Reduction of atrial natriuretic factor circulating levels by endogenous sympathetic activation in hypertensive patients

MASSIMO VOLPE, M.D., NICOLA DE LUCA, M.D., STEVEN A. ATLAS, M.D., MARIA J. CAMARGO, M.D., CIRO INDOLFI, M.D., GIUSEPPE LEMBO, M.D., BRUNO TRIMARCO, M.D., MARIO CONDORELLI, M.D., AND JOHN H. LARAGH, M.D.

ABSTRACT The effects of endogenous activation of sympathetic nervous system on systemic and regional hemodynamics and on plasma levels of atrial natriuretic factor (ANF) were studied in subjects with essential hypertension. Stimulation of sympathetic nervous system was reflex-induced by a selective deactivation of carotid baroreceptors obtained by increasing external neck-tissue pressure (NTP) by means of a neck chamber. The effects of graded levels ( +30, +45, and +60 mm Hg) and one single and sustained level (+45 mm Hg for 15 min) of NTP were studied. As expected, NTP caused reflex increases in blood pressure, heart rate, and forearm vascular resistance, whereas atrial pressures did not change significantly and cardiac output tended to increase. In the studies based on graded levels of NTP, immunoreactive ANF (irANF) progressively fell (from 31.7 ± 10 to 13.3 ± 4 fmol/ml; p < .05) and the changes in irANF were significantly correlated with those observed in FVR (r = -.671, p < .001). Both hemodynamic and irANF changes were prevented by adrenergic blockade (phenolamine + propranolol). During +45 mm Hg NTP for 15 min, the levels of irANF fell both in the pulmonary artery and in the inferior vena cava. The irANF arteriovenous difference also fell during this maneuver. These data show that, in hypertensive patients, factors other than atrial wall tension may influence ANF release. They also show that endogenous sympathetic activation may reduce ANF release.


A LARGE body of evidence indicates that the degree of atrial stretch or wall tension is a predominant factor in the regulation of atrial natriuretic factor (ANF) release in isolated heart preparations,1, 2 intact animals,3, 4 and human subjects.5, 6 Several maneuvers, including blood volume expansion,3, 5-7 mechanical distention of atria,4, 8 and increases in central blood volume induced by head-out water immersion9, 10 or head-down till11, 12 have been shown to raise ANF plasma levels. In addition, elevated circulating levels of the peptide have been found during atrial tachyarrhythmias13 and in other pathologic states associated with increased atrial filling pressure, such as congestive heart failure,14-16 renal failure with fluid retention,17 and primary aldosteronism.18

It is not clear to date whether factors other than atrial wall tension may be involved in the control of ANF release. In this regard, it has been shown that several hormonal agents,18, 19-22 cardiac nerve activity,8 and heart rate23 may also contribute to regulate ANF secretion. With regard to the possible influence of sympathetic nervous system on ANF release, the administration of pharmacologic doses of catecholamines has been shown to increase ANF levels,9, 21 but this effect could be explained by concurrent increases in atrial pressures. Under more physiologic conditions, the hemodynamic profile associated with activation of the sympathetic nervous system may be quite different from that observed during exogenous infusion of the agonists.

We therefore decided to investigate the effect of endogenous sympathetic activation on ANF release in man. For this purpose, we determined the levels of plasma ANF and the changes in systemic and regional hemodynamics during reflex activation of the sympa-
thetic nervous system induced by selective carotid baroreceptor unloading.

Methods

Patients. The study was performed in patients of both sexes with established mild or moderate essential hypertension. In all patients, the existence of major cardiac or systemic diseases other than hypertension had been previously excluded by accurate clinical, laboratory, ultrasound, and x-ray studies. Severe cardiovascular compromise caused by hypertension was excluded on the basis of clinical and ultrasound studies. Impairment of cardiovascular reflexes was also ruled out before the study by appropriate tests (Valsalva maneuver, tilt, and cold pressor test). Informed written consent was obtained in all cases and the protocol was approved by the Research Committee of our Institution. The subjects did not receive treatment for 3 weeks before the study.

