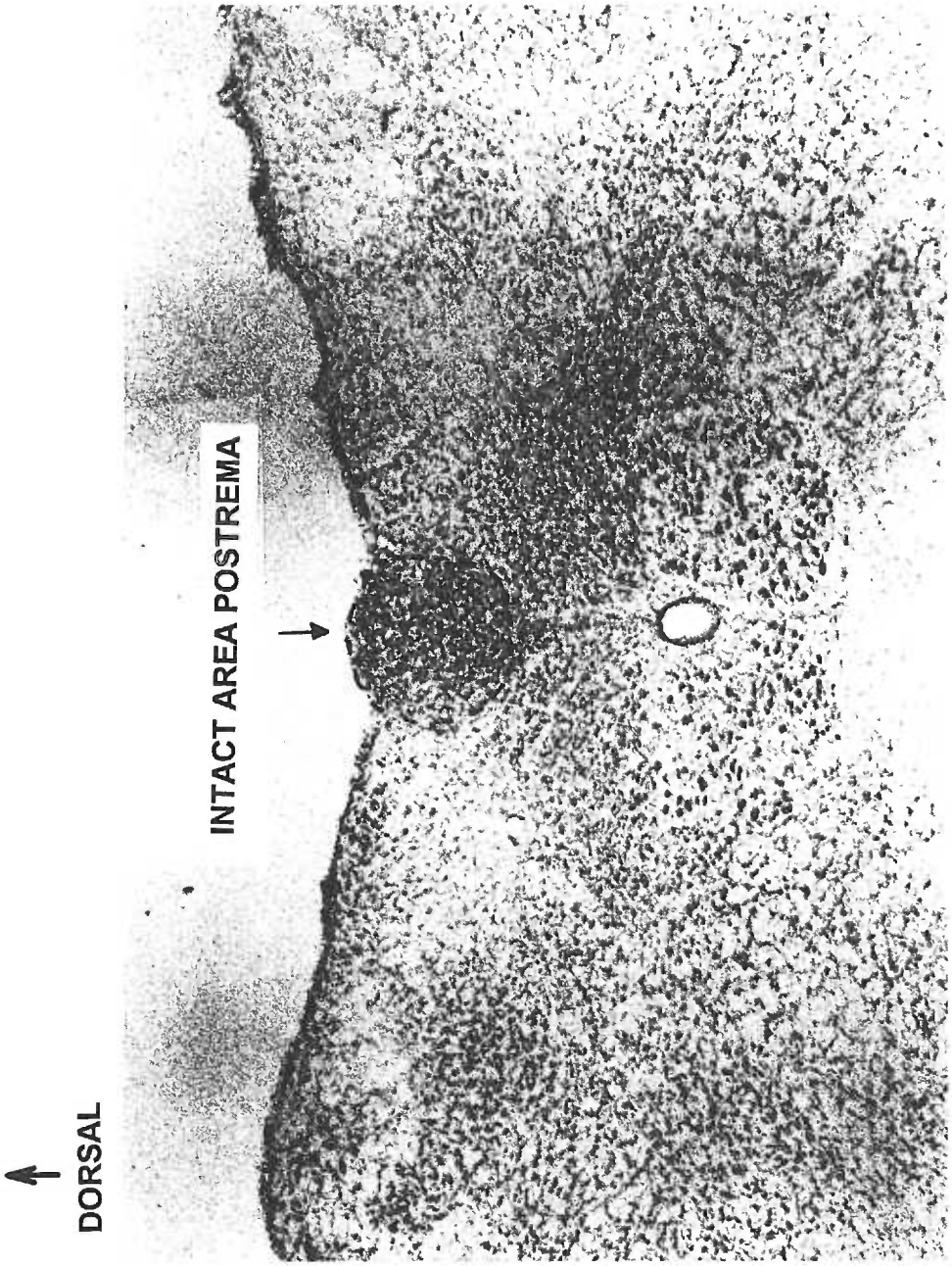

Figure 4.1a Typical histological section from a rat in area postrema sham lesion group, showing an intact area postrema.

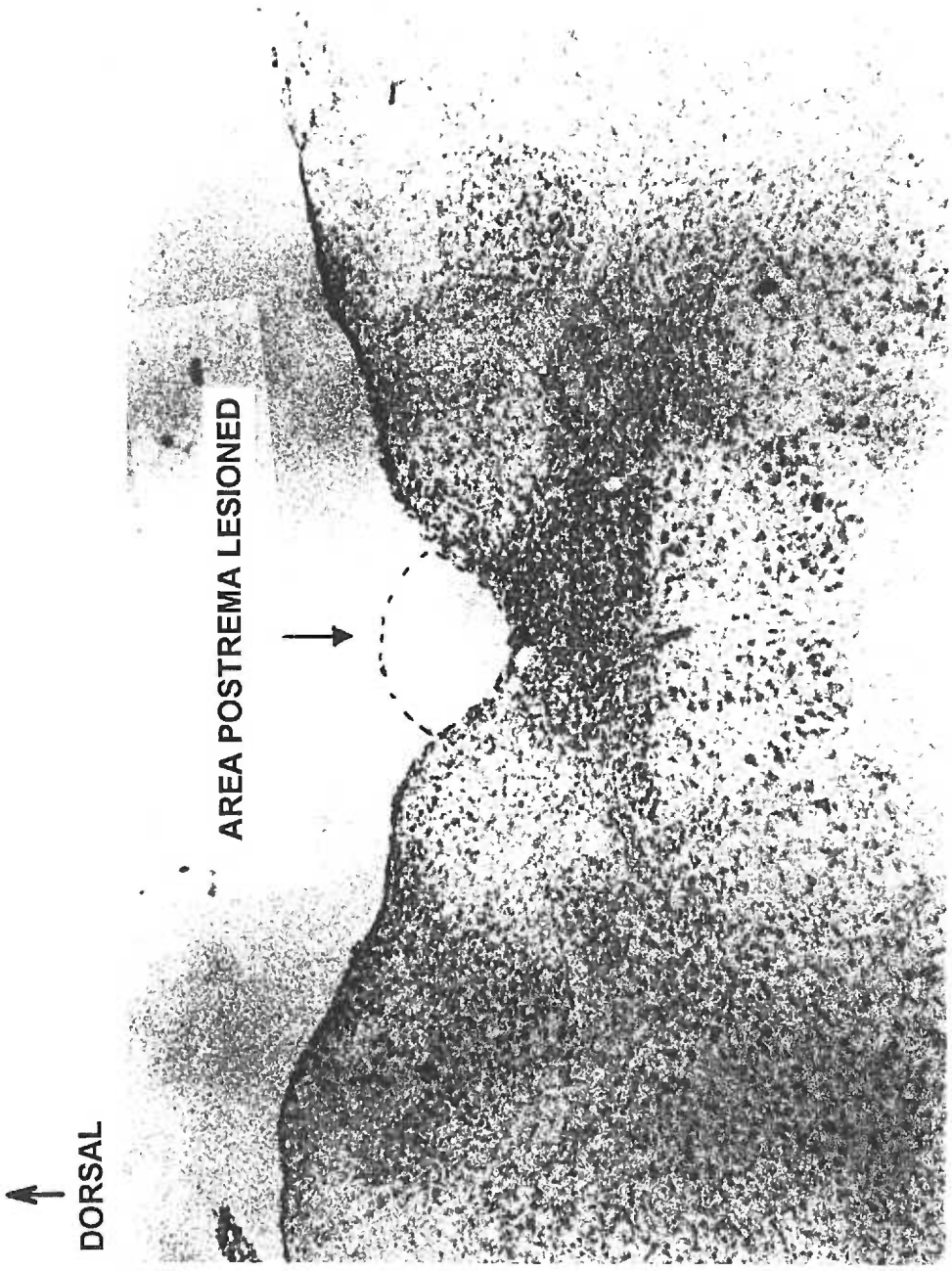

Figure 4.1b Typical histological section from a rat with area postrema lesion, showing absence of the area postrema.

