Atrial Natriuretic Factor and Arterial Baroreceptor Reflexes in Unanesthetized Rats

Alberto U. Ferrari, Anna Daffonchio, Carla Sala, Silvia Gerosa, and Giuseppe Mancia

The modulation exerted by atrial natriuretic factor (ANF) on the cardiac and vascular influences of arterial baroreceptors was investigated in two groups of unanesthetized, chronically instrumented normotensive rats. In group 1, the reflex control of heart rate was assessed by graded baroreceptor stimulations and deactivations obtained by intravenous boluses of phenylephrine and nitroprusside. Under either circumstance, baroreceptor reflex sensitivity was expressed as the linear regression slope relating the chronotropic responses to the drug-induced mean arterial pressure changes. In group 2, right common carotid occlusion was performed in rats with their aortic and left carotid sinus baroreceptors denervated to assess the baroreceptor control of blood pressure; the reflex response was quantitated as the peak blood pressure rise observed during the maneuver. The reflex studies were performed before and during atriopeptin III infusion (0.15–0.20 μg/kg/min for 60 minutes). ANF augmented the bradycardic response to phenylephrine by 102.5±29% (p<0.01), reduced the tachycardic response to nitroprusside by 67.7 ±6.4% (p<0.01), and failed to modify the pressor response to carotid occlusion (−6.8±2.1%, p=NS). In a separate group of rats infused with low dose nitroprusside, no change in the baroreceptor-heart rate reflex was observed. ANF infusion (0.20 μg/kg/min) performed in further separate groups of conscious rats raised plasma ANF to 480±58 fmol/ml. Values in control vehicle-infused rats were 50±8 fmol/ml. Vascular reactivity (pressor response to intravenous phenylephrine boluses in anesthetized, sinoaortic-denervated rats) was only minimally reduced by ANF. It is concluded that ANF at doses within the pathophysiological range exerts a complex modulatory effect on arterial baroreceptor reflexes, consisting of potentiation of the cardioinhibitory, no change of the vascular, and depression of the cardioexcitatory reflex influences. These data may have pathophysiological implications in high ANF release states. (Hypertension 1990;15:162–167)

The involvement of atrial natriuretic factor (ANF) in the neural control of circulation is suggested by several lines of evidence. First, ANF and ANF receptors are localized in the nucleus tractus solitarii, in the area postrema, in the anteromedial region of the third ventricle, and in other central sites known to be involved in cardiovascular regulation.1,2 Second, ANF-like substances activate vagal afferents capable of inhibiting efferent sympathetic nerve activity.3,4 Third, Volpe et al5 have shown in anesthetized rabbits that ANF enhances the bradycardic response to injection of a vasopressor drug, suggesting that this hormone potentiates the arterial baroreceptor reflex. However, in the same study other baroreceptor responses were unaffected by ANF. In addition, Ackermann et al6 observed in anesthetized rats that the hypotension induced by atrial extracts is not accompanied by the expected tachycardia, which suggests an impairment rather than a potentiation of the baroreceptor reflex by ANF.

Because of these discrepancies, we decided to reassess the effects of ANF on the arterial baroreceptor reflex in Sprague-Dawley rats. At variance with previous studies, the evaluation was conducted in unanesthetized rats with ANF doses that minimally affected baseline hemodynamics and increased plasma ANF levels within the range seen in cardiovascular diseases. Furthermore, the baroreceptor reflex was tested by several techniques so that not only the cardiac but also the vascular reflex responses could be assessed.

Methods

Surgical Procedures

The study was conducted in 35 normotensive Sprague-Dawley rats of either sex that were 11.5±0.8 weeks old (mean±SEM) and weighed 269±16 g.
In 16 rats (group 1), anesthesia was induced by ketamine HCl (80 mg/kg i.p.). Polyethylene catheters were placed in both femoral veins and in one femoral artery, passed subcutaneously, and exteriorized at the interscapular region. All catheters were periodically flushed with heparinized saline (0.01% solution) to keep them patent until the time of study (see below).

In another eight rats (group 2) similarly anesthetized by ketamine, catheters were placed in one femoral vein and in one femoral artery and exteriorized as in the previous group. The neck was opened via an anterior midline incision. The aortic and superior laryngeal nerves were cut bilaterally according to the technique described by Krieger. The carotid sinus area of the left side was denervated by stripping the vessel walls from their connections to the surrounding tissues, but the carotid sinus area of the contralateral side was left intact. A balloon-in-cuff occluder, consisting of an inflatable Silastix tube (Dow Corning, Midland, Michigan) and a rigid PE-320 cuff, was positioned around the right common carotid artery. The balloon was connected to a PE-50 extension that was passed subcutaneously and exteriorized at the interscapular region so that reversible common carotid artery occlusion could be performed by injection of 0.05–0.05 ml fluid into the occluder. The intravascular catheters were periodically flushed as in group 1.