Protocol. All subjects were on a daily diet containing 150 meq Na+, 60 meq K+, and 1500 ml of fluids for 5 days before the studies. On the day preceding the study the subjects avoided caffeine, smoking, and strenuous exercise and were made familiar with the procedure and the neck-chamber technique.

All studies were started at 9 A.M. with the subject in a supine position after an overnight fast. No premedication was used. Under local anesthesia with 2% lidocaine, a heparinized catheter was introduced percutaneously through a femoral artery for direct measurement of arterial blood pressure, which was continuously monitored by pressure transducers on a multichannel polygraph. Heart rate was also monitored through ECG lead II. Right atrial pressure was recorded through the proximal hole of a Swan-Ganz triple-lumen catheter introduced through the femoral vein. The tip of this catheter was positioned in the pulmonary artery to measure the cardiac output by the thermodilution technique. For this purpose, the catheter was connected to a thermodilution computer.

Finally, the tip of this catheter was periodically advanced to the pulmonary wedge position to obtain measurements of pulmonary capillary wedge pressure as an index of left atrial pressure. All tracings were recorded simultaneously at a paper speed of 25 mm/sec. To evaluate hemodynamic changes in the forearm vascular bed, two-dimensional Doppler flowmetry of the right brachial artery was also performed according to the technique previously described from our laboratory.24 Forearm vascular resistance was then calculated, as previously described24 and expressed in mm Hg/ml/sec.

Femoral blood samples for measurements of plasma renin activity (PRA), plasma norepinephrine (PNE), and immunoreactive ANF (irANF) were obtained during the last 30 sec of each experimental phase. Samples for irANF measurements were also obtained from the inferior vena cava and from the pulmonary artery in two of the experimental protocols, as described below.

Three protocols were performed. In protocol 1, effects of graded increases in neck-tissue pressure on peripheral venous levels of irANF were assessed. In protocol 2, effects of graded increases in neck-tissue pressure on the levels of irANF measured in the inferior vena cava and in the pulmonary artery with the simultaneous assessment of cardiac output were determined. The time-course of the hemodynamic and hormonal effects produced by a single, sustained increase in neck-tissue pressure was evaluated in protocol 3.

Protocol 1. This protocol was performed in 11 patients. After stabilization, baseline measurements of blood pressure, heart rate, forearm vascular resistance (FVR), right atrial and pulmonary wedge pressures, irANF, PRA, and PNE were obtained. Graded increases in external neck pressure (+30, +45, and +60 mm Hg) were then rapidly applied and maintained for 4 min each by means of a variable pressure pneumatic neck chamber similar to that previously described by Ludbrook et al.25 About 86% of this pressure is transmitted to the tissues adjacent to the carotid sinus and increases in neck pressure up to 60 mm Hg are not associated with changes in cerebral blood flow and do not involve chemoreceptors in the reflex effect.25 This procedure induces a proportional reduction in carotid transmural pressure, which produces baroreceptor deactivation with a consequent reflex sympathetic stimulation. Steady-state hemodynamic responses (the average value of 30 measurements obtained during the last 60 sec of each phase) were used for the analysis. After 15 min of recovery, hemodynamic and hormonal measurements were obtained again.

Protocol 2. Five patients were studied with this protocol. In these subjects the same neck-chamber procedure described above was performed. However, to further clarify the nature of the changes in irANF observed during the first protocol, the levels of irANF were determined on samples obtained simultaneously from the pulmonary artery and from the inferior vena cava. In addition, cardiac output was determined during the last minute of each experimental phase as the average of three to five measurements. The same protocol was repeated 5 min after the intravenous administration of propranolol (0.15 mg/kg) and phentolamine (0.2 mg/kg). These doses have been previously shown to produce an effective blockade of adrenergic receptors, which lasts for at least 20 min.24,25

Protocol 3. In five additional patients, a single prolonged (15 min) increase in neck-tissue pressure (+45 mm Hg) was applied. Samples from the inferior vena cava and pulmonary artery for measurements of irANF and hemodynamic measurements, including cardiac output, were obtained in control conditions and at 5, 10, and 15 min during the maneuver.