LSNA (or HR) data were fit to the logistic function to generate parameters a, b, c and d, using graphics software (Sigmaplot, Jandel Scientific, Corte Madera, CA). Constraints of maximum and minimum of LSNA or HR were set for the fitting process. The maximal or minimal values of LSNA and HR were determined in each experiment when the high, or low plateau of LSNA and HR were reached while MAP was still being decreased or increased by NP or PE infusion, respectively. The range of the baroreflex curve, e, was defined as (a-d), and the maximal gain of the baroreflex curve was calculated using the formula (-be/4). Means ± SEM of individual fit curve parameters were calculated, and statistical analysis was performed to determine within and between group differences in these parameters (Table 4.1 and 4.2). The averaged a, b, c and d were then used to generate averaged baroreflex curves (Fig. 4.3 and 4.5).

In addition, LSNA (% max) (or HR) from all rats in each group were pooled by calculating the mean and SEM of all data points collected within 5 mm Hg MAP increments. Multiple points at the same MAP in each animal were averaged before pooling. The means of pooled data were plotted with SEM of LSNA (or HR) and then fit to the logistic function (Fig. 4.4 and 4.6).

All data are presented as means ± SEM. For time course and baroreflex function studies, data were analyzed using two-factor ANOVA, repeated one way (time or drug) (161). For comparison of MAP lability, pressor responses to iv bolus of Ang II and changes in MAP50 or MAP100 after losartan, one-factor ANOVA was used. The Newman-Keuls post hoc test was used for multiple comparisons after

ANOVA (161). All analyses were performed using GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD). A significance level of $p<0.05$ was accepted.

RESULTS

Three groups of rats, APx (n=10), SFR (n=9) and SAL (n=9) were used in this study. In some rats, LSNA was lost either before or during the experiment but HR was recorded, or HR was not detected by the tachograph due to an inadequate trigger but LSNA was recorded. Therefore, the final number of rats included for data analysis and presentation in the figures and tables may be less than these totals.

Time course of changes in LSNA and HR after losartan

As shown in Fig. 4.2, basal LSNA, expressed as % max, was significantly higher ($P<0.05$) in APx rats (56±5 % max) than SFR and SAL rats (42±2 and 46±4 % max, respectively), but basal HR was not significantly different among groups (APx: 399±13; SFR: 386±10; SAL: 405±11; beats/min). Basal MAP in SAL rats (100±3 mm Hg) was significantly lower ($p<0.01$) than that in APx rats (107±3 mm Hg) and SFR rats (108±2 mm Hg).

After losartan administration, MAP was maintained by methoxamine infusion at pre-losartan levels in all groups (Fig. 4.2). Losartan decreased LSNA, and the decreases were not different among groups (Fig. 4.2). Forty min after losartan

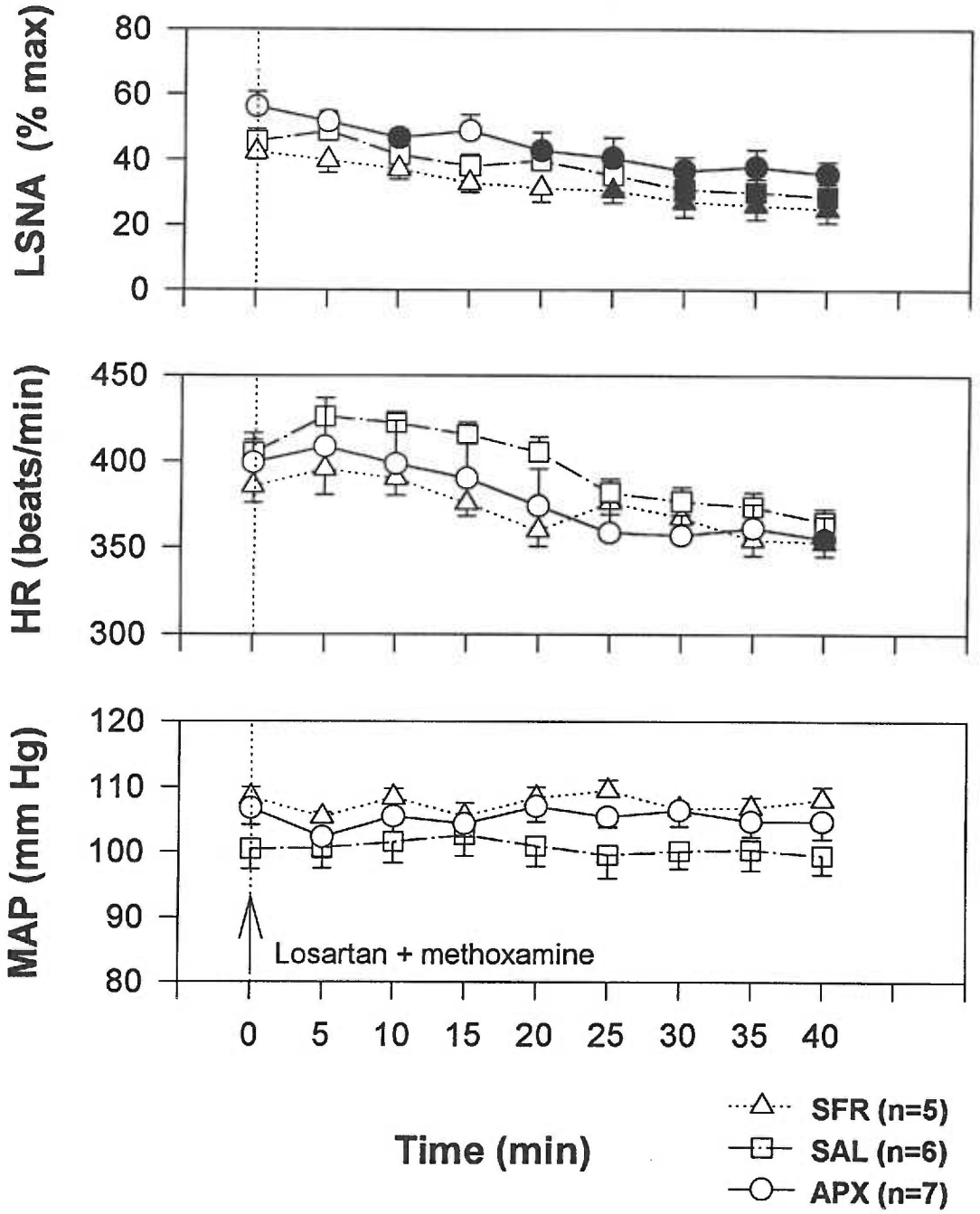

Figure 4.2 Time course of changes in lumbar sympathetic nerve activity (LSNA) and heart rate (HR) after iv injection of losartan, with post-losartan mean arterial pressure (MAP) maintained at pre-losartan basal levels by iv infusion of methoxamine in area postrema lesioned rats (APx) and sham operated rats with (SFR) or without (SAL) food restriction. Solid symbols indicate values that are significantly different ($P<0.05$) from the corresponding basal values.

administration, LSNA had fallen ($p<0.01$) to 36±4, 25±4, and 29±4 % max in APx, SFR and SAL rats, respectively.

