ANP and Bradycardic Reflexes in Hypertensive Rats
Influence of Cardiac Hypertrophy

Colleen J. Thomas, Geoffrey A. Head, Robyn L. Woods

Abstract—In previous studies we demonstrated that in normotensive rats, but not in spontaneously hypertensive rats (SHR), atrial natriuretic peptide (ANP) enhances bradycardic reflexes through an action on cardiac vagal afferent pathways. The present study aimed to determine whether cardiac hypertrophy, hypertension, or a nonreversible genetic factor accounted for the insensitivity of SHR to ANP action on cardiac reflex pathways. SHR were treated with the angiotensin-converting enzyme (ACE) inhibitor perindopril (3 mg/kg per day) for 6 weeks from 4 to 9 weeks of age (SHR-S, n=10) or for 9 weeks from 4 to 12 weeks of age (SHR-L, n=10) or were untreated (SHR, n=10) to produce differential effects on blood pressure and left ventricle/body weight ratio (LV/BW). Untreated normotensive Wistar-Kyoto rats (WKY, n=10) were also studied. At 13 weeks of age, all rats were instrumented with aortic and jugular catheters, and at 14 weeks we measured heart rate reflexes to rapid intravenous infusions of methoxamine (100 μg/kg, cardiac baroreflex) and serotonin (5 to 60 μg/kg, von Bezold-Jarisch cardiac chemosensitive reflex), with either α-rat ANP (150 ng/kg per minute IV) or saline vehicle (270 μL/h IV) infusion. Perindopril treatment for 6-week (SHR-S) and 9-week (SHR-L) durations maintained blood pressure at normotensive levels in both groups. SHR-S exhibited a small degree of cardiac hypertrophy (LV/BW was 8% higher than in WKY but 11% less than in untreated SHR), but LV/BW was normalized in SHR-L (to within 1% of WKY LV/BW). In WKY, ANP significantly (P<0.05) enhanced bradycardic responses to both the cardiac baroreflex (by 42±10%) and von Bezold-Jarisch chemosensitive reflex (by 17±5%) activation but had no effect in SHR. The cardiac reflex action of ANP was restored in SHR-L (ANP enhanced reflex bradycardia by 28±12% and 36±8%, baroreflex and von Bezold-Jarisch reflex, respectively; P<0.05), but SHR-S, which developed some cardiac hypertrophy, remained unresponsive to ANP. Our results suggest that the inability of ANP to sensitize cardiac vagal (nonarterial) afferents in SHR was not due to an inherited irreversible component, or the hypertension per se, but was associated with the presence of cardiac hypertrophy. A functional consequence of hypertension-induced cardiac hypertrophy may be the inhibition of the cardioprotective action of ANP through cardiac vagal reflexes. (Hypertension. 1998;32:548-555.)

Key Words: atrial natriuretic factor ■ baroreflex ■ reflex ■ hypertrophy, cardiac ■ perindopril ■ rats, inbred SHR

Atrial natriuretic peptide (ANP) does not appear to have direct negative chronotropic effects on the heart,1-3 but in recent years there has been growing evidence that the cardiac hormone influences one or more aspects of the reflex control of heart rate (HR).4-7 Studies in our laboratory have provided evidence that ANP acts preferentially on nonarterial or cardiopulmonary afferent pathways, leading to cardiac slowing.8,9 In conscious, instrumented normotensive rats, we observed that intravenously administered ANP enhanced reflex bradycardia when a rapid “ramp” rise in blood pressure (BP) was elicited with a vasoconstrictor agent8,9 and when chemosensitive receptors were activated by serotonin (von Bezold-Jarisch reflex) in the heart.9 We interpreted these findings to indicate a selective effect of ANP on cardiac vagal afferents of both mechanosensory and chemosensory pathways, since the “ramp” method invokes predominantly nonarterial baroreceptor input,10 and the von Bezold-Jarisch reflex activates cardiac chemosensitive receptors on vagal afferents.11

The abnormalities that are known to exist in the baroreflex control of HR of both animal and human models of hypertension have been attributed largely to a reduced cardiopulmonary or vagal contribution to the reflex.12-14 Spontaneously hypertensive rats (SHR) exhibit a reduced vagal component of the HR range, which is manifested as an attenuated bradycardic capacity in the face of increases in BP.15 Given our observations in normotensive rats, we speculated that ANP may be able to restore the vagal deficit in these animals. Surprisingly, ANP did not influence the reflex bradycardic responses in SHR to either baroreflex or von Bezold-Jarisch reflex activation.9

Possible explanations for this lack of sensitivity may include structural alterations in the heart associated with cardiac hypertrophy, elevated BP, or a component indepen-
dent of structure or BP. Cardiac hypertrophy is a common consequence of hypertension,16 and evidence from Minami and Head17 suggests that the diminished ability of the vagus to respond appropriately to changes in arterial pressure in SHR is highly correlated with the degree of cardiac hypertrophy. These workers administered an angiotensin-converting enzyme (ACE) inhibitor to young SHR for various long-term periods and showed that in addition to the BP-lowering actions, the duration of treatment determined the level of left ventricular hypertrophy (LVH).17 On the basis of results of the treatment regimens from that study,17 we treated 2 groups of young SHR with the ACE inhibitor perindopril for different periods so that some rats would have completely normal BP and heart size and others would have normal BP but some degree of LVH. The aim of our present study was to determine whether normalization of BP alone in SHR was sufficient to restore the cardioinhibitory actions of ANP, or whether the cardiac hypertrophy in these animals was responsible for preventing ANP-induced activation of cardiac sensory afferents resulting in reflex bradycardia.

