Effect of a Pressor Infusion of Angiotensin II on Sympathetic Activity and Heart Rate in Normal Humans

Steven R. Goldsmith and Gregory J. Hasking

We tested the hypothesis that pressor infusions of angiotensin II (AII) could stimulate the sympathetic nervous system as reflected by norepinephrine (NE) spillover in humans. AII was infused at 5 ng/kg/min in six healthy volunteers, with vehicle and phenylephrine infusions as controls, on 3 separate days. Heart rate, mean arterial pressure, plasma NE, NE clearance, and NE spillover were assessed before and after 30-minute infusions of AII, vehicle, or phenylephrine in the supine position and then after 15 minutes of head-up and 15 minutes of head-down tilt. Both AII and phenylephrine raised mean arterial pressure (88±9.6 to 103±14 mm Hg, p<0.001, and 91±7.6 to 104±9.2 mm Hg, p<0.001, respectively), whereas heart rate fell only with phenylephrine (60±6 to 51±6.3 beats/min, p<0.001). Neither plasma NE nor NE spillover was affected by either infusion, and NE clearance declined slightly with both. No changes occurred in any variable during vehicle infusions in the supine position. During upright tilt, NE spillover increases were attenuated by both AII and phenylephrine while NE clearance changes were slightly greater, leaving plasma NE increases similar on each day. During head-down tilt, NE and NE spillover declined comparably on each study day. We conclude that in healthy humans, using NE spillover as the measure, 1) pressor infusions of AII do not increase basal sympathetic activity, enhance sympathetic stimulation during baroreceptor unloading (upright tilt), or attenuate sympathetic inhibition during baroreceptor loading (head-down tilt) and 2) the absence of bradycardia during pressor infusions of AII cannot be attributed to global sympathetic stimulation. This suggests that AII may inhibit the efferent response to acute baroreceptor loading in humans. (Circulation Research 1991;68:263–268)

The peptide angiotensin II (AII), apart from its direct effect, a potent pressor action, has powerful and diverse indirect effects on cardiovascular regulation. These effects reportedly include activation of the sympathetic nervous system at central, ganglionic, and peripheral sites; potentiation of the vasopressor action of norepinephrine (NE) and central inhibition of the sinoaortic baroreflex. Most data regarding these indirect effects of AII have been gathered in animal studies; relatively little information has come from human investigation. At the same time, the success of angiotensin-converting enzyme inhibitor therapy for both hypertension and heart failure has focused attention on the need for a better understanding of the overall effect of AII on the human circulation, because the mechanisms by which these drugs produce sustained clinical benefit are not yet fully clear.

In this study, we investigated the possibility that pressor levels of AII might stimulate the sympathetic nervous system in normal humans, either at rest or in response to maneuvers that increase and decrease basal sympathetic tone. We also investigated whether the previously observed failure of pressor infusions of AII to cause bradycardia in humans could be accounted for by changes in sympathetic nervous system activity. To answer these questions, we relied on the assessment of plasma NE levels and NE spillover during time- and pressure-controlled infusions of AII in healthy human volunteers, both in the supine position and during head-up and head-down tilt. The results indicate that mildly pressor infusions of AII have little if any effect on global sympathetic nervous system activity in humans under these conditions. However, the results also suggest a potentially important influence of AII on the cardiac response to baroreflex stimulation.
Materials and Methods

Protocol

Six healthy normal humans aged 26–67 years (mean, 35 years) participated in the investigations. None had a history of hypertension, diabetes, heart disease, or any other significant systemic illness, and none was on any medication at the time of study. Studies were always conducted in the postabsorptive state.

Intravenous cannulas were placed in each forearm for infusion and blood sampling. Arterial pressure and heart rate were measured by an automatic sphygmomanometric device. Thirty minutes of supine rest on a tilt bed was allowed after placement of the intravenous cannulas to be sure that heart rate and arterial pressure were stable. Blood samples then were taken for measurement of plasma NE concentration and for plasma blanks for the assay of our tracer.

