Acute Sympathoexcitatory Action of Angiotensin II

In Conscious Baroreceptor Denervated Rats

Ling Xu and Alan F. Sved

Department of Neuroscience,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15260

Running Head: Angiotensin-evoked sympathoexcitation

address for editorial correspondence:
Dr. Alan F. Sved
Department of Neuroscience
University of Pittsburgh
446 Crawford Hall
Pittsburgh, PA  15260

Phone  412-624-6996
Fax      412-624-9198
e-mail  sved@pitt.edu

Copyright 2002 by the American Physiological Society.
Abstract

Angiotensin II (ANGII) has complex actions on the cardiovascular system. ANGII may act to increase sympathetic vasomotor outflow, but acutely the sympathoexcitatory actions of exogenous ANGII may be opposed by ANGII-induced increases in arterial pressure evoking baroreceptor-mediated decreases in sympathetic nerve activity. To examine this hypothesis, the effect of ANGII infusion on lumbar sympathetic nerve activity was measured in unanaesthetized chronic sino-aortic denervated rats. Chronic sino-aortic denervated rats had no reflex heart rate responses to pharmacologically evoked increases or decreases in arterial pressure. Similarly, in these denervated rats, nitroprusside-induced hypotension had no effect on lumbar sympathetic nerve activity; however, phenylephrine-induced increases in arterial pressure were still associated with transient decreases in sympathetic nerve activity. In control rats, intravenous infusion of 100 ng/kg/min ANGII increased arterial pressure and decreased heart rate and sympathetic nerve activity. In contrast, ANGII infusion caused an increase in both lumbar sympathetic nerve activity and heart rate in sino-aortic denervated rats. In rats that underwent sino-aortic denervation surgery but still had residual baroreceptor reflex-evoked changes in heart rate, the effect of ANGII on heart rate and sympathetic nerve activity were variable and correlated to the extent of baroreceptor reflex impairment. The present data suggest that pressor concentrations of ANGII in rats act rapidly to increase lumbar sympathetic nerve activity and heart rate, though baroreceptor reflexes normally mask these effects of ANGII. Furthermore, these studies highlight the importance of fully characterizing sino-aortic denervated rats used in experiments examining the role of baroreceptor reflexes.

Key words: sympathetic nervous system, arterial blood pressure, hypertension, renin
Introduction

Angiotensin II (ANGII) has complex actions on the cardiovascular system. Acutely, increases in circulating levels of ANGII produced by infusion of exogenous ANGII increase arterial pressure (AP) by acting directly to constrict vascular smooth muscle. However, accumulating evidence indicates that a chronically elevated circulating level of ANGII increases AP via an increase in sympathetic vasomotor tone (5, 6, 10, 39). For example, ganglionic blockade produces a greater depressor response during long-term infusion of ANGII compared to that observed during acute ANGII infusions in control animals (8, 23, 26, 52). Furthermore, doses of ANGII that do not increase AP during acute infusion (i.e., subpressor doses) do result in a delayed increase in AP that can be totally reversed with sympatholytic drugs (14, 36). Indeed, increases in sympathetic nerve activity (SNA) have been recorded in animals infused chronically with ANGII (29). These studies collectively suggest that chronically elevated levels of ANGII in the circulation may stimulate sympathetic outflow (5, 6).

Although ANGII at acutely subpressor doses seems to elicit a delayed sympathoexcitation as reflected by a delayed, apparently neurogenically-mediated, increase in AP (14, 36), the time course of the sympathoexcitatory action of pressor doses of ANGII is complicated by the direct vascular actions of ANGII. Specifically, ANGII evoked increases in AP would be expected to cause a baroreceptor-evoked decrease in SNS activity (39), thereby masking a sympathoexcitatory action of ANGII. Competing influences of ANGII-induced excitation opposing an indirect inhibitory effect of ANGII-induced increased AP have been carefully documented in the case of ANGII-evoked thirst (9, 45, 46).
Therefore, based on the available data, it appears that increases in circulating ANGII levels have two competing influences on the sympathetic outflow. ANGII acts to increase SNA, though the time-course of this action is unclear. On the other hand, ANGII acts indirectly, through increases in AP caused by its vasoconstrictor action, to stimulate baroreceptors and thereby inhibit sympathetic activity. Because infusion of pressor doses of ANGII increase AP and baroreceptor-evoked sympathoinhibition is quite powerful, sympathoinhibition evoked by ANGII predominates with acute administration of ANGII. However, with chronically elevated AP, baroreceptors reset to the higher pressure (39) and therefore provide less inhibition of sympathetic activity; under these conditions, the sympathoexcitatory influences of ANGII may instead predominate. Note, however, that Lohmeier et al. (28) have recently established that the effects of chronic ANGII infusion on renal sodium excretion in dogs are consistent with renal sympathoinhibition mediated via cardiopulmonary and arterial receptors that do not reset during ANGII-evoked hypertension. Alternatively, the mechanisms responsible for ANGII-induced sympathoexcitation may not operate acutely, and develop only in the chronic presence of ANGII. It has also been suggested that ANGII itself, independent of any change in AP, acts to reset the baroreceptor reflex (39), and such an action of ANGII would further complicate this issue. Therefore, in an animal with intact baroreceptor reflexes it would be difficult to distinguish between a resetting of the baroreceptor reflex and a shift in sympathetic vasomotor tone upon which the baroreceptor reflex acts. In an effort to clarify the direct actions of ANGII on sympathetic outflow, we determined the effects of a pressor dose of ANGII infused intravenously on lumbar sympathetic nerve activity (LSNA) in sino-aortic denervated rats. To avoid the possible confounds of anesthesia, these experiments were conducted in unanesthetized, unrestrained rats. Furthermore,
because we have previously highlighted the importance of completeness of baroreceptor
denervation for studies on the role of baroreceptor denervation on cardiovascular
regulation (44), we also considered extent of baroreceptor denervation as a variable.
Methods

