Acute sympathoexcitatory action of angiotensin II in conscious baroreceptor-denervated rats

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Angiotensin II (ANG II) has complex actions on the cardiovascular system. Acutely, increasing in circulating levels of ANG II produced by infusion of exogenous ANG II increase arterial pressure (AP) by acting directly to constrict vascular smooth muscle. However, accumulating evidence indicates that a chronically elevated circulating level of ANG II 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 ANG II than that observed during acute ANG II infusions in control animals (8, 23, 26, 52). Furthermore, doses of ANG II 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 ANG II (29). These studies collectively suggest that chronically elevated levels of ANG II in the circulation may stimulate sympathetic outflow (5, 6).

Although ANG II 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 ANG II is complicated by the direct vascular actions of ANG II. Specifically, ANG II-evoked increases in AP would be expected to cause a baroreceptor-evoked decrease in sympathetic nervous system activity (39), thereby masking a sympathoexcitatory action of ANG II. Competing influences of ANG II-induced excitation opposing an indirect inhibitory effect of ANG II-induced increased AP have been carefully documented in the case of ANG II-evoked thirst (9, 45, 46).

Therefore, on the basis of the available data, it appears that increases in circulating ANG II levels have two competing influences on the sympathetic outflow. ANG II acts to increase SNA, although the time course of this action is unclear. On the other hand, ANG II 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 ANG II increases AP, and baroreceptor-evoked sympathoinhibition is quite powerful, sympathoinhibition evoked by ANG II predominates with acute administration of ANG II. However, with chronically elevated AP, baroreceptors reset to a higher pressure (39) and, therefore, provide less inhibition of sympathetic activity; under these conditions, the sympathoexcitatory influences of ANG II may instead predominate. However, Lohmeier et al. (28) recently established that the effects of chronic ANG II infusion on renal sodium excretion in dogs are consistent with renal sympathoinhibition mediated via car-
diopulmonary and arterial receptors that do not reset during ANG II-evoked hypertension. Alternatively, the mechanisms responsible for ANG II-induced sympathoexcitation may not operate acutely and develop only in the chronic presence of ANG II. It has also been suggested that ANG II itself, independent of any change in AP, acts to reset the baroreceptor reflex (39), and such an action of ANG II would further complicate this issue. Therefore, in an animal with intact baroreceptors, this action of ANG II would further complicate a resetting of the baroreceptor reflex and a shift in sympathetic vasomotor tone on which the baroreceptor reflex acts. In an effort to clarify the direct actions of ANG II on sympathetic outflow, we determined the effects of a pressor dose of ANG II infused intravenously on lumbar SNA (LSNA) in sinoaortic-denervated rats. To avoid the possible confounds of anesthesia, these experiments were conducted in unanesthetized, unrestrained rats. Furthermore, because we previously highlighted the importance of completeness of baroreceptor denervation for studies on the role of baroreceptor denervation in cardiovascular regulation (44), we also considered the extent of baroreceptor denervation as a variable.

METHODS

Adult male Sprague-Dawley rats (Zivic Laboratories, Zeleinion, PA), initially weighing 225–300 g, were housed individually in wire-mesh hanging cages in a temperature-controlled colony room (22–24°C, lights on from 7 AM to 7 PM). Rats had ad libitum access to food (Purina 5001 Rat Chow) and tap water for ≥7 days before use in experiments.

Sinoaortic denervation. Rats were subjected to surgical sinoaortic denervation or sham surgery while anesthetized with 2% halothane in 100% O2 via a cone placed over the nose. Surgical sinoaortic denervation was performed as described previously (43). Briefly, a 2- to 3-cm midline incision was made in the ventral neck and, after retraction of 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 closed, and the halothane was terminated. Rats were injected with an antibiotic (penicillin G, 30,000 U im) and the ganglionic blocking drug chlorisondamine (5 mg/kg sc) to block the initial cardiovascular effects of sinoaortic denervation, as well as bronchial constriction and secretion. Because rats that have undergone sinoaortic denervation surgery often do not drink for a few days, they were injected subcutaneously with 10 ml of 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 sinoaortic denervation. Rats were allowed 2–4 wk for recovery before 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 intravenous infusion of methohexital sodium (4–6 µl/min of a 10 mg/ml solution). A silicone rubber-tipped catheter was implanted into the descending aorta via the femoral artery for measurement of AP and heart rate (HR).

