Converting Enzyme Inhibition Prevents the Effects of Atrial Natriuretic Factor on Baroreflex Responses in Humans

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The aim of this study was to assess the influence of atrial natriuretic factor (ANF) on arterial baroreflex chronotropic responses and to investigate whether this effect of ANF is affected by angiotensin converting enzyme inhibition (CEI). For this purpose, in 13 normal volunteers, the reflex chronotropic responses to arterial baroreceptor stimulation (phenylephrine, 25–100 μg i.v.) or deactivation (nitroglycerin, 25–100 μg i.v.) were evaluated in control conditions and during the steady-state phase of a sustained infusion of ANF (50 ng/kg/min) or placebo, before and during prolonged treatment with the converting enzyme inhibitor enalapril (20 mg p.o. for 5 days). ANF infusion, which raised plasma ANF levels from 48±19 to 1,765±203 pg/ml, was associated with a slight decrease in systemic blood pressure and no change in heart rate. In addition, it caused a significant increase of the regression slope obtained with phenylephrine (from 11.3±2 to 18.5±2 msec/mm Hg) and a significant reduction of slope of the nitroglycerin-produced regression line (from 9.3±1 to 5.6±0.6 msec/mm Hg). After sustained CEI, which raised plasma renin activity from 1.4±0.4 to 19.9±5 ng/ml/hr, ANF infusion induced an increase in plasma ANF levels and a reduction in blood pressure comparable to those observed in control conditions. During CEI, however, ANF infusion had no significant effect on the chronotropic baroreflex responses produced by phenylephrine or nitroglycerin. Chronotropic and pressor responses to cold exposure were unchanged after CEI and during ANF. Our results demonstrate that ANF, at this dosage, significantly modulates the chronotropic responses to baroreceptor manipulation so that bradycardic responses are enhanced and tachycardic responses blunted. They also show that CEI prevents this effect of ANF. Although other possibilities cannot be excluded, the interaction of atrial peptide with the baroreflex mechanisms may be accounted for by antagonism of the effects of angiotensin II on baroreflex pathways. (Circulation 1990;82:1214–1221)

Atrial natriuretic factor (ANF) is a polypeptide hormone secreted primarily by the cardiac atria that has potent effects on renal handling of sodium and water and on systemic hemodynamics.1–4

Several observations suggest that ANF exerts a complex influence on the neural control of circulation. Previous studies demonstrated the existence of ANF or receptors for ANF in the nucleus tractus solitarii, the area postrema, the anteroventral third ventricle region, and the aortic baroreceptors.5–8 In addition, exogenous infusion of ANF reduces blood pressure without raising heart rate9–12 and modulates the cardiovascular responses to cardiopulmonary or arterial baroreceptor manipulations.13–17

See p 1523

The mechanisms underlying the interference of the peptide with the neural control of the circulation are unclear. In this regard, Thoren et al18 reported that ANF may augment vagal afferent traffic and blunt baroreflex-mediated increases of sympathetic outflow and attributed this effect to sensitization of receptors with vagal afferents in the cardiopulmonary area.19,20 Studies performed in our laboratory suggested that ANF increases vagal influences on the circulation.14 Finally, the observations that ANF antagonizes many biological effects of angiotensin II (Ang II)21–25 and that the distribution of binding sites for the two hormones in the central nervous system shows a striking overlap7 have suggested that ANF may affect arterial baroreflexes by antagonizing the influence of

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Ang II on baroreceptor responses. Ang II, in fact, modulates the baroreflex control of circulation mostly through a vagolytic action at the central nervous system level.26–31

In the present study, the influence of exogenous infusion of ANF on arterial baroreflexes was assessed in humans. In addition, to investigate the mechanisms underlyinig the interactions of ANF with arterial baroreflexes, the effects of atrial peptide on baroreflex chronotropic responses were assessed again after sustained converting enzyme inhibition.

**Methods**

**Subjects and General Procedures**

The study was performed in 13 normal volunteers of both sexes whose ages ranged from 22 to 38 years (body weight, 66.5–72.5 kg). Informed, written consent was obtained from each subject before the study. The protocol was approved by the Research Committee of our institution. In the week preceding the first experimental session and throughout the study, the subjects were maintained on a standard daily diet containing 1,500 ml fluids, 130 meq sodium, and 60 meq potassium. Two days before the study, the subjects were made familiar with the experimental procedure, and the day preceding the study they were required to abstain from caffeine, alcohol, smoking, and strenuous exercise. The studies were always performed in the morning, starting between 10:30 and 11:30 AM after an overnight fast, with the subject in the supine position, in a quiet room with the temperature kept constant between 22° and 24° C.