Adult male Sprague-Dawley rats (Zivic Laboratories, Zelienople PA), initially weighing 225-300g, were used in these experiments. Rats were housed individually in wire-mesh hanging cages in a temperature-controlled colony room (22-24 °C, lights on from 7:00 a.m. to 7:00 p.m.). Rats had ad libitum access to food (Purina #5001 Rat Chow) and tap water for at least 7 days prior to use in experiments.

Sino-aortic denervation

Rats were subjected either to surgical sino-aortic denervation or sham surgery while anesthetized with halothane (2% in 100% O2 via a cone placed over the nose). Surgical sino-aortic denervation was preformed as described previously (43). Briefly, a 2-3 cm midline incision was made in the ventral neck, and after retracting the sternocleidomastoid muscle the carotid bifurcation was exposed on one side. With the aid of a dissecting microscope, the superior laryngeal nerve was identified and cut at its junction with the nodose ganglion. The superior cervical ganglion was then removed. Neural and connective tissue was stripped from the region of the carotid bifurcation and carotid sinus and this area was wiped with a solution of 10% phenol in ethanol. The denervation procedure was then performed on the opposite side. The neck wound was then close and the halothane terminated. Rats were injected with an antibiotic (Penicillin G, 30,000 units, im) and the ganglionic blocking drug chlorisondamine (5 mg/kg sc) to block the initial cardiovascular effects of sino-aortic denervation, as well as bronchiole constriction and secretion. Because rats that have undergone sino-aortic denervation surgery often do not drink for a few days, they were injected s.c. with 10 ml saline each day until daily spontaneous water intake exceeded 20 ml; this always occurred within 5
days. Control rats were subjected to sham denervation. In these rats the carotid bifurcation was exposed bilaterally, but no nerves were cut; animals were kept anesthetized for a period of time similar to that required for sino-aortic denervation. Rats were allowed 2-4 weeks for recovery prior to use in experiments.

Experimental Protocol

On the morning of the experiment, the rat was anesthetized with methohexital sodium (50 mg/kg ip; Brevital, Eli Lilly, Indianapolis IN). A catheter (PE-50 tubing) was inserted into the left femoral vein, and anesthesia was maintained by iv infusion of methohexital sodium (4-6 ul/min of a 10 mg/ml solution). A silastic-tipped catheter was implanted into the descending aorta via the femoral artery, for measurement of arterial pressure and heart rate.

A recording electrode was then placed on a lumbar nerve bundle, as described previously (51). Briefly, a 5-7 cm midline abdominal incision was made and the intestines were retracted. The lumbar nerve bundle was exposed and gently dissected from surrounding tissue. The nerve was then placed on a bipolar stainless steel wire electrode and the electrode was anchored in place using polyvinylsiloxane dental impression material (President Light Body, Coltene). A ground wire was placed subcutaneously. The electrode wires and catheters were tunneled subcutaneously to exit between the scapulae. All incisions were closed with 3-0 silk and the methohexital infusion was terminated. The rat was then placed in a test cage (10.5 in od Plexiglas cylinder with bedding) to recover. Rats regained consciousness within 15 minutes, and began to move around the cage.
Experiments were initiated 2-3 hours after completion of the surgery for electrode implantation, at which time the rats appeared undisturbed and resting quietly. Arterial pressure was monitored via the arterial catheter connected to a Statham pressure transducer and a Grass 7P preamplifier (Grass Instruments, Quincy MA). Heart rate (HR) was measured using a Grass 7P44 tachograph triggered by the arterial pulse wave. Lumbar sympathetic nerve activity (LSNA) was amplified (10,000-20,000X, BMA 831, CWE Inc., Ardmore PA), filtered (50-10,000 Hz), rectified, and integrated with a 1 sec reset time (Grass 7P10). Mean AP (MAP), HR, and integrated LSNA (iLSNA) were continuously recorded on chart paper using a Grass polygraph. In addition, these parameters were also sampled at 1000 Hz and recorded using a PC-based data acquisition system (LabView, National Instruments) for subsequent analysis. During baroreceptor reflex testing (see below) and at specified times during ANGII infusion, raw nerve activity was sampled at 10,000 Hz and recorded on the computer.