A recording electrode was then placed on a lumbar nerve bundle, as described previously (51). Briefly, a 5- to 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 a bedding of wood) to recover. Rats regained consciousness within 15 min and began to move around the cage.

Experiments were initiated 2–3 h after completion of the surgery for electrode implantation, at which time the rats appeared undisturbed and resting quietly. AP was monitored via the arterial catheter connected to a Statham pressure transducer and a preamplifier (model 7P, Grass Instruments, Quincy, MA). HR was measured using a tachograph (model 7P44, Grass Instruments) triggered by the arterial pulse wave. LSNA was amplified (10,000–20,000×; model BMA 831, CWE, Ardmore, PA), filtered (50–10,000 Hz), rectified, and integrated with a 1-s reset time (model 7P10, Grass Instruments). 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 1,000 Hz and recorded using a personal computer-based data acquisition system (LabView, National Instruments) for subsequent analysis. During baroreceptor reflex testing (see below) and at specified times during ANG II 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 intravenous injection of nitroprusside (5 µg/kg) and phenylephrine (5 µg/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 nitroprusside and phenylephrine were classified as completely sinoaortic denervated (SAD), whereas rats that had undergone the denervation procedure but had residual reflex changes in HR were considered to be partially sinoaortic denervated (pSAD) (44).

After completion of the baroreceptor reflex assessment, rats were left to stabilize for 30 min before the experiment was continued. Then baseline values for MAP, HR, and iLSNA were recorded for 30 min. An infusion of ANG II (100 ng·kg−1·min−1 iv in 10 µl/min; Sigma Chemical, St. Louis, MO) was then initiated and maintained for 120 min. MAP, HR, and iLSNA were recorded continuously during this period as well as for 10–60 min after termination of the ANG II 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 ≥30 min after death. This background noise level was subtracted from total nerve activity that was recorded during the experiment.

A total of 5 SAD rats, 10 pSAD rats, and 5 control rats yielded data for analysis. Baroreceptor reflex responses were evaluated using maximal change in HR and iLSNA (inte-
RESULTS

Baseline parameters. Before infusion of ANG II, baroreceptor reflex responses were assessed in all rats, so that rats subjected to sinoaortic denervation surgery could be classified on the basis of the extent of baroreceptor denervation. Of the 15 rats that underwent sinoaortic denervation surgery, five were found to have no reflex-evoked changes in HR in response to nitroprusside and phenylephrine (Table 1). In contrast, the 10 other rats displayed changes in HR in response to nitroprusside and phenylephrine, although these responses were considerably blunted compared with 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 sinoaortic denervation surgery were assessed as 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 than in control rats (Table 2); MAP in pSAD rats was not significantly different from that in 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 than in 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 phenylephrine and nitroprusside responses).

Nitroprusside injections decreased MAP in all rats, although the change in MAP was greater in SAD than in control rats (Table 1). The nitroprusside-evoked fall in MAP was considerably greater in pSAD than in control rats, although it was not quite as large as in SAD rats (Table 1). Phenylephrine injections increased MAP in all rats, and, again, the response was significantly larger in SAD than in 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). 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). Variable degrees of residual tachycardia and increased LSNA were observed in pSAD rats in response to nitroprusside, and these two parameters were highly correlated.