Losartan also similarly decreased HR in all groups (Fig. 4.2). At 40 min after losartan administration, HR was significantly suppressed to 355±13 beats/min in APx rats and to 353±8 beats/min in SFR rats ($p<0.05$). Although the HR suppression in SAL rats was not significant, HR at 40 min post-losartan (365±8 beats/min) was not significantly different from the corresponding HR values in APx or SFR rats.

Baroreflex control of LSNA

Effect of AP lesion. In general, the AP lesion did not alter baroreflex control of LSNA, except that the lesion decreased maximal LSNA. Before losartan administration, maximal gain of the LSNA/MAP curve in APx rats was not different from that in SFR or SAL rats either when nerve activity was expressed as % con or as % max (Fig. 4.3 and 4.4; Table 4.1). Thus, ablation of the AP did not change the sensitivity of baroreflex control of LSNA. MAP50 was also not different between groups (Table 4.1). When LSNA was expressed as % con, maximal LSNA in APx rats was significantly lower ($p<0.01$) than that in SFR and SAL rats, as was the range (Fig. 4.3, Table 4.1). The lesser maximal LSNA and range in APx rats was not revealed when LSNA was expressed as % max (Fig. 4.4, Table 4.1), because of the normalization.

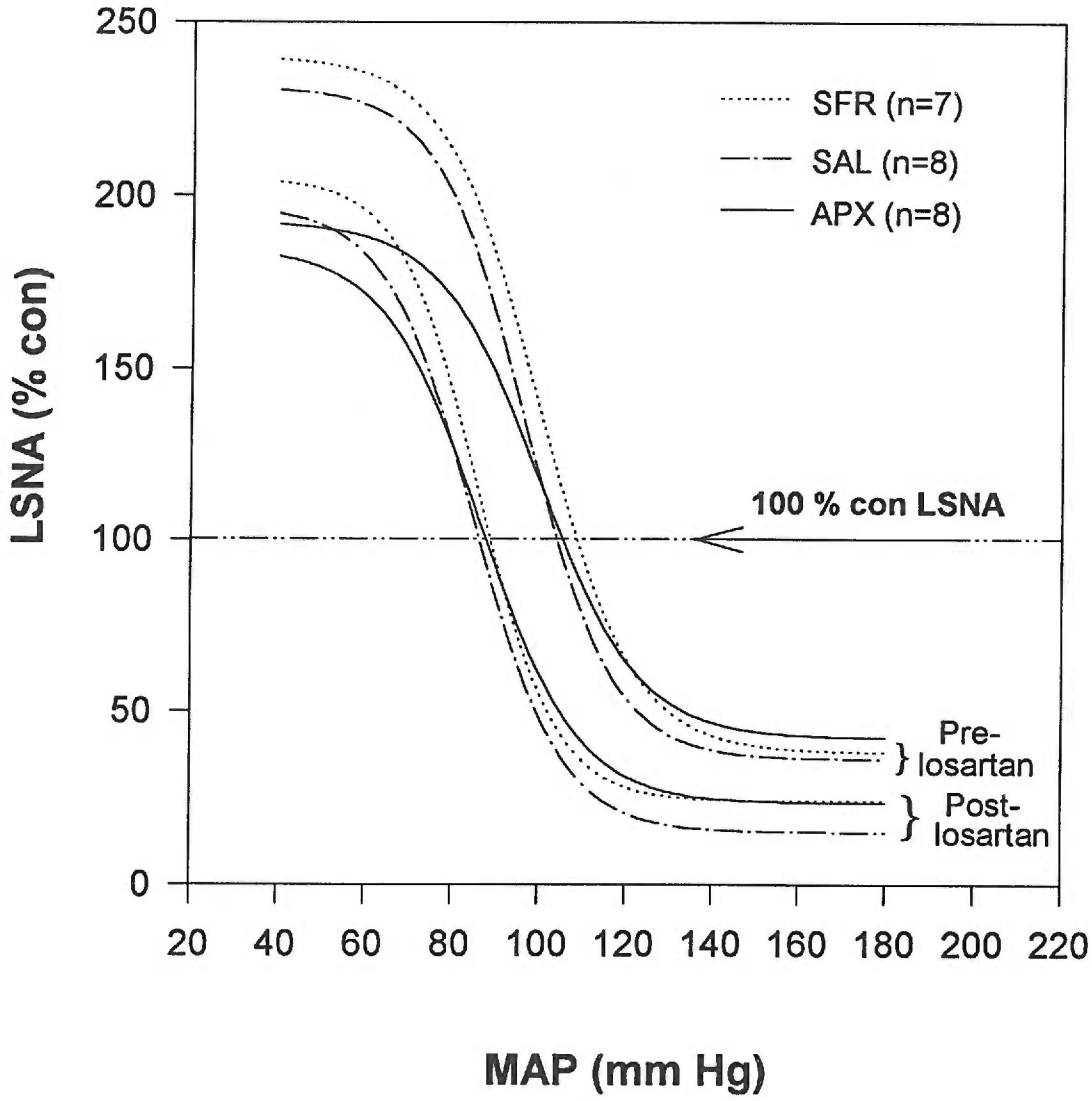

Figure 4.3 Baroreflex control of lumbar sympathetic nerve activity (LSNA) in area postrema lesioned rats (APx) and sham operated rats with (SFR) or without (SAL) food restriction before (Pre-losartan) and after (Post-losartan) iv injection of losartan. LSNA was normalized to the pre-losartan basal LSNA (% con) in each rat. Sigmoidal baroreflex curves were generated from the averaged parameters as described in the text. MAP, mean arterial pressure.