Baroreceptor reflexes were tested in all rats by measuring the HR responses to iv injection of nitroprusside (5 ug/kg) and phenylephrine (5 ug/kg). Peak change in HR divided by peak change in MAP evoked by these two drugs was used as an index of baroreceptor sensitivity. Rats lacking reflex changes in HR to both nitroprusside and phenylephrine were classified as complete sino-aortic denervated (SAD), whereas rats that had undergone the denervation procedure but had residual reflex changes in HR were considered to be partially sino-aortic denervated (pSAD) (44).

After completing the baroreceptor reflex assessment, rats were left to stabilize for 30 min before proceeding with the experiment. Then baseline values for MAP, HR, and iLSNA were recorded for 30 min. An intravenous infusion of ANGII (100 ng/kg/min in 10 ul/min; ANGII from Sigma Chem. Co. St. Louis MO) was then initiated and
maintained for 120 min. MAP, HR, and iLSNA were recording continuously during this period, as well as for 10-60 min after termination of the ANGII infusion.

At the end of the experiment, rats were given a lethal dose of methohexital (150 mg/kg iv). The noise level of the nerve recording was determined at least 30 min after death. This background noise level was subtracted from total nerve activity that was recorded during the experiment.

A total of 5 complete SAD rats, 10 pSAD rats, and 5 control rats yielded data for analysis. Baroreceptor reflex responses were evaluated using maximal change in HR and LSNA (integrated over 2 sec) in response to the maximal change in MAP evoked by either nitroprusside or phenylephrine. In addition, because sino-aortic denervation never eliminated phenylephrine-evoked sympathoinhibition, this response was further examined by integrating 4 second bins of activity taken at 20 second intervals during the phenylephrine-evoked increase in MAP. Baseline MAP, HR, and iLSNA were based on the average of 3 readings taken at 10 min intervals during the 30 min baseline period. For iLSNA the value at each point was the average of 120 sec of recording. Lability of MAP was measured as the standard deviation of MAP determined by 200 points taken at 6 sec intervals during the final 20 minutes of the baseline period and 90-110 minutes of ANGII infusion.

Values are presented as mean ± SEM. Baseline and baroreceptor reflex data were analyzed using one-way ANOVA, with post-hoc Tukey’s tests for between group comparisons. The effects of ANGII infusion were analyzed using a two-way ANOVA with repeated measures (group X time) with post-hoc comparisons performed using the Tukey HSD test. A significance level of p<0.05 was used for all analyses. Linear regression was used to evaluate the relationship between the extent of baroreceptor
responsiveness and other measured parameters. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago)
Results

Baseline Parameters

Prior to infusion of ANGII, baroreceptor reflex responses were assessed in all rats, so that rats subjected to sino-aortic denervation surgery could be classified based on the extent of baroreceptor denervation. Of the 15 rats that underwent sino-aortic denervation surgery, 5 were found to have no reflex-evoked changes in HR in response to both nitroprusside and phenylephrine (Table 1). In contrast, the 10 other rats displayed changes in HR in response to nitroprusside and phenylephrine, though these responses were considerably blunted compared to control rats (Table 1). In these denervated rats with residual reflexes, the baroreceptor reflex evoked change in HR assessed with nitroprusside was highly correlated to that assessed with phenylephrine (R=0.73; P<0.05).

Once rats that had undergone sino-aortic denervation surgery were classified as either complete SAD or pSAD on the criterion of HR responses to phenylephrine and nitroprusside, baseline parameters were compared between these two groups and control rats. Baseline MAP was higher in SAD rats than in control rats (Table 2); MAP in pSAD rats was not significantly different from either SAD or control rats. Baseline HR was similar across all three groups (Table 2); iLSNA also did not differ significantly among groups. Lability of MAP was greater in SAD rats compared to control rats, and was intermediate in pSAD rats (Table 2). Furthermore, the magnitude of the lability of MAP was significantly correlated with the sensitivity of baroreceptor reflex changes in HR (P<0.05 for both phenylephrine and nitroprusside responses).