A bolus injection of 12 μCi 1-[7-3H]norepinephrine ([3H]NE, Du Pont–New England Nuclear, Boston) then was given over 3 minutes, followed by a continuous infusion of 0.8 μCi/min for 90 minutes. Blood samples for plasma NE and [3H]NE concentrations were collected at 25 and 30 minutes after the initiation of the infusion to document steady-state conditions. At 30 minutes, while the [3H]NE infusion was maintained, a second infusion containing either AII (Hypertensin, Ciba-Geigy Corp., Edison, N.J.) or vehicle (5% dextrose in water) was begun at a rate calculated to produce an AII dose of 5 ng/kg/min. AII or vehicle infusions were continued for 30 minutes with arterial pressure and heart rate recorded at 5-minute intervals.

After 30 minutes of these infusions, blood samples were taken as control and after the first 30 minutes of the [3H]NE infusion. Subjects then were tilted 60° upright, and all variables were reassessed after 15 minutes in this position. After upright tilt, subjects were placed into a 30° head-down tilt, with all variables reassessed a final time 15 minutes later and the infusions discontinued.

For each subject, AII and vehicle infusions were given in a double-blind randomized fashion on two different study days at an interval of at least 1 week. After analysis of the results of the foregoing studies to determine the magnitude of the pressor effect of AII as compared with vehicle, five of the six subjects returned for a third day of study as a positive control experiment. The protocol was as before, except that phenylephrine was given in place of the vehicle or AII infusions. The initial dose was 10 μg/min and was titrated upward at 5-minute intervals to produce a mean arterial rise comparable to that seen with AII. In all subjects, this was easily accomplished in 15 minutes or less. The infusion then was maintained for 30 minutes as with vehicle and AII before the upright and head-down tilt.

Plasma Norepinephrine Kinetics

NE kinetics were assessed by the method of Esler et al.13 The method uses a steady-state infusion of [3H]NE in trace amounts. It assumes that, at steady state, endogenous NE and infused, radiolabeled NE are cleared at equivalent rates, and is predicated on the absence of recirculation of tracer in the body. If clearance of endogenous NE is equivalent to that of [3H]NE, the following relation is implied:

$$\text{NE "spillover"} = \frac{\text{Infusion rate} \times \text{[3H]NE infused}}{\text{Plasma [NE]}}$$

$$= \frac{\text{Plasma [(3H)NE]}}{\text{Plasma [(3H)NE]}}$$

Knowing the infusion rate for [3H]NE, the concentration of infused [3H]NE, the plasma concentration of [3H]NE, and the concentration of endogenous plasma NE, one then can calculate the spillover rate of endogenous NE.

$$\text{NE spillover, ng/ml} = \frac{\text{(Plasma [NE], pg/ml)} \times \text{(Clearance [3H]NE, l/min)}}{\text{Activity of [3H]NE is measured as disintegrations per minute per milliliter and plasma NE as picograms per milliliter. The units for NE spillover are nanograms per minute, because by convention clearance is expressed in liters per minute.}}$$

This technique now has been applied extensively in both animal and human investigations and, given the assumptions stated, is accepted as a measure of NE kinetics under steady-state conditions. Its key advantage is that one can make inferences about NE release and not rely solely on plasma NE concentration, which is affected independently by both release rates and clearance. It is important to realize, however, that spillover is still an indirect reflection of sympathetic nervous system activity, because it measures only NE release into the bloodstream, not direct synaptic cleft NE concentrations or nerve traffic. But it is clearly more attractive than relying solely on plasma NE assessment and has the additional advantage over localized nerve traffic recordings of assessing the overall systemic activity of the sympathetic nervous system.