**Instrumentation**

Pulsatile arterial blood pressure was measured by a short heparinized polyethylene cannula introduced percutaneously into the brachial artery (after local anesthesia with 2% procaine solution) and connected to a pressure transducer. The R-R interval was measured by monitoring lead II of an electrocardiogram at high speed (50 mm/sec) recording. A thin polyethylene catheter was introduced percutaneously into an antecubital vein for drug or fluid administration, while another heparinized polyethylene 5F catheter introduced through a contralateral antecubital vein was advanced to the right atrium. Intraarterial blood pressure, right atrial pressure, and heart rate were monitored continuously. Systemic and atrial pressures were measured through Statham P23db pressure transducers connected to a multi-channel polygraph.

Blood samples for measurement of plasma levels of immunoreactive ANF (irANF) and plasma renin activity (PRA) were obtained from the arterial line. The samples were collected in chilled tubes, rapidly spun in a cold centrifuge, and stored at −40° C.

**Techniques for Studying Arterial Baroreflexes**

The technique used to evaluate the arterial baroreflexes, which was suited for evaluating baroreceptor control of heart rate, was based on the chronotropic response to the pharmacological manipulation of blood pressure. Phenylephrine bolus injection (25–100 µg i.v.) was used to increase blood pressure by at least 20 mm Hg and nitroglycerin (25–150 µg i.v.) to lower it by at least 20 mm Hg. The drugs were injected during a period of 5–10 seconds in order to cause blood pressure and reflex heart rate changes that reached a maximum and were then sustained for 5–10 seconds. To analyze the effects of vasoactive drugs, we calculated the average systolic arterial and heart interval during the 10-second period before injection and during the ramp of the pressure changes. The responses were evaluated by calculating the slope of the regression lines obtained by plotting each R-R interval against the corresponding preceding value of systolic blood pressure, according to the method described by Smyth et al.32 To provide a more detailed analysis of the baroreflex responses, the gain of the baroreceptor reflex control of heart rate was also determined by the ratio of the percent change in R-R interval to the corresponding change in systolic arterial pressure.

**Protocol**

Thirty minutes after placement of intravascular catheters, hemodynamic (blood pressure, heart rate, and right atrial pressure) and hormonal (immunoreactive ANF, plasma renin activity) baseline measurements were obtained. Subsequently, repeated bolus (three to five) injections of phenylephrine and nitroglycerin were made in a random order, with each injection separated from the preceding one by at least 10 minutes. Injections of vehicle alone were also interposed and did not elicit any hemodynamic effect. The subjects were asked to breathe regularly and were unaware of the site of injections. To evaluate the vascular and chronotropic response to direct stimulation of sympathetic nervous system, after recovery a cold pressor test was performed. Exposure to cold was obtained by immersing one hand of the subject in water at 4° C for 90 seconds.

After recovery, measurements were repeated again to obtain a new baseline. A constant infusion of ANF (α-human-1-28 atrial natriuretic peptide, Bissendorf peptide GMBh, FRG) (50 ng/kg/min) was then started. After 15 minutes of infusion, that is, when the increase in plasma levels achieved equilibrium, hemodynamic and hormonal measurements were performed to evaluate the direct effects of the peptide. Finally, arterial baroreflexes were assessed again as described above, whereas the ANF infusion was continued.

On the day after the first experimental session, eight subjects started a treatment with the angiotensin converting enzyme inhibitor, enalapril (Enapren, Merck, Sharp & Dohme) at the dose of 20 mg p.o. at 8:30 AM for 5 consecutive days. A prolonged administration was chosen to avoid the consequences of the acute administration of enalapril. The dose of enalapril used induces a stable inhibition of the angio-
tensin converting enzyme activity and extremely low circulating levels of angiotensin II for about 24 hours. The peak effect of this dose on converting enzyme activity is achieved within 2 hours, and it is maintained for more than 6 hours. The pressor response to intravenous administration of angiotensin I is 20±3 mm Hg in control conditions and 2±1 mm Hg (p<0.001) 6 hours after 20 mg enalapril. Five separate subjects received one tablet of placebo at the same hour in the morning for 5 days.

On the fifth day of treatment with enalapril or placebo, a second experimental session, exactly reproducing the first, was held. No significant change in the hemodynamics and hormonal parameters or in the baroreflex responses was observed between the two sessions in the placebo group.