Nitroprusside injections decreased MAP in all rats, though the change in MAP was greater in SAD rats than in control rats (Table 1). In pSAD rats the nitroprusside-evoked fall in MAP was considerably greater than in control rats, though not quite as
large as observed in complete SAD rats (Table 1). Phenylephrine injections increased MAP in all rats, and, again, the response was significantly larger in SAD rats compared to control rats (Table 1). The phenylephrine-evoked increase in MAP was similar in SAD and pSAD rats.

As expected, nitroprusside injections were associated with marked increases in HR and iLSNA in control rats (Table 1). Complete SAD rats were defined as having no change in HR in response to nitroprusside, and these rats also had no increase in LSNA (Table 1). pSAD rats, had variable degrees of residual tachycardia and increased LSNA in response to nitroprusside, and these two parameters were highly correlated (R=0.75, p<0.01). Phenylephrine injections elicited reflex decreases in HR and LSNA in control rats (Table 1). However, the maximal phenylephrine-evoked inhibition of iLSNA was similar in all three groups of rats (Table 1). Nonetheless, the time course of phenylephrine-evoked inhibition of iLSNA was markedly blunted in SAD rats compared to control rats (Figure 1); the duration of the response was intermediate in pSAD rats.

**Effects of ANGII infusion**

In control rats intravenous infusion of 100 ng/kg/min ANGII rapidly and markedly increased MAP (Figures 2 and 3). The ANGII-induced increase in MAP was accompanied by bradycardia and sympathoinhibition (Figures 2 and 3). This initial decrease in HR and LSNA was comparable to that observed with phenylephrine-evoked increases in MAP (p>0.1, comparing ΔHR or ΔiLSNA/ΔMAP between treatments). Although the ANGII-induced increase in MAP remained stable for the entire 120 min infusion period, HR and LSNA slowly returned toward control levels, and by the end of the infusion period HR and LSNA were not significantly different from baseline values.
The pattern of responses was markedly different in SAD rats. In SAD rats, ANGII infusion caused an initial pressor response that was significantly larger than observed in control rats (Figure 2), though by 20 minutes into the infusion the values were more similar. However, the increase in MAP in SAD rats was accompanied by increases in HR and LSNA, rather than the decreases that were observed in control rats. In response to ANGII infusion in SAD rats, HR and LSNA increased within 10 min, and these increases were never preceded with decreases. LSNA remained elevated compared to baseline and to control rats throughout the entire 120 min infusion period (Figures 2 and 3). In contrast, HR gradually returned to baseline values during this time (Figures 2 and 3).

In pSAD rats, ANGII infusion increased MAP similar to what was observed in control rats (Figure 4). The initial HR response to ANGII in pSAD rats was quite variable, with HR decreasing in 4 rats and increasing in the other 6 (9±7 at 10 min; n=10; p>0.1). However, there was a tendency for this initial HR response to be correlated to the baroreceptor reflex index assessed by the response to nitroprusside (R=0.55; P<0.1). As a group, HR increased significantly in pSAD rats from 30-90 min of the infusion period, but was not different from baseline at 2 hr (Figure 4). In contrast to either control rats or SAD rats, the response of LSNA to ANGII infusion in pSAD was initially quite variable. As a group, the LSNA response to the ANGII infusion in pSAD was a gradual increase during the infusion period (Figure 4). By 60 min into the infusion period, LSNA had increased above baseline, to a similar degree as observed in complete SAD rats (Figure 4). In pSAD rats the ANGII-evoked increase in HR and iLSNA at 10 min into the ANGII infusion were highly correlated (R=0.73; p<0.05).
In control rats and pSAD rats, ANGII infusion did not significantly alter lability of MAP. In contrast, ANGII infusion significantly reduced the lability of MAP in SAD rats (6.72±0.61 to 5.08±0.67, n=5, p<0.05).

**Discussion**

The key observation of the present study is that acute infusion of a pressor dose of ANGII is associated with a rapid increase in LSNA in SAD rats. Previous studies have suggested that the sympathoexcitatory actions of ANGII may develop only slowly in rats, but these studies have been confounded by baroreceptor-evoked decreases in SNA. In addition, differences in the effects of ANGII in complete SAD rats and pSAD rats (i.e., rats subjected to sino-aortic baroreceptor denervation surgery but still displaying residual reflex responses) highlight the importance of carefully documenting the extent of baroreceptor denervation.