Table 1. Baroreceptor reflex responses in sham-operated, SAD, and pSAD rats

<table>
<thead>
<tr>
<th></th>
<th>Sham-Operated</th>
<th>SAD (n = 5)</th>
<th>pSAD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$ MAP, mmHg</td>
<td>Mean ± SE</td>
<td>46 ± 4</td>
<td>61 ± 3*</td>
</tr>
<tr>
<td>Range</td>
<td>37 to 60</td>
<td>50 to 65</td>
<td>49 to 70</td>
</tr>
<tr>
<td>$\Delta$HR, beats/min</td>
<td>Mean ± SE</td>
<td>−109 ± 23</td>
<td>0</td>
</tr>
<tr>
<td>Range</td>
<td>−60 to −175</td>
<td>0</td>
<td>−20 to −70</td>
</tr>
<tr>
<td>$\Delta$HR/Map</td>
<td>Mean ± SE</td>
<td>−2.5 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>$\Delta$LSNA %</td>
<td>Mean ± SE</td>
<td>−41 ± 5</td>
<td>−34 ± 5</td>
</tr>
<tr>
<td>Range</td>
<td>−29 to −55</td>
<td>−21 to −46</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Baseline MAP, HR, and LSNA in sham-operated, SAD, and pSAD rats

<table>
<thead>
<tr>
<th></th>
<th>Sham Operated</th>
<th>SAD (n = 5)</th>
<th>pSAD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>Mean ± SE</td>
<td>114 ± 3</td>
<td>128 ± 5*</td>
</tr>
<tr>
<td>MAP lability</td>
<td>Mean ± SE</td>
<td>1.71 ± 0.15</td>
<td>6.72 ± 0.61*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>Mean ± SE</td>
<td>373 ± 16</td>
<td>395 ± 14</td>
</tr>
<tr>
<td>LSNA, μV·s</td>
<td>Mean ± SE</td>
<td>54 ± 12</td>
<td>92 ± 20</td>
</tr>
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</table>

Values for MAP, MAP lability, HR, and LSNA are means ± SE from sham-operated, SAD, and pSAD rats recorded before infusion of ANG II. *Significantly different from sham operated, $P < 0.05$. †Significantly different from SAD, $P < 0.05$.
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 compared with control (Fig. 1); the duration of the response was intermediate in pSAD rats.

**Effects of ANG II infusion.** In control rats, infusion of ANG II (100 ng·kg⁻¹·min⁻¹ iv) rapidly and markedly increased MAP (Figs. 2 and 3). The ANG II-induced increase in MAP was accompanied by bradycardia and sympathoinhibition (Figs. 2 and 3). This initial decrease in HR and LSNA was comparable to that observed with phenylephrine-evoked increases in MAP (P > 0.1, comparison of ΔHR or ΔiLSNA/ΔMAP between treatments). Although the ANG II-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 (Fig. 2). The pattern of responses was markedly different in SAD rats. In SAD rats, ANG II infusion caused an initial pressor response that was significantly larger than that observed in control rats (Fig. 2), although by 20 min 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 ANG II infusion in SAD rats, HR and LSNA increased within 10 min, and these increases were never preceded by decreases. LSNA remained elevated compared with baseline and control rats throughout the entire 120-min infusion period (Figs. 2 and 3). In contrast, HR gradually returned to baseline values during this time (Figs. 2 and 3).

In pSAD rats, ANG II infusion increased MAP similar to that observed in control rats (Fig. 4). The initial HR response to ANG II in pSAD rats was quite variable, with HR decreasing in four rats and increasing in the other six animals (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 to 90 min of the infusion period but was not different from baseline at 2 h (Fig. 4). In contrast to control or SAD rats, the response of LSNA to ANG II infusion in pSAD rats was initially quite variable. As a group, the LSNA response to the ANG II infusion in pSAD rats was a gradual increase during...
the infusion period (Fig. 4). By 60 min into the infusion period, LSNA had increased above baseline to a degree similar to that observed in complete SAD rats (Fig. 4). In pSAD rats, the ANG II-evoked increases in HR and iLSNA at 10 min into the ANG II infusion were highly correlated ($R = 0.73, P < 0.05$).

In control and pSAD rats, ANG II infusion did not significantly alter lability of MAP. In contrast, ANG II infusion significantly reduced the lability of MAP in SAD rats ($6.72 \pm 0.61$ to $5.08 \pm 0.67$, $n = 5$, $P < 0.05$).

**DISCUSSION**

The key observation of the present study is that acute infusion of a pressor dose of ANG II is associated with a rapid increase in LSNA in SAD rats. Previous studies have suggested that the sympathoexcitatory actions of ANG II 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 ANG II in SAD and pSAD rats (i.e., rats subjected to sinoaortic baroreceptor denervation surgery but still displaying residual reflex responses) highlight the importance of carefully documenting the extent of baroreceptor denervation.