To examine the influence of venous pressure on the baroreflex responses, in four separate normal subjects (two men, two women; mean age 29±4 years) we studied the reflex responses to phenylephrine and nitroglycerin in the supine position and after the subjects had been sitting for 15 minutes, before and during ANF infusion (50 ng/kg/min for 15 minutes). In these subjects, central venous pressure, systemic blood pressure, and heart rate were measured.

Hormonal Assays

PRA was measured by radioimmunoassay (sensitivity: 50 pg/tube angiotensin I; intra-assay and interassay variability coefficients, 5.8% and 9.7%, respectively).

Plasma immunoreactive ANF levels were determined by radioimmunoassay using rabbit antiserum (Peninsula), iodinated human ANF-(99-126) (2,200 Ci/mmol, Amersham), and human ANF-(99-126) (Nova Biochem) as a standard. ANF was extracted from plasma with Sep-Pak C18 cartridges (Waters Associates, Milford, Mass.). The recoveries determined on each plasma sample ranged from 72% to 86%. The eluates were dried overnight in a Savant speed-vac evaporator and reconstituted in radioimmunoassay buffer. The assay was performed in disequilibrium conditions. Intra-assay and interassay variation coefficients were 6.2% and 10.1%, respectively. The radioimmunoassay sensitivity was 2.0 pg/tube.

Data Analysis

The data obtained by injection of vasoactive drugs calculated during the ramp phase of the pressure changes were analyzed by linear regression analysis. The average slope obtained in each subject was used for further analysis. Significant linear regression coefficients were always found between systolic blood pressure and the subsequent R-R interval (r was never less than 0.70, p was never greater than 0.01).

Student's t test for paired data was used for comparisons of data with normal distribution within the same subjects. Wilcoxon's nonparametric test was applied for the analysis of data with nonnormal distribution. Two-way analysis of variance was used for comparisons of the reflex gain. All data are reported as mean±SEM.

Table 1. During ANF Infusion Before and After Converting Enzyme Inhibition

<table>
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<th>Baseline</th>
<th>ANF</th>
<th>Control</th>
<th>CEI</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>85±4</td>
<td>79±4*</td>
<td>83±4</td>
<td>72±3*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>71±2</td>
<td>70±4</td>
<td>71±1</td>
<td>72±3</td>
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ANF, atrial natriuretic factor; CEI, converting enzyme inhibition; MAP, mean arterial pressure; HR, heart rate. *p<0.05 vs. control.

Results

Effects of ANF on Blood Pressure, Heart Rate, and Plasma Renin Activity in Control Conditions and After Converting Enzyme Inhibition

In control conditions, ANF infusion (50 ng/kg/min) raised plasma immunoreactive ANF levels from 48±19 to 1,765±203 pg/ml (p<0.001) after 15 minutes of infusion. During the steady-state phase of ANF infusion, mean blood pressure showed a slight but consistent reduction, whereas heart rate did not change significantly (Table 1). Also, plasma renin activity did not change during ANF (from 1.4±0.4 to 1.4±0.4 ng/ml/hr, NS).

Chronic converting enzyme inhibition induced by a 5-day treatment with enalapril (20 mg p.o.) significantly increased baseline plasma renin activity (19.9±5 ng/ml/hr, p<0.01 vs. pretreatment) and tended to reduce basal plasma ANF levels (34.1±12 pg/ml, NS vs. pretreatment). The plasma ANF levels

FIGURE 1. Mean values of systolic blood pressure (SBP) and heart rate period (HP) before (preinjection) (B) and at the peak effect of phenylephrine (PHE) and nitroglycerin (NG) in control conditions (solid line), during atrial natriuretic factor (ANF) infusion (dotted line), before and after converting enzyme inhibition (CEI). All changes caused by PHE or NG were significant. Standard errors were omitted for clarity. *p<0.05 vs. the corresponding values after ANF.
achieved during the steady state of the infusion were quite comparable to those obtained before enalapril (1,806±317 pg/ml, p<0.001 vs. baseline). The baseline levels of blood pressure and heart rate, as well as the responses of these variables to ANF after converting enzyme inhibition, were not different from those measured in control conditions (Table 1).

Finally, central venous pressure was significantly reduced by chronic enalapril (from 1.4±0.2 to 0.4±0.3 mm Hg, n=4, p<0.05).

Effects of ANF on Baroreflex Responses to Administration of Vasoactive Drugs Before and After Converting Enzyme Inhibition

Figure 1 depicts blood pressure and heart period values before and at the peak effect of phenylephrine and nitroglycerin administration in control conditions and during ANF infusion, before and during converting enzyme inhibition. Although the effect of vasoactive drugs on blood pressure was comparable in all situations, during ANF infusion, the bradycardic response to phenylephrine was accentuated and the tachycardic response to nitroglycerin was reduced. In contrast, after converting enzyme inhibition, the responses observed before and during ANF were similar.