PRA was measured by radioimmunoassay as previously described.29 PNE concentrations were measured by using cation-exchange high-performance liquid chromatography with electrochemical detection after purification and concentration with alumina.29 Plasma ANF was measured by radioimmunoassay according to the technique previously reported,10 with rabbit antiserum (RAS 8798, Peninsula Laboratories Europe), iodinated human ANF (200 Ci/mmol; Amersham), and α-hANP1-28 (Bissendorf Peptide GMBH) as a standard. Plasma samples were extracted by C-18 Sep-Pak cartridges (Waters Inc.) as previously described.10 Recoveries, determined for each plasma sample by adding a small amount of radiolabeled ANF, ranged from 67% to 86%. Intra-assay and interassay coefficients of variation were 6.6% and 10.5%, respectively. The sensitivity of the radioimmunoassay was 1 fmol/tube.

Analysis of data. Statistical analysis was performed by analysis of variance and Dunnett t test was used for post hoc comparisons. Linear regression analysis was used to plot hemodynamic changes vs the corresponding changes in ANF for each level of neck tissue pressure. Data are presented as mean ± SE.

Results

Clinical characteristics of the patients included in the study are presented in table 1.

Figure 1 shows the effects of graded levels of neck-tissue pressure (NTP) on hemodynamic variables and on plasma levels of ANF. The progressive reflex sympathetic activation was associated with a rise in blood pressure (F = 11.1, p < .01), heart rate (F = 9.17, p < .01), and FVR (F = 7.05, p < .01), which achieved significance at the lowest level of increase in
neck pressure. These variables tended to return toward baseline after withdrawal of the stimulus.

Right atrial pressure did not change significantly (F = 1.58, p = NS) but tended to increase slightly during NTP. Despite this, plasma levels of ANF fell progressively (F = 3.62, p < .05) (figure 1). As shown in table 2, left atrial pressure, as evaluated by pulmonary wedge pressure, also did not change significantly during NTP, nor did PNE or PRA levels.

Regression analysis performed between changes in hemodynamic variables and ANF during NTP showed a significant correlation between the baroreceptor-mediated changes in FVR and the changes in plasma ANF concentrations (r = .671, p < .001).

Since changes in cardiac output could conceivably lead to short-term alterations in plasma ANF levels unrelated to a change in secretion rate, in five additional patients we measured simultaneous arterial and venous ANF levels as well as cardiac output during graded NTP.

As shown in table 3, at each level of NTP the decreases in plasma ANF levels measured in the pulmonary artery exceeded those measured in the inferior vena cava, so that the arteriovenous difference decreased progressively with each increase in carotid baroreceptor deactivation. In addition, the magnitude of the decreases in arteriovenous difference consistently exceeded tendency (not significant) for cardiac output to increase. These data suggest a minimal influence of the hemodynamic component in the observed change in ANF and moreover suggest that secretion rate decreased.

To evaluate the influence of the baroreceptor-mediated sympathetic reflex activation on ANF release, in

| TABLE 1 |
| Clinical characteristics of the patients (n = 11) |
| -- | -- |
| Age years | 41.2 ± 2 |
| Systolic blood pressure (mm Hg) | 164.4 ± 5 |
| Diastolic blood pressure (mm Hg) | 102.6 ± 3 |
| Heart rate (beats/min) | 74.2 ± 2.8 |
| Weight (kg) | 72.8 ± 3.2 |
| PRA (ng/ml/hr) | 1.79 ± 0.4 |
| Plasma aldosterone (pg/ml) | 169 ± 18 |
| Urinary excretion of sodium (meq/24 hr) | 132 ± 7 |

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| TABLE 2 |
| Effects of graded carotid baroreceptor deactivation on pulmonary wedge pressure, PNE, and PRA |
| -- | -- |
| NTP (mm Hg) | 0 | +30 | +45 | +60 |
| Pulmonary wedge pressure (mm Hg) (n = 4) | 8.7 ± 0.8 | 8.2 ± 1 | 7.8 ± 0.5 | 7.9 ± 0.7 | 6.9 ± 0.8 |
| PNE (pg/ml) | 197 ± 31 | 202 ± 36 | 224 ± 42 | 239 ± 51 | 244 ± 42 |
| PRA (ng/ml/hr) | 2.7 ± 0.6 | 2.9 ± 0.7 | 2.8 ± 0.7 | 2.8 ± 0.7 | 2.7 ± 0.7 |