**Sinoaortic denervation and baroreceptor responses.** Schreihofer and Sved (44) argued that the regulation of AP in SAD rats (i.e., rats with no detectable changes in HR in response to 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 sinoaortic denervated on the basis of the absence of changes in HR in response to pharmacologically evoked increases and decreases in AP, although there
have been previous studies showing renal SNA responses in SAD rats with residual reflexes (i.e., pSAD rats) (2, 20). Interestingly, in SAD rats, nitroprusside-evoked decreases in AP were not associated with any change in LSNA. Furthermore, rats that underwent sinoaortic 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 sinoaortic denervated, injection of phenylephrine still evoked a decrease in SNA. Although 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 HR or LSNA is unclear. However, Minisi et al. (37) demonstrated that phenylephrine increases pulmonary AP and could, therefore, decrease renal SNA by stimulating cardiopulmonary baroreceptor afferents; they noted that phenylephrine-evoked suppression of renal SNA in sinoaortic-denervated dogs was eliminated by vagotomy. 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 sinoaortic denervation must be carefully evaluated for the extent of residual reflexes, and rats with no residual changes in HR to 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. On the basis of reports in the literature, MAP in chronic SAD rats is normal or slightly elevated (1, 3, 7, 12, 38, 49), although 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 before 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 sinoaortic-denervated rats (although they were not completely denervated by the present 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 ( > 75% with the present group sizes) to have been statistically significant.

Effects of ANG II on cardiovascular regulation and sympathetic outflow. The acute cardiovascular actions of ANG II have been well studied in experimental animals. ANG II, infused in doses exceeding ~20 ng·kg⁻¹·min⁻¹ 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 ANG II does not influence baroreceptor-mediated inhibition of renal SNA (25, 34). However, other studies suggest that ANG II may shift the baroreceptor reflex curve or increase the activity of other sympathetic nerves (24, 32, 48). For example, ANG II-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 ANG II was counteracted by coinfusion of nitroprusside, ANG II elicited an increase in muscle SNA (32, 33). Acute ANG II-evoked reflex bradycardia is less than that caused by other pressor substances (39), suggesting that ANG II has additional effects on the control of the heart (see below).

In contrast to observations in baroreceptor-intact rats, intravenous infusion of ANG II in SAD rats was accompanied by an increase in HR and LSNA. The increase in LSNA occurred rapidly, being elevated by ≥ 20% within 10 min in four of the five SAD rats studied. In the other rat, LSNA did not increase to the same degree, although as a group the increase was 23 ± 7% (P < 0.05 compared with baseline). This is in marked contrast to the decrease in LSNA of ≥ 15% observed in each of the five control rats (~40 ± 7%). The rapid increase in LSNA evoked by intravenous infusion of ANG II in unanesthetized SAD rats is a novel observation and suggests that the sympathoexcitatory actions of ANG II 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⁻¹·min⁻¹. The observation that ANG II-evoked increases in regional vascular resistance were potentiated by sinoaortic denervation to a much greater extent in the hindlimb than in the renal or mesenteric circulation (3) is also consistent with this action of ANG II on LSNA and further suggests that this effect of ANG II may be specific for certain sympathetic nerves. Interestingly, in pSAD rats, infusion of ANG II still increased LSNA, although 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 required for exogenous ANG II to increase sympathetic outflow.