To analyze the baroreflex responses, regression lines were obtained relating the drug-induced changes in systolic blood pressure and the R-R interval. As shown in Figure 2, the average regression slope obtained by increasing systolic blood pressure by phenylephrine was significantly greater (p<0.05) during ANF infusion (18.5±2 msec/mm Hg) compared with that obtained in basal conditions (11.3±2 msec/mm Hg). This is illustrated in detail by the analysis of the average baroreceptor reflex gain for phenylephrine presented in Figure 2 (upper panel), which shows a greater reflex gain at elevated than at normal plasma immunoreactive ANF levels for each level of pressure change.

Figure 2. Effects of atrial natriuretic factor (ANF) on R-R interval vs. systolic pressure slopes before and during treatment with enalapril (CEI). PHE, phenylephrine; NTG, nitroglycerin. *p<0.05 vs. control. †p<0.05 vs. the corresponding value obtained before CEI.

Figure 3. Comparison of the average baroreceptor reflex gain (±SEM) with changes of systolic blood pressure induced by phenylephrine studied at normal and elevated atrial natriuretic factor (ANF) levels. Upper panel: baseline conditions. Lower panel: during converting enzyme inhibition (CEI). Analysis of variance showed significant difference between ANF and control only in basal conditions (F=4.215). ANF enhanced the reflex gain especially at low levels of reflex bradycardia.
The average regression slope obtained by reducing systolic blood pressure by nitroglycerin showed a slight, but significant, reduction after ANF infusion (from $-9.3\pm1$ to $-5.6\pm0.6$ msec/mm Hg, $p<0.05$) (Figure 1). Inspection of the reflex gain confirmed that ANF administration tended to depress the reflex tachycardia for each level of nitroglycerin-induced hypotension (Figure 3, upper panel).

After chronic converting enzyme inhibition with enalapril, the baseline reflex chronotropic response to phenylephrine was not different from that observed before enalapril (Figure 1). In contrast, the average regression slope of the response to nitroglycerin was significantly reduced ($-5.9\pm0.5$, $p<0.05$ vs. pretreatment baseline value) (Figure 1).

ANF infusion during converting enzyme inhibition was unable to modify both chronotropic baroreflex responses. In particular, after enalapril, ANF did not enhance the bradycardic response to phenylephrine (Figures 1 and 2, lower panel) and did not blunt the reflex response to nitroglycerin (Figures 1 and 3, lower panel) as observed in control conditions.

To evaluate the possible influence of venous pressure, the effects of ANF on baroreflex responses were studied also in the supine and sitting positions. As shown in Table 2, ANF enhanced the bradycardic response to phenylephrine and attenuated the tachycardic response to nitroglycerin independently of central venous pressure level.

**Cold Pressor Test**

Cold exposure caused significant increases in mean blood pressure and in heart rate that were similar before and during converting enzyme inhibition and were unaltered during the steady state of ANF infusion in both conditions (Figure 5).

The reproducibility of the reflex responses evoked by both the vasoactive drugs was assessed in a separate group of five subjects, both within the same session and in two different sessions separated by 5 days of placebo administration. The intrastudy variability for the regression slope obtained with phen-
Glycerin injection of ANF was 12.2% and 13.4%, respectively. The interstudy variability for the two regression slopes was 14.5% for phenylephrine and 15.3% for nitroglycerin.

Discussion

In the present study, the interactions of ANF with the baroreflex control of heart rate were examined in humans under control conditions and after converting enzyme inhibition.

Our major findings were 1) baroreflex-mediated bradycardia in response to phenylephrine administration is enhanced during ANF infusion, but this effect is abolished by converting enzyme inhibition, 2) baroreflex-mediated tachycardia caused by nitroglycerin injection is attenuated by ANF in control conditions but not after converting enzyme inhibition, 3) ANF does not affect the pressor and chronotropic responses to direct sympathetic stimulation evoked by cold exposure.

These results indicate that ANF, at the doses used in this study, modifies the arterial baroreflex responses and that this effect is prevented by converting enzyme inhibition.

It must be pointed out that the rate of infusion of ANF adopted in our protocol resulted in circulating levels of the peptide well above the physiological range. Therefore, our current data describe the influence of a pharmacological dose of ANF on arterial baroreflexes. To the extent that these findings can be extrapolated to pathophysiological conditions, it may be hypothesized that elevated ANF levels counteract the impairment of the baroreceptor-mediated chronotropic reflex observed in congestive heart failure as well as during water immersion or volume loadings, thus antagonizing an excessive degree of tachycardia.