FIGURE 1. Effects of graded increases in NTP on FVR, heart rate (HR), mean blood pressure (MBP), right atrial pressure (RAP), and plasma ANF (IR-ANF). * p < .05 vs baseline.

this group of patients the three levels of NTP were applied again after adrenergic blockade. As shown in table 3, after propranolol plus phentolamine, a progressive increase in external NTP failed to reduce plasma levels of ANF in blood from either the pulmonary artery or inferior vena cava.

Control experiments performed to evaluate the specificity of the changes observed during NTP demonstrated that both hemodynamic variables and ANF plasma levels were stable during repeated measurements performed at 5 min intervals in the absence of any stimulus over a 30 min period (mean blood pressure − initial value, 120 ± 3 mm Hg, final value, 122 ± 4 mm Hg, n = 6, p = NS; irANF − initial value, 33 ± 7 fmol/ml, final value, 31 ± 8 fmol/ml, n = 6, p = NS).
TABLE 3
Effects of graded increases in external NTP on ANF levels (fmol/ml) in pulmonary artery, inferior vena cava, arteriovenous difference, and cardiac output (liters/min) in five hypertensive subjects before and after adrenergic blockade (propranolol 0.1 mg/kg + phenolamine 0.15 mg/kg iv)

<table>
<thead>
<tr>
<th>Group</th>
<th>NTP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>30.8±9.5</td>
</tr>
<tr>
<td>IVC</td>
<td>16.1±4.7</td>
</tr>
<tr>
<td>AV</td>
<td>14.7±5.0</td>
</tr>
<tr>
<td>CO</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>Adrenergic blockade</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>19.7±7.4</td>
</tr>
<tr>
<td>IVC</td>
<td>15.9±5.9</td>
</tr>
<tr>
<td>AV</td>
<td>5.1±2.9</td>
</tr>
<tr>
<td>CO</td>
<td>4.5±0.6</td>
</tr>
</tbody>
</table>

PA = pulmonary artery; IVC = inferior vena cava; AV = arteriovenous difference; CO = cardiac output.

To more fully characterize the time course of the changes in ANF levels during NTP, in five additional patients we investigated the hemodynamic effects and the changes in arterial and venous ANF concentrations during a sustained increase in NTP (+ 45 mm Hg for 15 min). As shown in figure 2, in these subjects plasma levels of ANF decreased promptly in response to carotid baroreceptor deactivation and the reduction in the circulating levels of the peptide persisted throughout the maneuver. In addition, no change in atrial pressure was observed in this group of subjects.

Discussion

The evidence provided by the present studies suggests that endogenous activation of the sympathetic nervous system may lead to a decrease in ANF secretion independent of changes in atrial pressure.

This conclusion is in contrast with those of earlier studies in which the effects of exogenous catecholamines were studied in vitro or in intact animals. However, the protocol used in these latter studies did not permit one to determine whether the observed increase in plasma ANF was a direct effect of the exposure to high levels of neuromediators or whether it was an indirect effect of the concomitant changes in atrial wall tension. In this regard, a large number of experimental studies and clinical observations clearly indicates that the level of atrial wall tension is a predominant factor in the regulation of ANF release. The experimental approach adopted in the present study represents a more physiologic attempt to study the peripheral effects induced by changes in effrent sympathetic discharge to the heart. In contrast to the exogenous infusion of sympathetic agonists, the magnitude of sympathetic stimulation produced by these levels of reduction in carotid transmural pressure did...
not produce changes in atrial wall tension, as suggested by lack of significant changes in atrial pressures during the studies.