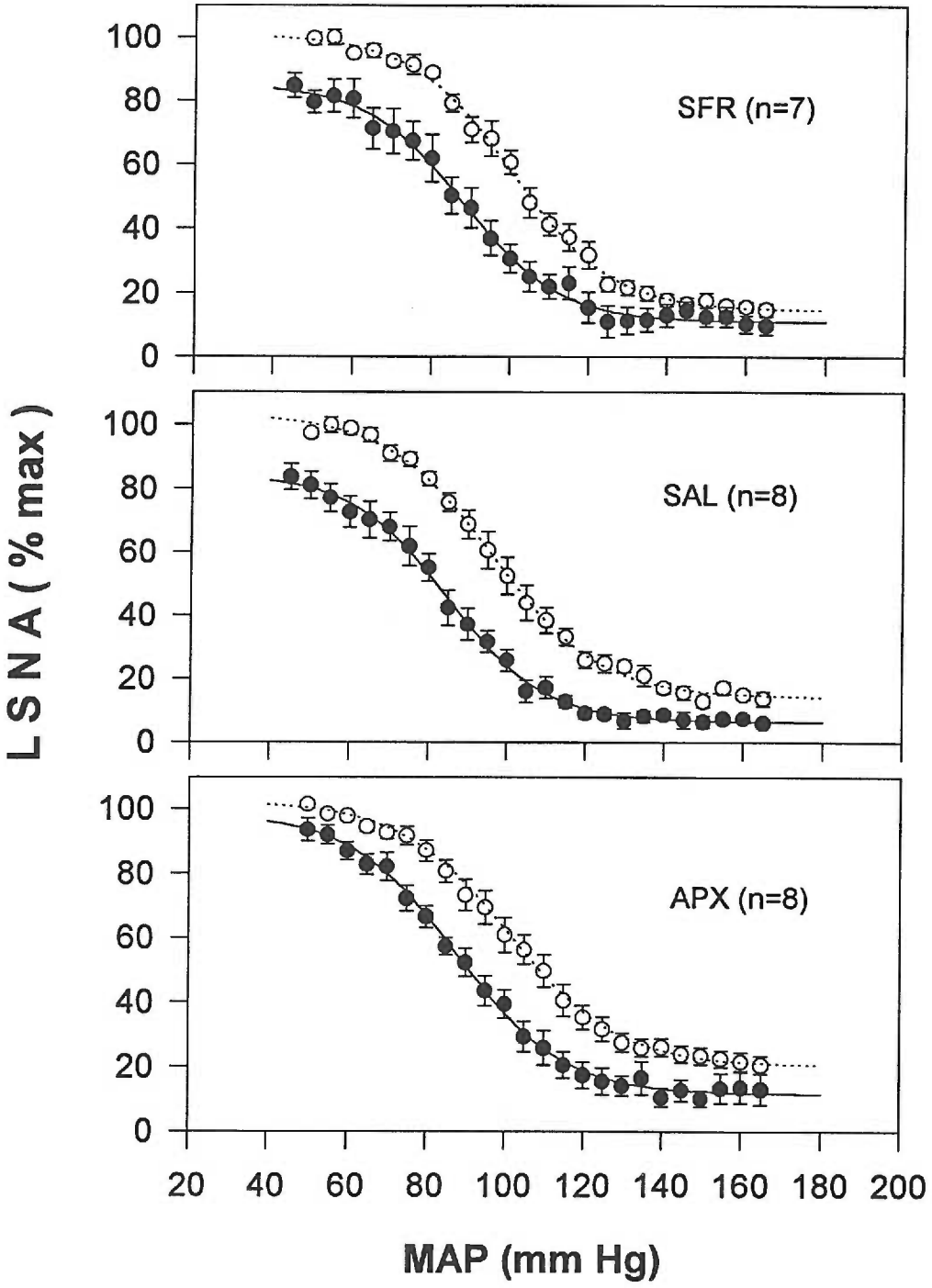

Figure 4.4 Baroreflex control of lumbar sympathetic nerve activity (LSNA) in area postrema lesioned rats (APx, bottom panel) and sham operated rats with (SFR, top panel) or without (SAL, middle panel) food restriction before (open circle) and after (solid circle) iv injection of losartan. In each rat, LSNA was normalized to the pre-losartan baroreflex-mediated maximum LSNA (% max). Data were pooled as described in the text. MAP: mean arterial pressure.

Table 4.1 Effects of Losartan on Baroreflex Control of Lumbar Sympathetic Nerve Activity in Area Postrema Lesioned Rats (APx) and Sham Operated Rats with (SFR) or without (SAL) Food Restriction.

	SFR (n=7)		SAL (n=8)		APx (n=8)	
	Pre-losartan	Post-losartan	Pre-losartan	Post-losartan	Pre-losartan	Post-losartan
LSNA (% con)						
Maximum	240±13	205±15 **	231±21	197±26 **	193±10 ††	185±8
Slope Coefficient	0.092±0.010	0.110±0.013	0.099±0.013	0.098±0.013	0.087±0.006	0.089±0.007
MAP50	100±3	86±5 **	97±3	85±3 **	100±3	87±3 **
Minimum	38±6	24±7 *	36±4	15±3 **	42±5	24±5 **
Range	202±11	181±20	195±18	182±25	150±11 ††	162±10
Maximal Gain	-4.72±0.64	-4.93±0.88	-4.96±0.89	-4.39±0.69	-3.20±0.20	-3.69±0.46
LSNA (% max)						
Maximum	101±1	86±4 **	101±1	85±4 **	102±1	97±3 ✧
Slope Coefficient	0.088±0.011	0.110±0.014	0.096±0.013	0.096±0.013	0.080±0.004	0.086±0.006
MAP50	100±3	86±5 **	97±3	84±3 **	100±3	87±3
Minimum	13±3	10±3	15±2	6±1 **	21±3 †	12±4 **
Range	88±4	76±7	86±1	79±5	81±3	85±3
Maximal Gain	-1.88±0.18	-2.01±0.0.28	-2.05±0.27	-1.88±0.25	-1.63±0.10	-1.85±0.18

Values are means ± SEM of baroreflex logistic curve parameters. Data were analyzed with ANOVA and Newman-Keuls test.

* $p<0.05$, ** $p<0.01$, post- vs pre-losartan in each group.

† $p<0.05$, †† $p<0.01$, APx vs SFR and SAL during pre-losartan period.

✧ $p<0.05$, APx vs SFR and SAL during post-losartan period.

Effect of losartan. Losartan shifted baroreflex control of LSNA to similarly lower MAP levels in all groups of rats without altering the sensitivity. Losartan did not affect maximal gain in any group of rats, regardless of whether the nerve activity was expressed as % max or as % con (Fig. 4.3 and 4.4; Table 4.1). MAP50 was similarly decreased ($p<0.01$) by losartan in all three groups, when LSNA was expressed as either % con (Fig. 4.3, Table 4.1) or % max (Fig. 4.4, Table 4.1). Another index of the position of the LSNA/MAP curve, MAP100, which is the MAP at 100% con LSNA (Fig. 4.3), was also similarly decreased ($p<0.01$) by losartan in all groups (APx: 106±2 to 88±2; SFR: 108±2 to 89±5; SAL: 103±3 to 84±5; mm Hg). Minimal LSNA was similarly suppressed ($p<0.05$) in all groups when nerve activity was expressed as % con, but no significant suppression in SFR rats was detected when LSNA was expressed as % max (Table 4.1). Finally, losartan suppressed ($p<0.01$) maximal LSNA in SFR and SAL rats but not APx rats (Fig. 4.3 and 4.4, Table 4.1).

In summary, the data suggest that in sodium deprived rats, lesion of the AP largely does not affect baroreflex control of LSNA, nor does it prevent losartan's action to suppress LSNA over most except low levels of MAP.

Baroreflex control of HR

Effect of the AP lesion. The AP lesion generally did not affect baroreflex control of HR (Fig. 4.5 and 4.6, Table 4.2). Before losartan administration, MAP50, maximal HR and minimal HR in APx rats were not significantly different from those

in SFR or SAL rats, indicating that AP lesion did not shift the HR/MAP curve (Table 4.2). However, while maximal gain in APx rats was not significantly different from that in SAL rats, it was larger ($p<0.05$) than that in SFR rats (Table 4.2).

Effect of losartan. Maximal gain was significantly decreased ($p<0.05$) by losartan in APx rats but not SFR or SAL rats (Table 4.2). Losartan shifted the HR/MAP curve to a lower MAP level in all groups (Fig. 4.5 and 4.6, Table 4.2). Losartan also significantly decreased ($p<0.05$) MAP50 in APx and SFR but not in SAL rats (Table 4.2); however, the decrease in MAP50 in SAL rats (-13±4 mm Hg) was similar to that in APx (-11±4 mm Hg) and SFR rats (-12±4 mm Hg), indicating that the HR/MAP curve was similarly shifted to a lower MAP level in all groups. Minimal HR was not altered in any group, but maximal HR was similarly suppressed in all groups ($p<0.05$; Table 4.2).

In summary, the data suggest that in sodium deprived rats, the AP lesion does not alter baroreflex control of HR, nor does it prevent losartan's action to suppress HR at all but high MAP levels.

Pressor response to Ang II

Before losartan administration, the pressor response to an Ang II bolus (100ng/kg, iv) in APx rats (24±3 mm Hg, n=6) was significantly smaller ($p<0.05$) than in SFR (44±4 mm Hg, n=9) but not SAL rats (37±4 mm Hg, n=8). At the end of each experiment, the same dose of Ang II did not change MAP in any rat, indicating complete blockade of AT1 receptors.