Our results are substantially consistent with previous findings obtained by ourselves in the anesthetized rabbit and by other investigations in the conscious rat. They are also largely consistent with the results obtained in a recent report by other investigators showing that lower doses of ANF (15-25 ng/kg/min) attenuated the baroreflex-mediated cardioacceleration and only tended to augment reflex bradycardic responses in humans. In that study, the reflex chronotropic responses were evoked by brief (5 seconds) and selective manipulations of the carotid baroreceptors, and it is possible that the slight discrepancies between the two studies are accounted for by the different techniques (as well as by different dosages of ANF). In fact, the two techniques for assessing arterial baroreflexes provide information on different aspects of baroreflex function. The technique for studying arterial baroreflexes adopted in our study was developed at the Oxford Center in 1969; it has been widely tested and provides a valid estimate of the gain of the R-R interval-systolic pressure relation. Furthermore, it has been recently found consistent with the closed-loop approach for studying arterial baroreflexes described by Pagani et al. A more detailed analysis of the baroreflex responses was also performed in our study by providing the behavior of the reflex gain in response to the graded pressure variations for both phenylephrine and nitroglycerin. This analysis is independent from the magnitude of the blood pressure response.

Our current findings show that the influence of ANF on baroreflex responses is complex because augmentation of bradycardic and attenuation of
tachycardic reflex responses were found. The mechanisms underlying the effect of ANF on arterial baroreflexes are unclear.

This effect of ANF might be related to the described interference of the peptide with cardiopulmonary receptors with vagal afferents, which modulate arterial baroreflex activity. ANF, however, sensitizes cardiac vagal afferents, thus increasing the influence of cardiac mechanoreceptors reflexes. This should result in an attenuation of arterial baroreflexes, rather than an augmentation, as observed for bradycardic reflex responses in our study. On the other hand, an involvement of cardiac mechanoreceptors in the baroreflex response to ANF based on the hemodynamic effect of the peptide appears to be unlikely, because ANF produces a decrease in cardiac filling pressure and does not increase the inotropic state.

An alternative mechanism is that ANF increases vagal influences on the circulation. Such a possibility is suggested by previous observations from our laboratory, showing that in the anesthetized rabbit the effects of ANF on systemic hemodynamics and baroreflex responses are prevented by vagotomy. The potentiation of vagal tone by ANF would explain the asymmetric influence on arterial baroreflexes observed in our study, that is, enhancement of bradycardia and reduction of tachycardia. The mechanisms by which ANF may increase vagal influences on the heart are not known. Because ANF acts as a functional antagonist of angiotensin II, we have hypothesized that this effect of ANF on vagal tone might be related to antagonism of the effects of angiotensin II. In fact, Ang II has a remarkable effect on the sensitivity of the baroreceptor–heart rate reflex, which is a consequence of its vagolytic action. Although alternative possibilities related to other effects of converting enzyme inhibitors cannot be excluded, the observation that enalapril abolished the influence of ANF on baroreflex responses supports this hypothesis. In this regard, the possibility that a nonspecific resetting of low pressure baroreceptors caused by a decreased cardiac preload during converting enzyme inhibition might have been responsible for our observations with enalapril is unlikely. In fact, ANF caused similar changes of baroreflex responses independently of central venous pressure changes produced by postural maneuvers. Similarly, it appears unlikely that the influence of sustained converting enzyme inhibition on baroreceptor responses was mediated by an interaction with the sympathetic nervous system. In fact, the hemodynamic response to cold was comparable before and during enalapril.

Finally, the observation that ANF did not affect the blood pressure responses to vasoactive agents or the sympathetically mediated reflex response to cold minimizes the possibility that the effects of ANF on baroreceptor responses are mediated by peripheral vascular effects or interactions with the direct sympathetic responses.

In conclusion, it seems reasonable to speculate that the influence of ANF on reflex chronotropic responses to baroreceptor manipulation might be related to functional antagonism of Ang II actions on baroreflex pathways. Our experiments cannot clarify at which level ANF may antagonize the effects of Ang II. The observation that the distribution of ANF-containing neurons and receptors in the central nervous system overlap with that observed for Ang II and that intracerebroventricular administration of ANF antagonizes the biological effects of Ang II may suggest an interaction of the two peptides at the central nervous system level.

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KEY WORDS • angiotensin II • atrial peptides • baroreceptors • congestive heart failure
Converting enzyme inhibition prevents the effects of atrial natriuretic factor on baroreflex responses in humans.
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