Assays

Blood samples from the assays were kept on ice, centrifuged at 4°C immediately after the study, and frozen at −70°C until assay. Plasma NE was measured by radioenzymatic methods using the Cat-a-Kit (Amersham Corp., Kalamazoo, Mich.). [3H]NE concentrations (disintegrations per minute per milliliter) were measured by adsorption of plasma catecholamines onto acid-washed alumina, elution with 0.2N HCl acid, and direct scintillation counting of the eluates. Infusion rates were calculated from the
TABLE 1. Response of Norepinephrine Spillover and Clearance, Plasma Norepinephrine, Heart Rate, and Mean Arterial Pressure to Angiotensin II, Vehicle, and Phenylephrine at Rest and During Head-Up and Head-Down Tilt

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Infusion</th>
<th>Head-up tilt</th>
<th>Head-down tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE spillover (ng/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>640±350</td>
<td>518.2±169.4</td>
<td>562.3±167.5</td>
<td>370.2±139.4†</td>
</tr>
<tr>
<td>Vehicle</td>
<td>635±245.4</td>
<td>593.5±253.6</td>
<td>781.3±304.8*</td>
<td>466.3±99†</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>535±255</td>
<td>469.8±249</td>
<td>539.4±301</td>
<td>376.8±161.5†</td>
</tr>
<tr>
<td>NE clearance (l/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>2.4±0.6</td>
<td>1.8±0.5*</td>
<td>1.3±0.3*</td>
<td>1.6±0.4*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.2±0.4</td>
<td>2.0±0.4</td>
<td>1.8±0.3*</td>
<td>1.8±0.2*</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>2.2±0.5</td>
<td>1.9±0.6*</td>
<td>1.6±0.4*</td>
<td>1.6±0.5*</td>
</tr>
<tr>
<td>NE concentration (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>All</td>
<td>264±63.4</td>
<td>286.7±62.2</td>
<td>416.5±73*</td>
<td>236.3±74.5†</td>
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<tr>
<td>Vehicle</td>
<td>288±110.3</td>
<td>272.4±100.8</td>
<td>413.2±134.7*</td>
<td>256.6±59.4†</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>243±62.7</td>
<td>246.4±91.8</td>
<td>323±148*</td>
<td>229±64.5†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>63.1±9.4</td>
<td>62.8±10</td>
<td>66.9±10.1</td>
<td>62.4±7.8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>69.6±16.2</td>
<td>64.6±9.1</td>
<td>69.3±11</td>
<td>63.5±7.5</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>60.1±6</td>
<td>51.4±6.3*†</td>
<td>55.6±5.4*‡</td>
<td>49±5.9*‡</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>87.9±9.6</td>
<td>103.3±13.6*</td>
<td>107.8±14.4*</td>
<td>101.1±9*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>84.4±11.9</td>
<td>89.5±11.1†</td>
<td>93.6±9.8‡</td>
<td>88.1±9†</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>90.6±7.6</td>
<td>104.1±9.2*</td>
<td>105.3±10.4*</td>
<td>102.1±11.8*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Control values are averaged results of measurements made 25 and 30 minutes after initiation of tritiated norepinephrine infusion. NE, norepinephrine; AII, angiotensin II.

*p<0.05 relative to control on the same day.
†p<0.05 relative to values for head-up tilt.
‡p<0.05 relative to values at the same time point on the other days.

The data in Table 1 indicate first that there was no significant effect of either AII or phenylephrine on NE spillover as compared with vehicle control in the supine position. There was a tendency for spillover to fall after both AII and phenylephrine, but these changes did not attain significance and, importantly, were not different compared with each other. Clearance declined slightly on each day, and plasma NE levels remained unchanged. Mean arterial pressure rose comparably with both AII and phenylephrine, but heart rate fell only during phenylephrine.

During head-up tilt, the rise in NE spillover was comparably attenuated by both AII and phenylephrine, while clearance declined further, increasing plasma NE comparably on each day. Compared with vehicle, mean arterial pressure remained higher after both AII and phenylephrine, whereas heart rate remained lower only after phenylephrine.

During head-down tilt, NE spillover fell on all 3 study days to levels below control; again, there were no differences between vehicle, AII, and phenylephrine. Clearance remained comparably low relative to control on each day. Plasma NE fell to similar levels, but not different than control because of the fall in clearance. Mean arterial pressure and heart rate returned toward their pretitl levels on each day, with the higher pressure persisting with both AII

activity of [3H]NE in the infusate and the volume rate of infusion. All samples from an individual subject were assayed in duplicate in the same assay. In this group of subjects, the recovery of [3H]NE added to plasma in the concentration range produced by the experiment was 56.2±8%. Variation between duplicate samples in the same assay averaged 3%, and the interassay variation for duplicate samples was ±8%.