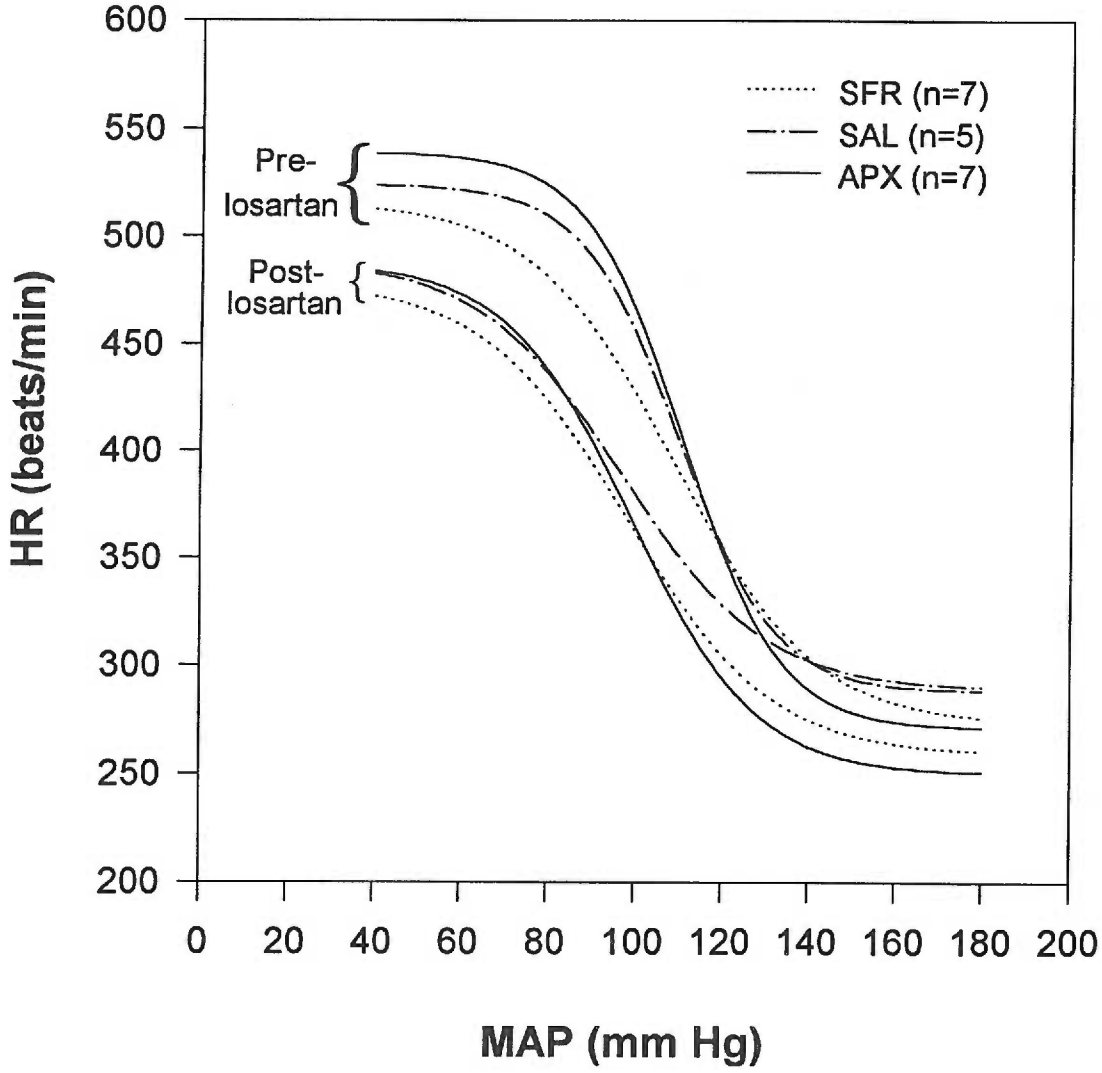

Figure 4.5 Baroreflex control of heart rate (HR) in area postrema lesioned rats (APx) and sham operated rats with (SFR) or without (SAL) food restriction, before (Pre-losartan) and after (Post-losartan) iv injection of losartan. Sigmoidal baroreflex curves were generated from the averaged parameters as described in the text. MAP: mean arterial pressure.

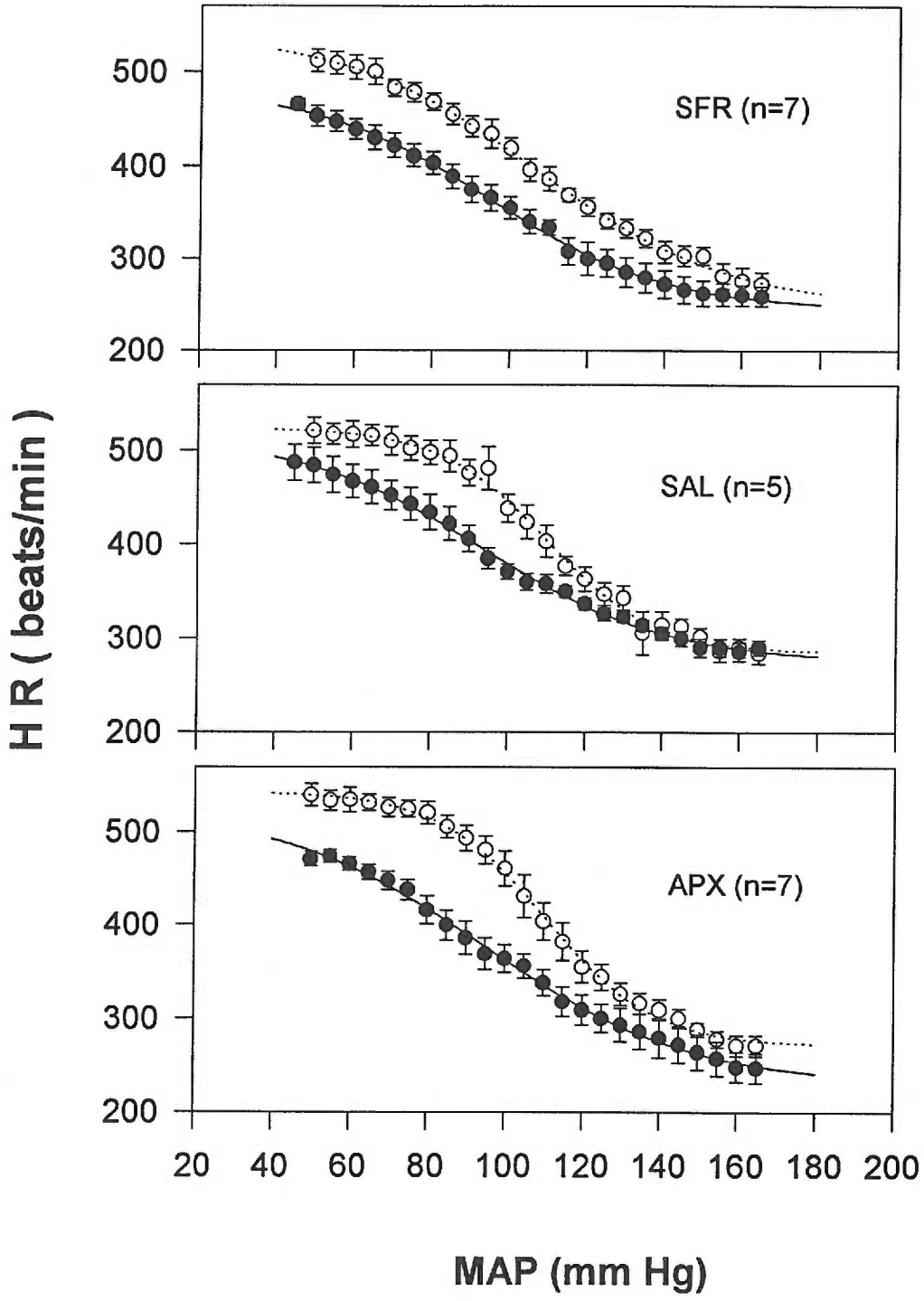

Figure 4.6 Baroreflex control of heart rate (HR) in area postrema lesioned rats (APx, bottom panel) and sham operated rats with (SFR, top panel) or without (SAL, middle panel) food restriction before (open circle) and after (solid circle) losartan administration. Data were pooled as described in the text. MAP: mean arterial pressure.