In a further 11 rats (group 3), ketamine anesthesia was used to implant a PE-50 catheter in one femoral artery and a thin PE-10 catheter in one femoral vein. The catheters were exteriorized dorsally as in the previous groups and subsequently used for rapid collection of arterial blood and for intravenous infusion.

**Protocols**

After surgery, each rat was individually housed in a wide plastic box with free access to food and water until after the end of the experimental protocol. All rats were allowed 48 hours to recover from surgery and anesthesia and to become accustomed to the environment.

Arterial blood pressure was continuously recorded via a Statham P23Dc transducer (Gould-Statham, Oxnard, California) and displayed on a Grass polygraph (Grass Instr. Co., Quincy, Massachusetts). Mean arterial pressure was obtained by electronic damping of the pulsatile signal, and heart rate was continuously measured by a cardiotachometer triggered by the pulse pressure wave.

In group 1, four doses of phenylephrine and four doses of nitroprusside (0.5, 1, 2, and 4 μg/kg for both drugs) were administered as intravenous boluses to respectively stimulate and deactivate arterial baroreceptors, thus eliciting reflex bradycardic and tachycardic responses. In nine rats, the injections were performed in the control condition and in the last 20–30 minutes of a 1-hour intravenous infusion of atriopeptin III (Sigma Chemical Co., St. Louis, Missouri). The infusion rate was 0.2 μg/kg/min, a dose that in preliminary experiments had been shown to raise plasma ANF levels to pathophysiological values without grossly altering arterial blood pressure and heart rate. The phenylephrine and nitroprusside injections were performed 3 minutes apart in random order. In the remaining seven rats, the protocol was the same, but atriopeptin III infusion was replaced by an infusion of nitroprusside at a dose of 0.2–0.3 μg/kg/min. This dose was selected to match the small hypotension induced by atriopeptin III and allowed us to determine whether the effects on the baroreceptor reflex observed with this peptide could be reproduced by a nonspecific vasodilator.

In group 2, three to five occlusions of the right common carotid artery were performed to deactivate right carotid baroreceptors, thus eliciting a reflex pressor response. The occlusions were performed in the control condition and during the last 20–30 minutes of a 1-hour infusion of atriopeptin III. The infusion rate was 0.15 μg/kg/min because, after partial baroreceptor denervation, higher ANF infusion rates induced a pronounced blood pressure fall. Each carotid occlusion was maintained for 10–20 seconds and was separated from the preceding one by a 3-minute interval.

In group 3, atriopeptin III (0.2 μg/kg/min in 0.3–0.5 ml total fluid, five rats) or vehicle (0.5 ml/hour, six rats) was infused for 45 minutes. Blood samples (1.0–1.5 ml) were drawn from the arterial catheter during the final 5 minutes of the infusion. Plasma was collected in prechilled tubes containing EDTA, centrifuged at 4°C, and stored at −80°C until assayed. Plasma ANF was measured by radioimmunoassay after extraction on C18 Sep-Pak Cartridges (Waters Chromatography Division, Milford, Massachusetts). The assay used an antiserum raised against human ANF-(99–126) (Peninsula Lab. Europe, St. Helens, UK), which has 100% cross-reactivity with rat ANF-(99–126) and with atriopeptin III. The sensitivity of the assay was 0.5 fmol/tube with an IC50 of 6.84 fmol/tube. The intra-assay and interassay variations were 7.5% and 11%, respectively.

**Studies on Effects of Atrial Natriuretic Factor on Vascular Reactivity**

In a further group of eight rats anesthetized by ketamine HCl, the aortic nerves were bilaterally cut and both carotid sinuses were denervated according to the procedures described above. Catheters were implanted in one femoral artery and in one femoral vein to allow measurements of the pressor effects of intravenous boluses of phenylephrine (1 and 2 μg/kg) before and during an infusion of atriopeptin III at the dose of 0.15 μg/kg/min. This was done to examine whether ANF interferes with the vasoconstrictor responses to α-adrenergic stimuli, thereby affecting the responses to reflex stimuli at a peripheral level.