Reflex stimulation of the sympathetic nervous system is the most prominent effect of carotid baroreceptor deactivation, and the increases in heart rate, blood pressure, and FVR observed during this maneuver provide evidence for sympathetic nervous system activation. Although the observation that plasma norepinephrine concentrations did not rise during the increase in external neck tissue pressure might speak against the postulated sympathetic stimulation, there is evidence that the manipulation of a single reflexogenic area, such as carotid baroreceptors, is not sufficient to alter the level of circulating catecholamines, even though it is capable of inducing significant sympathetically mediated hemodynamic effects. A more generalized involvement of arterial baroreflexes, as induced by tilting, exercise, or exposure to cold, is likely required to increase circulating levels of catecholamines, reflecting a more pronounced stimulation of the sympathetic nervous system.

The experiments based on graded increases in external NTP, which resulted in a progressive stimulation of the sympathetic nervous system, demonstrated a progressive reduction in the levels of irANF measured both in peripheral venous as well as pulmonary arterial blood, with a significant reduction in the arteriovenous difference across the heart. These changes induced by carotid baroreceptor deactivation were prevented by adrenergic blockade (intravenous phenolamine + propranolol), this latter observation further supporting a relationship between sympathetic drive to the heart and ANF plasma concentrations. Our results not only suggest that sympathetic discharge can alter plasma ANF levels but also suggest indirectly that the changes in ANF concentration observed during reflex sympathetic activation are related to a reduced secretion of the peptide.

For a hormone secreted by the heart via the coronary sinus, secretion rate can be estimated under steady-state conditions by the arteriovenous difference across the heart (e.g., pulmonary arterial concentration minus mixed venous blood before the coronary sinus) times cardiac output, since this term is equal to whole body clearance rate (which should equal secretion rate at steady state). In the case of ANF, however, it is difficult to determine the proper mixed venous sample, since the concentration in the superior vena cava may be greater than that in the inferior vena cava, suggesting that clearance by the lower portion of the body is greater. Therefore it would not be correct to calculate secretion rate from our data. If one assumes, however, that proportional falls in venous blood levels of ANF-like immunoreactivity were to occur in superior and inferior venae cavae (as seems likely), then the fact that the arteriovenous difference fell, not only during the graded increase in NTP but also during the sustained increase to a single level, can be taken as probable evidence of reduced secretion. This interpretation is reinforced by the observation that the associated increases in cardiac output (which could theoretically lead to a transient decrease in pulmonary arterial ANF concentration) were very small compared with the decreases in arteriovenous difference.

With regard to the intrinsic mechanisms involved in the ANF response to carotid baroreceptor deactivation, it should be mentioned that vagal inhibition might have contributed to decrease the circulating levels of the peptide. In fact, it has been suggested that acetylcholine may release ANF via the stimulation of muscarinic receptors. However, it is known that arterial baroreceptor-induced cardiovascular changes largely depend on modulation of sympathetic discharge. Therefore, it seems reasonable to postulate that the changes in ANF release are also related to sympathetic activation. Recent indirect observations are consistent with the possibility that adrenergic stimulation can reduce ANF secretion. In experiments performed in superfused beating atria, Schiebinger has shown that dibutyryl-cAMP reduces ANF secretion. In addition, Raine et al. reported reduced ANF plasma levels during ventricular pacing associated with systemic hypotension and possibly, with reflex sympathetic stimulation in man. Also Cody et al. found a significant inverse correlation between the changes in PNE and ANF levels during maximal exercise in hypertensive patients and suggested that adrenergic stimulation may counterregulate the anticipated ANF release associated with increased atrial pressure during maximal exercise. Finally, the observation that in anesthetized and vagotomized dogs, sympathetic stimulation induced by maximum electrical stimulation of the right ansa subclavia failed to produce any change in irANF despite an increase in heart rate of about 100 beats/min might further support the hypothesis that sympathetic stimulation per se reduces ANF secretion.

Further study is required to determine the physiologic relevance of our findings. It could be hypothesized that in conditions characterized by sympathetic stimulation, such as orthostatic stress exercise, shock, or marked hypotension, reduced secretion of ANF might be important functionally in maintaining blood
volume and pressure homeostasis through a reduced loss of fluids from the intravascular space.

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