Statistics

Analysis of variance for repeated measures compared the responses of each variable assessed at each available time point on the 3 days and between study days. If significant variation was found from control at any point within each day or between days, the individual means were compared by paired t tests with Fisher’s least significant difference method used to establish significance between individual means. A value of p<0.05 was accepted as significant.

Results

There were no differences between the 25- and 30-minute measurements of heart rate, arterial pressure, plasma NE, NE spillover, or NE clearance on any study day before vehicle, AII, or phenylephrine infusions. The average of these two values is therefore included in Table 1 as the control point.
and phenylephrine, and the lower heart rate only with phenylephrine.

Discussion

This protocol permitted us to determine the effects of a 5-ng/kg/min infusion of AII on heart rate, blood pressure, plasma NE, and NE kinetics, both in the basal supine position and in response to maneuvers that increase and decrease baseline sympathetic tone in healthy human volunteers. We included an assessment of the effects of AII during the baroreceptor loading and unloading maneuvers because there have been suggestions that the effects of AII on the sympathetic nervous system are more readily apparent if the system is stimulated before study.14 Our intent, therefore, was to determine whether a mildly pressor infusion of AII activated the sympathetic nervous system in the basal state, enhanced the normal degree of stimulation during a maneuver that unloaded both cardiopulmonary and sinoaortic baroreceptors, or attenuated the inhibition of sympathetic nervous system activity occurring during a maneuver that loaded these same receptors. Our major index of sympathetic activity was NE spillover to plasma, using the kinetic techniques developed by Esler et al.13

We feel that our data permit two major conclusions: 1) mildly pressor infusions of AII do not increase basal sympathetic nervous system activity, do not enhance the increase in activity of the sympathetic nervous system during upright tilt, and do not attenuate the decline in sympathetic nervous system activity seen with head-down tilt, and 2) the failure of heart rate to decline after a pressor infusion of AII cannot be attributed to an increase in global sympathetic activity. The second conclusion implies an inhibitory effect of AII on the heart rate response to baroreflex stimulation.

In the supine position, NE spillover was not different during AII infusions, as compared with vehicle and phenylephrine. Slight decreases in clearance, which were not statistically different from each other, occurred on each day. This may have represented additional time dependence in the whole body clearance of [3H]NE, despite 30 minutes rest before the [3H]NE infusion and 30 minutes of [3H]NE infusion before the interventions. Plasma NE remained essentially stable during this phase of the study.

These findings demonstrate first that there was no increase in activity of the sympathetic nervous system during AII. Second, if there were any baroreflex-mediated fall in activity as a consequence of the rise in arterial pressure, it was not different than that during a comparable rise in pressure with phenylephrine. Third, because heart rate declined only during phenylephrine, the absence of this response during AII cannot be attributed to a difference in overall sympathetic activity. This implies that AII inhibited the reflex response of heart rate to a rise in arterial pressure. Such an effect of AII has been well documented in experimental animals9,10 and implied by other studies in humans,11,12,15 but without direct measures of sympathetic activity. The only alternative explanation would be a direct chronotropic effect of AII, which is unlikely,16–20 or a highly selective effect of AII on purely cardiac sympathetic activity (difficult to exclude in humans but without an a priori or experimental basis).

During upright tilt, the expected reflex-related rise in NE spillover was attenuated by both AII and phenylephrine, probably because of their effects on baseline arterial pressure, but no difference in the response was evident between days. There were also no differences in the fall in NE spillover produced by the reflex adjustments to head-down tilt. These results clearly imply that AII did not enhance the sympathetic response to unloading baroreceptors or inhibit the sympathetic response to loading baroreceptors, again compared with both vehicle and equipressor controls.

The major practical limitation to interpretation of our data is reliance on NE kinetics as measured from a systemic vein. Sampling in this fashion does measure the integrated sympathetic response to a given stimulus but probably emphasizes it in the regional bed most closely associated with the sampling site. We did not measure nerve traffic, although in general, increases and decreases in nerve activity should parallel changes in spillover unless, perhaps, changes in nerve traffic are too subtle to be reflected in spillover. However, we do not mean to imply that our data in any way exclude a role for AII in the local regulation of sympathetic tone in a specific neurovascular bed, only that AII does not alter overall sympathetic activity at rest or during postural changes.