*Sino-aortic denervation and baroreceptor responses*

Schreihofer and Sved (44) have previously argued that the regulation of AP in complete SAD rats (i.e., rats with no detectable changes in HR in response to either evoked increases or decreases in AP) is qualitatively different from that in pSAD rats. That chronic SAD rats with essentially no baroreceptor reflex mediated changes in HR can be produced has been carefully documented (44). To our knowledge, the present study is the first to examine the effects of phenylephrine and nitroprusside on SNA in unanesthetized chronic SAD rats that are classified as completely sino-aortic denervated based on the absence of changes in HR in response to pharmacologically-evoked increases and decreases in AP, though there have been previous studies showing renal
SNA responses in SAD rats with residual reflexes (i.e., pSAD rats) (2, 20). Interestingly, in complete SAD rats nitroprusside-evoked decreases in AP were not associated with any change in LSNA. Furthermore, rats that underwent sino-aortic denervation surgery but still had residual nitroprusside-evoked changes in HR also had residual LSNA responses, and the magnitude of these two responses was significantly correlated.

In contrast to the lack of reflex effects evoked by nitroprusside in SAD rats, in rats that were classified as completely sino-aortic denervated injection of phenylephrine still evoked a decrease in SNA. Though this phenylephrine evoked sympathoinhibition in SAD rats was as large and rapid in onset as that observed in baroreceptor-intact rats, the duration of the response was considerably shorter. The mechanism underlying this residual phenylephrine-evoked sympathoinhibition in rats that show no phenylephrine-evoked bradycardia and no nitroprusside-evoked change in either HR or LSNA is unclear at present. However, Minisi et al. (37) demonstrated that phenylephrine increases pulmonary arterial pressure, and could therefore decrease renal SNA by stimulating cardiopulmonary baroreceptor afferents; they noted that phenylephrine evoked suppression of renal SNA in SAD dogs was eliminated by vagotony. Although this would appear to be the likely explanation for the present results, an effect mediated by baroreceptors in coronary arteries (50) or unrelated to its peripheral vasoconstrictor action (19) is also possible. This residual response may also reflect a small degree of residual aortic or carotid baroreceptor reflex function that is undetected by the other reflex tests. Whatever the explanation for the residual transient phenylephrine-evoked sympathoinhibition in SAD rats, the present data support the argument that rats subjected to sino-aortic denervation must be carefully evaluated for the extent of residual reflexes, and rats with no residual changes in HR to both increases and decreases in AP should be
considered separately from those rats that have even minimal residual reflex responses (44).

Baseline MAP of SAD rats in the present study was significantly higher than MAP in control rats. Based on reports in the literature, MAP in chronic SAD rats is either normal or slightly elevated (1, 3, 7, 12, 38, 49), though in previous studies in this laboratory MAP in chronic SAD rats has not been significantly different from MAP in control rats (41, 44). The slightly higher baseline MAP values in SAD rats in the present study might be a result of the experimental protocol, which involved surgical manipulation of the rat a few hours prior to study. Increased lability of MAP, a characteristic of SAD animals (1, 47), was also noted in these rats. Despite the higher baseline MAP in SAD rats, baseline LSNA was not significantly different between SAD and control rats. This is consistent with previous reports, in which renal SNA in SAD rats (though they were not completely denervated by the current criteria) was similar to that in control rats (2, 20). Nonetheless, in the present study, as a result of variability in baseline LSNA among animals the difference between SAD and control rats would have needed to be rather large (in excess of 75% with the current group sizes) in order to have been statistically significant.

Effects of ANGII on cardiovascular regulation and sympathetic outflow

The acute cardiovascular actions of ANGII have been well studied in experimental animals. ANGII, infused in doses exceeding approximately 20 ng/kg/min in conscious rats, elicits a rapid increase in AP that is accompanied by baroreceptor reflex-mediated bradycardia. Previous studies in conscious rabbits have shown that ANGII does not influence baroreceptor-mediated inhibition of renal SNA (25, 34). However, other
studies suggest that ANGII may shift the baroreceptor reflex curve or increase the activity of other sympathetic nerves (24, 32, 48). For example, ANGII-evoked increases in AP decrease muscle SNA in human subjects to a lesser extent than do increases in AP evoked by phenylephrine (32). Furthermore, when the pressor effect of ANGII was counteracted by co-infusion of nitroprusside, ANGII elicited an increase in muscle SNA (32, 33). Acute ANGII-evoked reflex bradycardia is less than that caused by other pressor substances (39), suggesting that ANGII has additional effects on the control of the heart (as discussed in more detail below).