Table 4.2 Effects of Losartan on Baroreflex Control of Heart Rate in Area Postrema Lesioned Rats (APx) and Sham Operated Rats with (SFR) or without (SAL) Food Restriction.

	SFR (n=7)		SAL (n=5)		APx (n=7)	
	Pre-losartan	Post-losartan	Pre-losartan	Post-losartan	Pre-losartan	Post-losartan
Maximum	516±11	479±14 *	524±13	489±17 *	539±12	488±9 *
Slope Coefficient	0.062±0.007	0.060±0.009	0.090±0.021	0.061±0.007	0.090±0.013 †	0.070±0.010
MAP50	109±5	98±3 *	110±4	97±3	111±3	99±6 *
Minimum	273±12	259±11	288±13	289±10	271±11	250±18
Range	243±16	220±14	236±14	200±17	268±19	238±22 ✧
Maximal Gain	-3.71±0.36	-3.28±0.43	-5.11±0.98	-3.05±0.40	-5.76±0.58 †	-3.93±0.44 *

Values are means ± SEM of baroreflex logistic curve parameters. Data were analyzed with ANOVA and Newman-Keuls test.

* $p<0.05$ post- vs pre-losartan in each group.

† $p<0.05$ APx vs SFR during pre-losartan period.

✧$p<0.05$ APx vs SAL during post-losartan period.

DISCUSSION

The present study is the first to demonstrate that ablation of the AP does not have major impact on the suppression of LSNA and HR produced by acute iv losartan at most MAP levels in conscious rats. The important new findings are that the effects of losartan to decrease LSNA and HR at basal MAP, to suppress baroreflex mediated changes in LSNA at any but low MAP levels and to suppress baroreflex mediated changes in HR were similar in AP lesioned and sham rats. Thus, in contrast to our hypothesis, the data suggest that the AP is not necessary for endogenous Ang II to chronically maintain LSNA, except at low MAP levels that are corresponding to maximal LSNA, in sodium deprived rats. The AP is also not necessary for endogenous Ang II to maintain HR.

Basal MAP and HR in APx rats were not significantly different from those in SFR rats, suggesting that the AP is not critical in the maintenance of basal MAP or HR during sodium deprivation. The results are similar to those of previous studies suggesting that the AP plays little or no role in the maintenance of MAP in sodium replete rats (19,24,53,54,70,89,107,166), dogs (80,126,129) and rabbits (108), or HR in rats (42,53,107), dogs (80,126) and rabbits (108). However, hypotension (51,142,143) and bradycardia (24,89,142,143) following AP lesion has also been reported in animals with normal sodium intake. One explanation for the differences in basal MAP and HR could be the length of time allowed for recovery after AP lesion. Feeding behavior is depressed a few weeks after AP lesions (75,76,89),

and this could affect cardiovascular regulation. For example, Skoog et al reported basal MAP and HR were lower in rats one week after AP ablation, compared with sham-operated rats (142,143). In the present and previous (19,42,53,107,166) studies, APx animals were allowed more time to regain steady state feeding and growth rates (24,75). On the other hand, it could be argued that the lack of AP-lesion-induced hypotension and bradycardia in the present study was because rats were studied 1-2 days after the stress of surgery for nerve electrode implantation. The stress could eliminate group differences in MAP or HR. However, sodium replete AP lesioned rats studied well after the AP lesion and surgery for catheter implantation are also found to have normal arterial pressure and HR (Collister and Osborn, unpublished data).

Consistent with findings in previous studies (35,162,163), losartan decreased LSNA and HR over the entire range of MAP in AP intact rats, indicating that endogenous Ang II chronically maintains sympathetic outflow during sodium deprivation. However, AP lesions did not alter the magnitude or time course of the suppression of LSNA and HR by losartan at pre-losartan basal MAP levels (Fig. 4.2), nor did it affect the suppression of baroreflex mediated LSNA and HR at most levels of MAP. Interestingly, the lesion did prevent the ability of losartan to decrease baroreflex-mediated maximal LSNA. These data indicate that the AP is not necessary for the action of endogenous Ang II to maintain sympathetic outflow at all but low MAP levels in sodium deprived rats.

These results may seem surprising given reports that AP lesions reduce

MAP in rats with hypertension induced by elevated circulating Ang II levels or insertion of the mouse mRen-2^d gene into the rat genome (5,52,53). Moreover, the hypotensive effect of chronic losartan infusion is reduced in rats with AP lesions (24). However, the anti-hypertensive effect of the AP lesion may not be due to a loss of a sympathoexcitatory action of circulating Ang II. Indeed, the AP lesion has also been shown to eliminate DOCA-salt hypertension in rats (54), which is a low renin model of hypertension. Alternatively, the AP may act as a mediator of sympathoexcitatory effect of circulating Ang II in these models of hypertension, but not during sodium deprivation. In support of this idea is a study showing that Ang II binding sites in the rat AP decrease during sodium deprivation (164).

Maximal LSNA, expressed as % con, was lower in AP lesioned rats compared with sham rats (Fig. 4.3, Table 4.1). This lower maximal LSNA may be due to an elevated absolute basal LSNA, a decreased absolute maximal LSNA, or both, since LSNA was normalized to basal LSNA. However, it seems unlikely that absolute basal sympathetic outflow would be increased after the AP lesion because basal HR were not increased in AP lesioned rats in both the present study and studies by others (19,24,53,54,89,107,143,166). Therefore, a decreased absolute maximal LSNA, with a smaller or no decrease in absolute basal LSNA in the AP lesion rats is likely. Moreover, because losartan abolished the difference in maximal LSNA between AP lesioned and sham rats (Fig. 4.3, Table 4.1), it appears that the smaller maximal LSNA in APx rats is due to the loss of a chronic effect of Ang II to maintain LSNA at low arterial pressure levels. In agreement with this

conclusion is the study of Skoog et al (142), in which hemorrhage reduced MAP to lower levels in AP lesioned rats than in sham rats. In addition, c-fos expression (a marker of activated neurons in the brain) in the rat AP, induced by hypotension (~50 mm Hg) (22,64,144), is blunted by iv administration of the Ang II antagonist ([Sar^1, Ile^8]-Ang II) (22). Therefore, the present data suggest that circulating Ang II may act at the AP to maintain maximal sympathetic outflow during hypotension in rats.

To our knowledge, the role of the AP in baroreflex control of sympathetic nerve activity has not been studied before in conscious rats. In the present study, the maximal gain of baroreflex control of LSNA was not altered by the AP lesion (Fig. 4.3 and 4.4, Table 4.1). Our data suggest that the AP may not be necessary for maintaining the sensitivity of baroreflex control of sympathetic outflow in sodium deprived rats. However, it may be required for maintaining the sensitivity of baroreflex control of HR in sodium deprived rats (Fig. 4.6) and normal sodium intake rabbits (108). Considering the evidence that the rat AP has connections with both NTS and other parts of the brain such as paraventricular nucleus (94,127,140,156), it is possible that in rats, the AP may participate in other functions influenced by baroreceptor input or circulating Ang II, such as water and sodium intake (76) and vasopressin release (78).