The failure of phenylephrine to suppress sympathetic activity requires a comment as a possible theoretical objection to the interpretation of our results. One might have expected NE spillover to decline in response to a rise in pressure with this agent, making the failure of AII to change NE spillover in fact a positive result. However, few data are available concerning the sympathetic response to small pressure changes with phenylephrine, and what is available tends to support our findings. Grossman et al21 reported plasma NE levels after phenylephrine doses that produced larger increases in both mean and diastolic pressures than we did, and they found small and inconsistent effects on neurotransmitter levels. Inspection of their data show very little if any effect of phenylephrine on plasma NE at lower doses. In fact, the largest decreases in NE are in association with no pressure change and vice versa, making the lack of a time control in that study a serious flaw. Eckberg et al,22 measuring muscle sympathetic nerve activity after phenylephrine, also without a time control, found that a large fall in sympathetic nerve traffic occurred between 0.4 and 0.8 µg/kg/min phenylephrine, despite a rise in diastolic pressure of only 3 mm Hg over the 0.4–µg/kg/min infusion. We gave on average 0.18 µg/kg/min phenylephrine and also raised pressure less, because our mean increased by 13
mm Hg versus their diastolic change of 11 mm Hg. Eckberg et al22 saw essentially no sympathosuppression at the lower pressures and phenylephrine doses, so these data are consistent with the absence of change in NE spillover in the current study. They also raise the possibility that the higher doses of phenylephrine might have decreased sympathetic activity via α2-receptor activation, because the pressure change was so small between the 0.4- and 0.8-μg/kg/min doses. It therefore seems that over this range of small pressure increases, the mechanism of heart rate decrease after phenylephrine is more likely to be parasympathetic stimulation, not sympathetic withdrawal.

There are few other human data dealing with AII and the sympathetic nervous system with which to compare the results of this investigation. Seidelin et al23 did not find any enhancement of the plasma NE response to several stimuli in the presence of subpressor AII infusions. We also have found that subpressor AII infusions do not increase basal plasma NE levels or enhance the plasma NE response to tilt and have extended these observations to include NE spillover and clearance, further suggesting that sympathetic activation during the basal state or baroreceptor unloading is not enhanced by low levels of AII.24 These studies together support the present conclusion that pressor infusions of this peptide also do not enhance overall sympathetic nervous system activity in humans. On the other hand, Webb et al25 have reported that subpressor AII infusions may enhance the vasoconstrictor response to baroreceptor unloading maneuvers in the isolated forearm. These investigators did not, however, entirely exclude the possibility that a postsynaptic interaction could have accounted for the results. Because other studies also support the possibility that AII may potentiate the effects of NE in humans,6,7,26 such an explanation also may be relevant to the results of Webb et al. Therefore, among the small number of studies presently available in humans, the consensus is that AII may facilitate the effects of released NE, but it does not stimulate NE release either at subpressor or mildly pressor levels.

The findings in this study are potentially relevant to understanding the mechanisms underlying the decreased levels of parasympathetic tone and impaired responsiveness of heart rate to baroreceptor loading that characterizes congestive heart failure.27 If in fact AII inhibits the heart rate response to baroreflex stimulation, high levels of the peptide could contribute to this abnormality in this disease. From a therapeutic standpoint, angiotensin-converting enzyme inhibitors uniquely improve hemodynamics in heart failure while decreasing heart rate and plasma NE levels.28,29 The data from the current study make it less likely that the decrease in heart rate and plasma NE levels are due to decreased sympathetic stimulation from AII. Other possibilities requiring investigation are improvements in baroreflex function and improved clearance of NE due to better organ blood flow after administration of these drugs. Resolution of these matters must await the appropriate studies in patients with this disease.

Acknowledgments

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References


**KEY WORDS** • angiotensin II • sympathetic nervous system • baroreflexes
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