In contrast to what was observed in baroreceptor intact rats, intravenous infusion of ANGII in complete SAD rats was accompanied by an increase in HR and LSNA. The increase in LSNA occurred rapidly, being elevated by at least 20% within 10 min in 4 of the 5 SAD rats studied. In the other rat, LSNA did not increase to the same degree, though as a group the increase was 23±7% (p<0.05 compared to baseline). This is in marked contrast to the decrease in LSNA of at least 15% observed in each of the 5 control rats (-40±7%). The rapid increase in LSNA evoked by intravenous infusion of ANGII in unanesthetized SAD rats is a novel observation, and suggests that the sympathoexcitatory actions of ANGII in rats can be quite rapid. Guo et al. (15) reported similar data in anesthetized rabbits, with LSNA increasing by an average of 28% with a dose of 100 ng/kg/min. The observation that ANGII-evoked increases in regional vascular resistance were potentiated by sino-aortic denervation to a much greater extent in the hindlimb than in either the renal or mesenteric circulations (3) is also consistent with this action of ANGII on LSNA, and further suggests that this effect of ANGII may be specific for certain sympathetic nerves. Interestingly, in pSAD rats infusion of ANGII still increased LSNA, though not quite as rapidly as in SAD rats. This observation suggests that with
increasing impairment of baroreceptor reflex function there is a reduction in the time it takes for exogenous ANGII to increase sympathetic outflow.

Additional evidence of rapid ANGII-evoked sympathoexcitation can be found in other studies in which ANGII increased muscle SNA in human subjects when the pressor effects of ANGII were prevented by co-infusion of nitroprusside (32, 33). Similarly, Kooner et al. (24) noted that ANGII-evoked rapid decreases in rabbit ear blood flow were eliminated by either clonidine or ganglionic blockade, suggesting that ANGII increased sympathetic outflow to cutaneous ear blood vessels. Because cutaneous sympathetic vasoconstrictor nerves are not influenced by baroreceptor input (21), these data are consistent with the notion that ANGII causes sympathoexcitation in the absence of baroreceptor input.

Previous studies in rat, as well as other species, have emphasized the point that the sympathoexcitatory actions of ANGII take a long time to develop (5). However, that conclusion has been based on studies in animals infused chronically with low doses of ANGII that are not acutely pressor (10) or infused with pressor doses of ANGII with baroreceptors intact (8, 23, 26, 52). In those studies, the doses were likely either too small to elicit rapid actions on the sympathetic nervous system or the sympathoexcitatory actions of ANGII were likely obscured by baroreceptor-mediated sympathoinhibition, and became apparent only as baroreceptors reset. Luft et al. (29) measured increased splanchnic SNA in rats receiving a chronic infusion of ANGII, though that study did not examine the acute effects of ANGII on SNA. Lohmeier et al. (28) have demonstrated that renal handling of sodium in response to a pressor dose of ANGII in split bladder dogs with unilateral renal denervation is consistent with renal sympathoinhibition that is maintained for at least 5 days of ANGII infusion. Furthermore, they have shown that in
dogs with combined sino-aortic and cardiopulmonary denervation, ANGII apparently causes renal sympathoexcitation that takes a few days to develop (28).

Infusion of ANGII in complete SAD rats increased HR in addition to LSNA; increased HR in response to ANGII infusion in SAD rats was also noted in another recent study (46). A similar ANGII induced tachycardia has been previously noted in completely baroreceptor denervated rats produced by destruction of the medial nucleus tractus solitarius (42), the brain stem site of termination of baroreceptor afferent nerves, as well as in baroreceptor denervated dogs (13, 18, 28). As with ANGII-induced increases in LSNA, the tachycardia occurs rapidly in response to ANGII infusion, and the rapid onset of tachycardia does not occur in pSAD rats.

Methodological Issues

The present studies compared the effects of intravenous infusion of 100 ng/kg/min of ANGII between control, SAD, and pSAD rats. This protocol relies on the assumption that an infusion rate of 100 ng/kg/min produces circulating levels of ANGII that are physiologically (or at least pathophysiologically) relevant, and that it causes similar increases in circulating ANGII levels in each group. Previous studies, including a recent report from this laboratory, have indicated that infusion of ANGII at this rate into control rats results in plasma ANGII levels in the range of 500 pg/ml; such levels are similar to levels that occur during severe hypotension (22, 31, 46). Furthermore, infusion of ANGII in chronic SAD rats and control rats results in equivalent increases in plasma ANGII levels (46).

Another methodological issue relates to the animal preparation. The present studies were conducted on conscious instrumented rats that had undergone surgery several hours prior to study. SNA may be elevated under these conditions, and this may
possibly have influenced the results. This preparation was chosen to avoid the confounding factors of anesthesia. The limited success rate of recording LSNA in chronically instrumented rats prompted us to conduct these studies in a more acute preparation. Despite the potential impact of surgery several hours prior to study on sympathetic function, MAP and HR of rats used in this study were rather similar to those observed in more chronically instrumented animals, suggesting that sympathetic function was relatively normal.