In the present study, we found that blockade of endogenous Ang II with losartan decreased maximal gain of baroreflex control of HR in APx but not sham rats (Table 4.2). Our result is consistent with previous findings indicating that Ang II infusion may increase the slope of cardiac baroreflex curves in conscious AP

lesioned but not AP intact animals (107,108). In addition, losartan similarly shifted the cardiac baroreflex curve to the left and similarly suppressed maximal HR without affecting minimal HR in APx and sham rats, suggesting that the AP does not play a major role, if any, in the maintenance of HR in sodium deprived rats at most arterial pressure levels.

A diminished pressor response to iv Ang II bolus was observed in APx rats compared with sham rats before losartan administration in the present study, in agreement with previous studies in dogs (51) and rabbits (108). Although we do not have a clear explanation, the decreased pressor response may be due to a higher sensitivity of baroreflex control of HR in APx rats (Fig. 4.6; (107,108)). It is not clear whether the total number of vascular Ang II receptors is down-regulated after AP ablation. If this is true, it could, at least in part, explain the lower Ang II pressor response in APx rats. However, there is also a report showing that short term (5-10 min) iv infusion of Ang II increases MAP identically in APx and sham rats (53). The difference may be due to different methods for Ang II administration, e.g. bolus vs infusion.

Baroreflex control of LSNA and HR is apparently mediated by different neuropathways in rats, since the AP lesion differentially affected LSNA and HR in the present study. The AP lesion decreased maximal LSNA but not maximal HR, and increased maximal gain of the HR/MAP curve but not that of the LSNA/MAP curve. In addition, losartan affected baroreflex-mediated LSNA and HR differently in APx rats. For example, losartan did not significantly affect maximal LSNA or

maximal gain of LSNA/MAP curve but decreased maximal HR and maximal gain of the HR/MAP curve in APx rats. These differences may be explained in part by the fact that HR is influenced by both sympathetic and parasympathetic drive to the heart, or that sympathetic drive to the lumbar nerve and the heart is different, or both.

If circulating Ang II does not act at the AP to increase sympathetic activity and HR in sodium deprived rats, then where else could it act? Forebrain CVO's could be involved, because these areas, including the organum vasculosum of the lamina terminalis and the subfornical organ, like the AP, have numerous Ang II receptors and connections with other brain regions that participate in the regulation of sympathetic outflow (for review, see reference (128)). Sympathetic outflow in sodium deprived rats could also be maintained by local brain Ang II acting at sites behind the blood brain barrier, since losartan can pass across the blood brain barrier to block AT1 receptors (103,167).

It is not clear, from data in this study, whether the absolute sympathetic activity is altered by chronic AP lesion. The direct nerve activity recording technique is only suitable for assessing changes in, but not absolute, nerve activity, because the recorded nerve activity depends on the contact conditions between the nerve branch and the electrode wire. However, indirect evidence supports the view that sympathetic outflow is not changed after the lesion because basal MAP and HR are similar between AP lesioned and sham rats, as discussed before. If this is true, then, the nerve activity data in this study suggest that the AP is not necessary for

Ang II to maintain absolute sympathetic nerve activity, because the suppression on LSNA and HR by losartan is similar between AP lesioned and sham operated LS rats. However, it is also possible that the central nervous system adapts after chronic AP lesion, because food and water intake behavior is changed after acute AP lesion and recovered several weeks after the lesion (76). For example, it is possible that after the chronic AP lesion, other brain regions may change their influence on the sympathetic outflow, which may, or may not be necessarily maintained by circulating Ang II. In addition, the lesion of AP could also eliminate fibers in this area, thus, changing the function of other brain regions which have projections passing through the AP to affect sympathetic outflow.

In conclusion, results in the present study suggest that the area postrema is not necessary for endogenous Ang II to chronically maintain LSNA at most except low levels of MAP, nor is it necessary for Ang II to chronically maintain HR in conscious, sodium deprived rats.

Chapter 5

Summary and Conclusions

The studies presented in this dissertation tested the hypothesis that endogenous Ang II maintains sympathetic nerve activity through an action in the area postrema in conscious sodium deprived rats. These studies demonstrate that in rats during sodium deprivation, endogenous Ang II chronically maintains MAP, LSNA, RSNA and HR through AT1 but not AT2 receptors. However, in contrast to the hypothesis, the studies suggest that the area postrema is not required for this action of Ang II. The studies also show that the sensitivity of baroreflex control of sympathetic nerve activity, at least LSNA, is not affected by Ang II, nor is it maintained by the area postrema.

Studies presented in Chapter 2 show that in LS rats, acute iv administration of losartan decreases MAP in two phases: fast and then slow. The fast reduction in MAP can be due to both the loss of action of Ang II as a direct vasoconstrictor and the withdrawal of Ang II action in the maintenance of sympathetic activity. The slow phase of MAP reduction is more likely due to the latter, because complete blockade of vascular Ang II receptors occurs within minutes and remains 24 hr after iv losartan injection (62). Losartan must have worked through a pathway with slow time course to cause this slow MAP reduction.

It is likely that the action sites of losartan are located somewhere in the sympathetic nervous system or somewhere that can influence sympathetic nervous

system outflow. The finding that LSNA decreases toward control level regardless a further slow fall in MAP (Chapter 2) demonstrates that losartan decreases sympathetic nerve activity, at least LSNA, slowly. It can be argued that the decrease of LSNA during MAP reduction is due to baroreceptor adaption. However, evidence shows that rapid or acute baroreceptor resetting is less than 40% and this resetting remains constant within a few minutes up to 6 hr after onset of hypertension (90,91,111-113). The acute baroreceptor resetting 15 min after hypotension is about 21% (134). Thus, it is unlikely that decrease of LSNA towards control levels in less than 40 min is completely due to the baroreceptor adaption. Considering that Ang II can not be acting at the baroreceptor to influence sympathetic outflow (165), the decrease of LSNA after losartan could be due to its action in the brain, the preganglionic neurons in spinal cord, the ganglionic neurons in the sympathetic chain, or in all of the three places. In the studies of this dissertation, no effort has been made to separate pre- from post- ganglionic sympathetic nerve activity since lumbar nerve branch contains both pre- and post-ganglionic nerves. In future studies, it would be interesting to examine if the ganglionic neurons are involved in the action of Ang II by comparing changes of sympathetic activity of a pre-ganglionic nerve and a post-ganglionic nerve after losartan.

RSNA and HR remain elevated at constant levels despite the further slow reduction in MAP (Chapter 2). This may be due to several possibilities. The baroreceptors may be adapted to the low MAP, the elevated RSNA and HR could

have reached the maximal levels, or losartan may slowly reach its action sites to decrease sympathetic outflow and counteract a possible increase in sympathetic outflow due to a further MAP reduction. However, the action of losartan on LSNA, as discussed in the previous paragraph, supports the idea that the action of losartan to directly decrease RSNA and HR counteracts, at least in part, a further increase in RSNA and HR that is caused by the slow MAP reduction through the baroreflex pathway.

The slow phase reduction of MAP after losartan in LS rats could be due to a slow withdrawal of neurogenic vascular tone. LSNA is a representative of the sympathetic drive to the hindquarter to affect the hindquarter blood vessel resistance. Decreased LSNA can reduce the hindquarter resistance, hence, the total peripheral resistance. MAP, as a product of cardiac output and total peripheral resistance, can be reduced by a decrease in the total peripheral resistance. This possibility was not examined in the studies of this dissertation. Thus, to test this possibility, further studies need to be done to simultaneously examine changes in LSNA, hindquarter resistance, total peripheral resistance, cardiac output and MAP after losartan.