**Data Analysis**

In each rat, data on baroreceptor control of heart rate were treated as linear regressions, separately for...
Table 1. Effects of Atriopeptin III Infusion on Baseline Hemodynamics in Unanesthetized Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>AP III (0.2 µg/kg/min)</th>
<th>Control</th>
<th>AP III (0.15 µg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>141.8±5.0</td>
<td>129.3±4.5*</td>
<td>149.6±4.4</td>
<td>136.3±5.9*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84.4±4.2</td>
<td>85.1±5.4</td>
<td>96.5±3.7</td>
<td>93.3±4.5</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>105.2±4.8</td>
<td>100.0±5.0*</td>
<td>116.9±3.9</td>
<td>109.7±3.4*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>363.5±9.8</td>
<td>363.4±14.0</td>
<td>407.1±6.2</td>
<td>409.3±6.4</td>
</tr>
</tbody>
</table>

Data are mean±SEM of nine rats in group 1 and of eight animals in group 2. Data from group 1 and 2 are shown separately. AP III, atriopeptin III; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

*p<0.05 during AP III vs. control.

Atrial Natriuretic Factor and Baroreceptor–Heart Rate Reflex

As shown in Table 1, ANF (0.2 µg/kg/min) slightly lowered systolic and mean arterial pressure in group 1 rats (nine rats). Diastolic pressure was unaffected by the infusion of this substance, which also failed to modify heart rate.

However, these minor hemodynamic effects were accompanied by clearcut alterations of the baroreceptor–heart rate reflex. As illustrated in the examples of Figure 1, the bradycardic responses to baroreceptor stimulation induced by phenylephrine was markedly potentiated by ANF. In contrast, the tachycardic responses to baroreceptor deactivation by nitroprusside were markedly blunted. These changes were observed in virtually all rats and were significant in the group as a whole, amounting to an average increase and reduction in baroreceptor reflex sensitivity of 102.5% and 67.7%, respectively (Figure 2).

In the seven rats receiving nitroprusside injection, the decrease in blood pressure was as small as in the rats receiving ANF. Heart rate showed a small and nonsignificant increase, and the baroreceptor reflex sensitivities observed during baroreceptor stimulation and deactivation were similar before and during the infusion of the vasodilator (Table 2).

Atrial Natriuretic Factor and Baroreceptor–Blood Pressure Reflex

As shown in Table 1, baseline blood pressure and heart rate were slightly higher in group 2 rats than in group 1 rats, presumably as a result of the aortic baroreceptor denervation and the unilateral stripping of the carotid sinus. Similar to group 1, ANF infusion (0.15 µg/kg/min) caused a slight reduction in systolic and mean arterial pressure with no effects
on diastolic pressure and heart rate. In contrast to the prominent changes of the baroreceptor–heart rate reflex observed in group 1, however, in these rats the pressor response to common carotid artery occlusion was left totally unaffected by ANF as documented in the example of Figure 3 and in the mean group data shown in Figure 4.

Effects of Atrial Natriuretic Factor Infusion on Plasma Atrial Natriuretic Factor Levels

The unanesthetized rats subjected to vehicle infusion (group 3) had plasma ANF levels of 50±8 fmol/ml. In contrast, in the rats receiving atriopeptin III infusion at the rate of 0.2 μg/kg/min (i.e., the higher rate used in the studies on baroreceptor reflexes), plasma ANF levels were 480±58 fmol/ml (i.e., they were nine to 10 times higher).

Effects of Atrial Natriuretic Factor on Vascular Reactivity

As shown in Figure 5, in the eight sinoaortic-denervated rats that were given intravenous phenylephrine the rises in blood pressure were slightly less during ANF infusion as compared with before ANF infusion. This was the case for both the smaller and the larger dose of the vasopressor drug.

Discussion

Our data show in the unanesthetized rat that the reflex effects elicited by altering the activity of arterial baroreceptors is markedly modified by ANF infused at doses devoid of major blood pressure and heart rate influences. They further show that this is a specific effect because nitroprusside is unable to affect the ability of baroreceptors to modulate the sinus node. These findings strengthen the concept that ANF affects the cardiovascular system not only directly but also via modulation of neural cardiovascular control.

TABLE 2. Effects of Low Dose Nitroprusside Infusion on Baseline Hemodynamics and on Baroreceptor Control of Heart Rate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Nitroprusside infusion</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>107.6±3.8</td>
<td>102.4±3.1</td>
<td>0.05</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>360.9±8.5</td>
<td>389.0±11.3</td>
<td>NS</td>
</tr>
<tr>
<td>PNE BRS (msec/mm Hg)</td>
<td>0.76±0.1</td>
<td>0.88±0.1*</td>
<td>NS</td>
</tr>
<tr>
<td>NP BRS (msec/mm Hg)</td>
<td>0.91±0.2</td>
<td>0.83±0.1*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data (mean±SEM) refer to observations in seven unanesthetized rats before (control) and during a 0.2–0.3 μg/kg/min intravenous infusion of nitroprusside. Baroreceptor reflex sensitivity (BRS) was separately calculated from the bradycardic response to baroreceptor stimulation and the tachycardic responses to baroreceptor deactivation obtained by intravenous bolus injections of phenylephrine (PNE) and nitroprusside (NP), respectively. MAP, mean arterial pressure; HR, heart rate. p values refer to the significance of the changes observed during the infusion.