**Mechanism of ANGII evoked increases in LSNA and HR**

There are several sites at which ANGII could conceivably act to rapidly increase LNSA and HR. ANGII has been reported to act directly at the heart to increase HR (27), though this seems to require higher concentrations of ANGII in the heart than were likely attained in the present study. Alternatively, ANGII might increase HR by either increasing sympathetic neural activity to the heart and/or decreasing parasympathetic neural activity to the heart (39). Because the ANGII-induced increase in HR was accompanied by an increase in LSNA and the magnitude of these two responses was highly correlated in animals subjected to sino-aortic denervation surgery, it seems likely that these two responses reflect a single mechanism. Since LSNA was recorded from postganglionic fibers and it is known that ANGII can depolarize postganglionic neurons (16, 39), ANGII might act at the level of sympathetic ganglia. However, the concentration of ANGII needed to depolarize sympathetic postganglionic neurons likely exceeds the concentrations that existed in the present experiment. For example, in a recent study by Ma et al. (30), iv injection of 160 ng/kg ANGII was observed to increase RSNA in anesthetized mice by acting directly on the postganglionic neuron. The plasma
levels of ANGII in those animals were likely more than 10-fold greater than produced in the present study. Interestingly, this response observed by Ma et al. (30) appeared to result from an increase in low amplitude electrical activity. In contrast, the increase in LSNA observed in the present study with much smaller doses of ANGII infused in conscious rats appeared to result from an increase in the frequency of large amplitude spikes (e.g., see Figure 3). Thus, ANGII may act to increase sympathetic outflow from the CNS (39). For example, ANGII might act on sensory receptors to evoke an increase in SNA, as ANGII has been reported to act on cardiac receptors to produce such an effect (4). Alternatively, ANGII might act on regions of the CNS that lack a blood-brain barrier such as the area postrema or subfornical organ (6, 39). Of particular relevance to the present study, is the report that destruction of the area postrema eliminates the delayed increase in AP caused by infusion of small doses of ANGII (11). Furthermore, because ANGII infusions markedly increase AP and therefore might disrupt the blood brain barrier (35), circulating ANGII might gain access to regions of the central nervous system where it could act to increase SNA (10).

It must also be noted that these experiments were conducted in chronic SAD rats and that some compensations may occur in response to surgical sino-aortic denervation that might change how the animal responds to ANGII. For example, the number of angiotensin binding sites in the nucleus tractus solitarius is reduced following sino-aortic denervation (17, 40). However, Barron et al. (3) noted that the marked potentiation of ANGII-evoked pressor responses that is present in chronic SAD rats occurs acutely.
Summary

In summary, the present studies show that in chronic SAD rats, intravenous infusion of ANGII results in a rapid increase in LSNA and HR. These data indicate that ANGII can produce a rapid sympathoexcitation, at least under certain conditions.
Acknowledgments

These studies were supported by a grant from the U.S. National Institutes of Health (HL-55687).
References


Figure Legends

Figure 1. Time course of the effect of phenylephrine on MAP and LSNA in control and SAD rats. The effect of an intravenous bolus injection of phenylephrine (5 ug/kg) on MAP and LSNA was measured in control rats (open bars; n=5), SAD rats (closed bars; n=5), and pSAD rats (gray bars; n=7, data from 3 pSAD rats were not included because of movement artifact during some portion of the test period). Data are expressed as change from baseline at 0-4 sec, 20-24 sec, and 40-44 sec post injection. Although the initial change in LSNA was similar in the three groups (see also Table 1), LSNA continued to be decreased in control rats whereas LSNA was not significantly different from baseline levels by 20 sec in SAD rats and by 40 sec in pSAD rats. * indicates significant difference from control group, p<0.05.

Figure 2. Effect of ANGII infusion on MAP, HR, and LSNA in control and SAD rats. MAP, HR, and LSNA (mean ±SE) for sham control rats (n=5) and complete SAD rats (n=5) at 10-min intervals prior to infusion of ANGII are shown in the panels on the left. The average of these pre-infusion values are taken as the basal value, represented by the open symbols in the panels on the right. MAP, HR, and LSNA are then shown at regular intervals after the initiation of an intravenous infusion of ANGII (100 ng/kg/min). * indicates significance difference from basal value, p<0.05. # indicates significant difference from the sham control group, p<0.05.

Figure 3. Records of the effects of ANGII on MAP, HR, and LSNA in representative Sham and SAD rats. Records are taken just prior to infusion of ANGII (A and C) and at
the end of 120 min of ANGII infusion (B and D) in a Sham rat and an SAD rat. These recordings are representative of the group data presented in Figure 2.