The increase of both RSNA and HR after losartan in LS rats (Chapter 2) demonstrates that hypotension after iv Ang II blockade is powerful enough to increase sympathetic outflow through the baroreflex pathway to mask any possible reduction in sympathetic activity that is maintained by Ang II prior to the blockade. The findings further indicate that experiments studying sympathetic outflow should

be carefully designed to take into account of the baroreflex activation or inhibition of sympathetic outflow after changes in MAP.

LSNA changes differently compared with RSNA and HR after losartan: LSNA decreases toward control level after an initial increase, but RSNA and HR remains elevated at constant levels after losartan (chapter 2). The results suggest that sympathetic drive to different nerve branch is not necessary to be similarly regulated and can be affected differently by either Ang II blockade or MAP reduction. This information further supports the idea that plasma norepinephrine level is not a good index for study of sympathetic system outflow to regional areas. Although norepinephrine spill-over, another index of the sympathetic outflow, can be used in replace of plasma norepinephrine level to measure sympathetic drive to a specific organ or area, the accuracy of this method remains questionable. norepinephrine released to the blood stream could be different from that released from the nerve endings due to the diffusion distant from the nerve endings to the blood vessels. In addition, the release of norepinephrine from the nerve endings can be affected by many manipulators including Ang II. Thus, direct nerve recording, as the direct index of the sympathetic nervous system activity is better than both plasma norepinephrine level and norepinephrine spill-over in studying this system. This method in fact is the only way to study sympathetic drive to a specific sympathetic nerve branch.

After MAP was restored to pre-losartan basal levels in LS rats, LSNA, RSNA and HR are all suppressed to below pre-losartan basal levels (Chapter 2). The

results indicate that endogenous Ang II has a broad action in maintaining sympathetic drive to different organs and areas, including the hindquarters, the kidneys and the heart. In addition, losartan causes similar but smaller changes in MAP, RSNA and HR in CS rats compared with LS rats (Chapter 2), suggesting that even normal levels of endogenous Ang II maintain basal MAP in part by maintaining sympathetic outflow. The lack of changes in MAP, RSNA, LSNA and HR after losartan in HS rats (Chapter 2) further demonstrates that the action of losartan in LS and CS rats is specifically due to blockade of Ang II type 1 receptors.

In LS rats, acute iv injection of losartan suppresses LSNA when MAP is prevented from falling immediately after losartan administration (Chapter 3). The result further confirms the findings described in Chapter 2 that losartan maintains sympathetic outflow at basal MAP. In addition, losartan decreases maximal and minimal LSNA and shifts baroreflex control of LSNA to a lower MAP level without altering maximal gain. Thus, at each given level of MAP within baroreflex range, losartan decreases LSNA. Losartan also decreases maximal but not minimal HR and shifts baroreflex control of HR to a lower MAP level with decreased maximal gain. Thus, losartan decreases HR at most except high levels of MAP within the baroreflex range. Taken together, these results suggest that endogenous Ang II maintains sympathetic outflow over the entire baroreflex range of MAP in LS rats.

PD123319, the AT2 antagonist, has no effects on the variables examined in LS rats (Chapter 3). Considering the results obtained with losartan, this result indicates that endogenous Ang II chronically maintains LSNA and HR through AT1

but not AT2 receptors.

Either changes in sodium intake or blockade of Ang II with losartan does not alter the maximal gain of baroreflex control of LSNA (Chapter 3 and 4), suggesting that Ang II does not have action on the sensitivity of baroreflex control of sympathetic activity. Furthermore, studies showing a similar maximal gain of baroreflex control of LSNA between rats with and without the area postrema (Chapter 4) suggest that the area postrema is not involved in regulating sensitivity of baroreflex control of sympathetic activity, at least in rats during sodium deprivation. Thus, no difference in maximal gain of baroreflex control of LSNA has been observed between LS and HS rats (Chapter 3), before and after losartan in LS rats (Chapter 3 and 4), or between AP lesioned and sham operated LS rats (Chapter 4). The results indicate that neither Ang II nor the area postrema is involved functionally or anatomically, in the pathway that influences the sensitivity of baroreflex control of sympathetic activity.

One of the important findings in the study presented in Chapter 4 is that the area postrema is not required to maintain basal HR and MAP, nor is it required to maintain the set-point, or position, of baroreflex control of LSNA and HR in LS rats. No differences in basal MAP and HR have been observed between LS APx and SFR rats. In addition, the suppression of LSNA and HR by losartan at basal MAP and the shift of baroreflex control of LSNA or HR to a lower MAP level after losartan are also similar between APx and sham rats. The finding is important not only because it is opposite to what many researchers have speculated but also in that

it indicates that future studies concerning sites of central action of circulating Ang II in rats should be focused on other structures in the brain instead of the area postrema.

Another important finding in the study of Chapter 4 is that maximal LSNA in APx rats is lower than that in sham rats, and maximal LSNA can not be suppressed by losartan in APx rats as it is in sham rats. Losartan abolishes the difference in maximal LSNA between rats with and without the area postrema. The results indicate that the area postrema is necessary for endogenous Ang II to maintain maximal LSNA in LS rats.

It is not clear, from the data in this dissertation, whether the absolute sympathetic activity is altered by changes in sodium intake. The recorded nerve activity is subject to the number of nerve fibers from which nerve activity is recorded, and the contact condition between the nerve and electrode. Thus, nerve activity recording is not suitable for measurement of absolute nerve activity. However, many other studies, using measurement of plasma norepinephrine concentrations or norepinephrine spill-over as an indirect index of sympathetic activity, indicate that sympathetic outflow is increased during sodium deprivation (56,118,160). In studies of this dissertation, losartan decreases LSNA, RSNA and HR more in LS than in CS rats (Chapter 2). Taken together, it is suggested that circulating Ang II could increase sympathetic nerve activity to maintain normal levels of MAP during sodium deprivation.

In conclusion, studies in this dissertation provide direct evidence that

endogenous Ang II chronically maintains sympathetic activity over the entire baroreflex range of MAP in conscious, sodium deprived rats, by acting through AT1 but not AT2 receptors. This neurogenic action of Ang II may contribute to the maintenance of normal arterial pressure during sodium deprivation. The studies also suggest that the area postrema is not required for this neurogenic action of Ang II over most except low levels of MAP.

Perspective:

It remains unclear where circulating Ang II acts to maintain sympathetic system activity. However, at least two sites are unlikely. One is baroreceptors (165) and another is the area postrema (Chapter 4). The other possible sites for the neurogenic action of Ang II could be other CVOs, preganglionic neurons in the spinal cord or ganglionic neurons. Further studies need to be done to access the role of these possible sites.

It is also possible that Ang II acts at sites within the blood brain barrier. Although it is very unlikely for circulating Ang II to cross the blood brain barrier due to the large molecular weight and negative charges of this peptide, it does not mean that Ang II can not get to sites behind the blood brain barrier. In addition, possible changes in the local renin-angiotensin system as well as the number of Ang II receptors in the brain due to changes in sodium intake may also affect the sympathetic outflow regulation.

Since plasma concentration of vasopressin was not monitored in studies of

this dissertation, it is not clear if this hormone contributes to changes of nerve activity and HR after losartan as well as after MAP restoration with methoxamine. To avoid any possible effect of changes in vasopressin in sympathetic activity, similar studies could be repeated in Brattleboro rats, a strain that lacks the ability to produce vasopressin.

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