* P≤5.
However, the main result of the present study is that ANF affects the arterial baroreceptor reflex in a diversified fashion. In our rats, ANF had no effect on the pressor response to common carotid occlusion. This could not be ascribed to the fact that ANF-independent changes in the baroreceptor reflex arch were masked by an opposite effect of this hormone on the peripheral vasculature because the pressor responses to phenylephrine were only minimally affected by ANF infusion. It thus reflected a substantial preservation of the baroreceptor–blood pressure control during an increase in the circulating level of the hormone.

On the other hand, this increased level profoundly modified the baroreceptor–heart rate reflex. In line with previous data, the ability of baroreceptor stimulation to slow the heart was markedly enhanced by ANF. In contrast to previous data, however, this was accompanied by a pronounced depression of the ability of a baroreceptor deactivation to induce cardioacceleration. Thus, ANF resets the baroreceptor control of the heart in a way that favors cardioinhibition and opposes cardioexcitation. In our experimental conditions, this occurred with little change in blood pressure and no alteration in heart rate, suggesting that ANF modulation of reflex cardiac control may intervene before any more direct effect of the hormone on the circulation.

Although the plasma concentration of ANF produced by our infusions was almost 10 times higher than normal, it was still within the level that can be observed in cardiovascular diseases characterized by stimulation of ANF secretion. Therefore, our observations have pathophysiological implications: 1) Favoring reflex cardioinhibition and opposing reflex cardioexcitation implies that ANF can attenuate the autonomic stimulation of the heart that characterizes congestive heart failure and perhaps contributes to its progression. 2) If the above modification of the baroreceptor reflex includes its powerful control of the atrioventricular node, ANF might help depress arteriovenous conduction and terminate an episode of paroxysmal tachycardia. 3) The pronounced attenuation of the chronotropic response to baroreceptor deactivation induced by ANF offers an explanation for the absence of tachycardia in response to the acute hypotensive effect of exogenous doses of this hormone. 4) It might be that the pronounced potentiation of the bradycardic influence of the baroreceptor reflex induced by ANF is involved in the syncpe experienced by heart failure patients treated with angiotensin converting enzyme inhibitors. This might result from removal of the restraining influence of angiotensin II on the baroreceptor reflex leaving the potentiating influence of ANF unopposed.

Our study design did not allow identification of the mechanisms underlying the alterations of the baroreceptor reflex induced by ANF. Because vagal afferents are activated by atriopeptins, these alterations can result from the ability of cardiopulmonary receptors to modulate arterial baroreceptor influences on the heart and the peripheral circulation; however, the modulation consists of a depression (i.e., a phenomenon different from that observed in our study).

Another possibility is that ANF sensitizes baroreceptor afferents with an increase in their responsiveness during stimulation and a maintenance of their discharge during deactivation. However, in anesthetized rabbits, exogenous ANF has been shown to have no effect on aortic baroreceptor activity. Furthermore, baroreceptor sensitization would account only for the alterations in the heart rate influences of the reflex without explaining why the blood pressure influences were not affected. A third possibility is that ANF increases vagal influences on the heart by a central action, a peripheral action, or both, exaggerating and opposing, respectively, the effects of stimuli that tend to increase and reduce vagal tone. This would explain why ANF modified a vagally mediated function such as the baroreceptor–heart rate reflex but not a sympathetically mediated function such as the baroreceptor–blood pressure reflex. We are not aware of any evidence for or against this explanation, which may be indirectly supported, however, by the fact that ANF antagonizes angiotensin II, one action of which is to reduce the vagal control of the heart.

Finally, our data allow a methodological consideration to be made. It is clear from the diversified influence of ANF on the cardiac and blood pressure responses to baroreceptor stimulation and deactivation that studying the baroreceptor reflex over a limited portion of its stimulus-response curve and taking into account one target only can lead to erroneous conclusions. This indicates that, in either animal or human studies, it might be important to use all available technical approaches so that multiple baroreceptor reflex functions can be explored.
References


KEY WORDS • atrial natriuretic factor • baroreceptor reflex • cardiovascular reactivity • central nervous system • rat studies
Atrial natriuretic factor and arterial baroreceptor reflexes in unanesthetized rats.
A U Ferrari, A Daffonchio, C Sala, S Gerosa and G Mancia

Hypertension. 1990;15:162-167
doi: 10.1161/01.HYP.15.2.162

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/15/2/162