Figure 4. Effect of ANGII infusion on MAP, HR, and LSNA in control and pSAD rats. MAP, HR, and LSNA (mean ±SE) for sham control rats (n=5) and pSAD rats (n=10) at 10-min intervals prior to infusion of ANGII are shown in the panels on the left; the data for the sham rats are the same data included in Figure 2, and are re-plotted here to facilitate comparison. The average of these pre-infusion values are taken as the basal value, represented by the open symbols in the panels on the right. MAP, HR, and LSNA are then shown at regular intervals after the initiation of an intravenous infusion of ANGII (100 ng/kg/min). * indicates significance difference from basal value, p<0.05. # indicates significant difference from the sham control group, p<0.05.
Table 1. Baroreceptor reflex responses in intact control rats (Sham), complete sino-aortic denervated rats (SAD), and partial sino-aortic denervated rats (pSAD).

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=5)</th>
<th>SAD (n=5)</th>
<th>pSAD (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenylephrine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔMAP (mm Hg)</td>
<td>46±4</td>
<td>61±3*</td>
<td>60±2*</td>
</tr>
<tr>
<td>ΔMAP range</td>
<td>37 – 60</td>
<td>50 – 65</td>
<td>49 – 70</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>-109±23</td>
<td>0</td>
<td>-38±6</td>
</tr>
<tr>
<td>ΔHR range</td>
<td>-60 – -175</td>
<td>0</td>
<td>-20 – 70</td>
</tr>
<tr>
<td>ΔHR/ΔMAP</td>
<td>-2.5±0.6</td>
<td>0</td>
<td>-0.6±0.01*</td>
</tr>
<tr>
<td>ΔLSNA (%)</td>
<td>-41±5</td>
<td>-34±5</td>
<td>-34±7</td>
</tr>
<tr>
<td>ΔLSNA range</td>
<td>-29 – -55</td>
<td>-21 – -46</td>
<td>0 – -71</td>
</tr>
<tr>
<td>ΔLSNA/ΔMAP</td>
<td>-0.9±0.1</td>
<td>-0.6±0.1</td>
<td>-0.6±0.1</td>
</tr>
<tr>
<td><strong>Nitroprusside</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔMAP (mm Hg)</td>
<td>-15±2</td>
<td>-67±3*</td>
<td>-52±4*#</td>
</tr>
<tr>
<td>ΔMAP range</td>
<td>-10 – -20</td>
<td>-62 – -80</td>
<td>-35 – -80</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>72±6</td>
<td>0</td>
<td>21±6*</td>
</tr>
<tr>
<td>ΔHR range</td>
<td>60 – 90</td>
<td>0</td>
<td>0 – 50</td>
</tr>
<tr>
<td>ΔHR/ΔMAP</td>
<td>-4.8±0.4</td>
<td>0</td>
<td>-0.5±0.1*</td>
</tr>
</tbody>
</table>
ΔLSNA (%)  
67±7  0  19±6

ΔLSNA range  
43 – 83  0  0 – 45

ΔLSNA/ΔMAP  
-4.7±1.0  0  -0.4±0.1*

Values are means ± SE from Sham, SAD, and pSAD rats. Values represent the maximal changes in MAP, HR, and LSNA evoked by injection of either phenylephrine (5 ug/kg iv) or nitroprusside (5 ug/kg iv). * indicates significant difference from the Sham group, p<0.05. # indicates significant difference between the SAD and pSAD groups, p<0.05. Values of 0 in the SAD group are different from both Sham and pSAD groups. Baseline values of MAP, HR, and LSNA are presented in Table 2.
<table>
<thead>
<tr>
<th></th>
<th>Sham (n=5)</th>
<th>SAD (n=5)</th>
<th>pSAD (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP (mm Hg)</strong></td>
<td>114±3</td>
<td>128±5*</td>
<td>117±2</td>
</tr>
<tr>
<td><strong>MAP lability (SD)</strong></td>
<td>1.71±0.15</td>
<td>6.72±0.61*</td>
<td>4.43±0.44*#</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>373±16</td>
<td>395±14</td>
<td>380±13</td>
</tr>
<tr>
<td><strong>LSNA (uV * s)</strong></td>
<td>54±12</td>
<td>92±20</td>
<td>54±5</td>
</tr>
</tbody>
</table>

Values are means ± SE from Sham, SAD, and pSAD rats recorded prior to infusion of ANGI II. * indicates significant difference from the Sham group, p<0.05. # indicates significant difference between the SAD and pSAD groups, p<0.05.
LSNA (µV)  HR (bpm)  AP (mm Hg)

A: Basal

SAD Rat

C: Basal

LSNA (µV)  HR (bpm)  AP (mm Hg)

D: Ang II

B: Ang II