Additional evidence of rapid ANG II-evoked sympathoexcitation can be found in other studies in which ANG II increased muscle SNA in human subjects when the pressor effects of ANG II were prevented by coinfusion of nitroprusside (32, 33). Similarly, Kooner et al.
(24) noted that ANG II-evoked rapid decreases in rabbit ear blood flow were eliminated by clonidine or ganglionic blockade, suggesting that ANG II 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 ANG II 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 ANG II take a long time to develop (5). However, that conclusion has been based on studies in animals infused chronically with low doses of ANG II that are not acutely pressor (10) or with pressor doses of ANG II with baroreceptors intact (8, 23, 26, 52). In those studies, the doses were likely too small to elicit rapid actions on the sympathetic nervous system or the sympathoexcitatory actions of ANG II were likely obscured by baroreceptor-mediated sympathoinhibition and became apparent only as baroreceptors reset. Luft et al. (29) measured increased splanchic SNA in rats receiving a chronic infusion of ANG II, although they did not examine the acute effects of ANG II on SNA. Lohmeier et al. (28) demonstrated that renal handling of sodium in response to a pressor dose of ANG II in split-bladder dogs with unilateral renal denervation is consistent with renal sympathoinhibition that is maintained for >5 days of ANG II infusion. Furthermore, they have shown that, in dogs with combined sinoaortic and cardiopulmonary denervation, ANG II apparently causes renal sympathoexcitation that takes a few days to develop (28).

Infusion of ANG II in SAD rats increased HR in addition to LSNA; increased HR in response to ANG II infusion in SAD rats was also noted in another recent study (46). A similar ANG II-induced tachycardia has been 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 ANG II-induced increases in LSNA, the tachycardia occurs rapidly in response to ANG II infusion, and the rapid onset of tachycardia does not occur in pSAD rats.

Methodological issues. The present studies compared the effects of infusion of ANG II (100 ng·kg⁻¹·min⁻¹ iv) between control, SAD, and pSAD rats. This protocol relies on the assumption that an infusion rate of 100 ng·kg⁻¹·min⁻¹ produces circulating levels of ANG II that are physiologically (or at least pathophysiologically) relevant and that it causes similar increases in circulating ANG II levels in each group. Previous studies, including a recent report from this laboratory, have indicated that infusion of ANG II at this rate into control rats results in plasma ANG II levels of ~500 pg/ml, which is similar to that during severe hypotension (22, 31, 46). Furthermore, infusion of ANG II in chronic SAD and control rats results in equivalent increases in plasma ANG II 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 before 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 before 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 ANG II-evoked increases in LSNA and HR. There are several sites at which ANG II could conceivably act to rapidly increase LSNA and HR. ANG II has been reported to act directly at the heart to increase HR (27), although this seems to require higher concentrations of ANG II in the heart than were likely attained in the present study. Alternatively, ANG II might increase HR by increasing sympathetic neural activity to the heart and/or decreasing parasympathetic neural activity to the heart (39). Because the ANG II-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 sinoaortic denervation surgery, it seems likely that these two responses reflect a single mechanism. Because LSNA was recorded from postganglionic fibers and it is known that ANG II can depolarize postganglionic neurons (16, 39), ANG II might act at the level of sympathetic ganglia. However, the concentration of ANG II 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), intravenous injection of 160 ng/kg ANG II increased renal SNA in anesthetized mice by acting directly on the postganglionic neuron. The plasma levels of ANG II in those animals were likely >10-fold greater than produced in the present study. Interestingly, this response observed by Ma et al. 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 ANG II infused in conscious rats appeared to result from an increase in the frequency of large-amplitude spikes (Fig. 3). Thus ANG II may act to increase sympathetic outflow from the central nervous system (39). For example, ANG II might act on sensory receptors to evoke an increase in SNA, inasmuch as ANG II has been reported to act on cardiac receptors to produce such an effect (4). Alternatively, ANG II might act on regions of the central nervous system 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 ANG II (11). Furthermore, because ANG II infusions
markedly increase AP and, therefore, might disrupt the blood-brain barrier (35), circulating ANG II 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 complications may occur in response to surgical sinoaortic denervation that might change how the animal responds to ANG II. For example, the number of angiotensin-binding sites in the nucleus tractus solitarius is reduced after sinoaortic denervation (17, 40). However, Barron et al. (3) noted that the marked potentiation of ANG II-evoked pressor responses that is present in chronic SAD rats occurs acutely.

**Summary.** The present studies show that, in chronic SAD rats, intravenous infusion of ANG II results in a rapid increase in LSNA and HR. These data indicate that ANG II can produce a rapid sympathoexcitation, at least under certain conditions.

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