Methods

Animals

The experimental protocol for this study was approved by the Alfred Hospital/Baker Medical Research Institute Animal Experimentation Committee. Studies were carried out in male WKY and SHR bred at the Baker Medical Research Institute. All rats were housed individually in a temperature-controlled room (23°C to 25°C) with a 12-hour day/night cycle and had free access to pelleted rat chow and water.

ACE Inhibitor Treatment

Animals entered the study at 4 weeks of age. There was 1 group of Wistar-Kyoto rats (WKY) and 3 treatment groups of SHR. SHR were treated with the ACE inhibitor perindopril (S9490-3; Institute de Recherches Internationales Servier, Courbevoie, France; 3 mg/kg daily in drinking water, ad libitum) for 6 weeks (from 4 to 9 weeks of age, n=10; SHR-S) or 9 weeks (from 4 to 12 weeks of age, n=10; SHR-L) or were given vehicle (tap water, n=10; SHR). During the treatment regimen, the concentration of the perindopril solution was adjusted on a biweekly basis to account for individual body weight and water intake variations. The administration of perindopril (dissolved in distilled water and added to the drinking water) was preceded in the weeks before and followed in the weeks after by normal tap water. The group of untreated WKY was included in the study as normotensive controls with normal left ventricle to body weight ratio (LV/BW) for comparison. These animals were provided with tap water ad libitum from 4 weeks of age (n=10, WKY). Body weight and water intake were measured in all rats 2 times a week from 4 to 13 weeks of age.

Surgical Preparation: Arterial and Venous Catheters

When all rats reached 13 weeks of age, aortic and jugular catheters were implanted using a surgical procedure that we have described previously.3,18 Briefly, the animals were anesthetized intraperitoneally with a mixture of methohexisone sodium (Brietal Sodium, Eli Lilly; 40 mg/kg), pentobarbitone (Nembutal, Boehringer Ingelheim; 30 mg/kg), and atropine sulfate (Astra; 0.5 mg/kg). For direct recording of arterial pressure and HR, a Teflon-tipped catheter (OD, 0.45 mm; ID, 0.3 mm; Small Parts) was inserted into the abdominal aorta through a midline incision in the abdominal wall. An incision was also made in the skin lateral to the larynx to insert a triple-lumen catheter (OD, 1.5 mm; ID, 0.5 mm; TV4, Dural Plastics) down the right jugular vein to the vena cava, with the tip at the level of the heart. This triple-lumen catheter allowed for simultaneous injections and infusions of very small volumes. The free ends of the catheters were filled with heparinized saline (100 IU/mL), passed subcutaneously to emerge at the back of the neck, secured, and occluded with pins.

Hemodynamic Measurements and Experimental Protocol

In rats at 14 weeks of age, approximately 7 days after the arterial and venous catheters were implanted, HR reflex responses were assessed. The average weight of the animals in the 4 groups at this age was 294±3 g (weight range, 250 to 335 g). On the experimental days, the arterial catheter was connected to a Cobe disposable pressure transducer (Lakewood, New Jersey). Systemic arterial BP, and the signals were recorded continuously on an 8-channel Graphtec recorder (Linearcorde WR3310). HR was measured using a tachometer (Baker Medical Research Institute) triggered by the arterial pulse pressure signal. In addition, both BP and HR data for the ramp experiments were digitized and recorded continuously with a Metrabyte DAS-8 analog-digital card and a data acquisition program (NewAD, Baker Medical Research Institute) on an Olivetti M-280 computer at 0.5-second intervals. The rats were left for ~1 hour before the experimental protocol was begun to ensure that BP and HR had stabilized.

On 1 experimental day, steady-state baroreflex curves were constructed. On an alternate experimental day, baroreflex responses to the rapid ramp technique and the von Bezold-Jarisch reflex were measured. Each of the 3 methods was tested in the presence of vehicle (0.9% saline, 270 µL/H) or α-ant ANP (28 amino acids; Peninsula Laboratories; 150 ng/kg per minute) via intravenous infusions, with the order of vehicle or ANP on each day alternated in each rat. There was an interval of at least 30 minutes between the end of the first infusion (either ANP or vehicle) and the beginning of the second infusion (alternate solution).

Baroreflex Stimulation

Two methods of stimulation were used for baroreceptor-HR reflex measurement. The steady-state method involved alternate intravenous injections of 1 to 50 µL of methoxamine hydrochloride (2 to 100 µg/kg doses; Wellcome Research Laboratories) and sodium nitroprusside (1 to 50 µg/kg doses; Nipride, Roche Products) to produce a series of stepped increases and decreases in mean BP (from ±2 to 60 mm Hg) in each rat.8,9 The steady-state changes in mean BP and HR, measured over 10 to 15 seconds, were fitted to a sigmoid logistic equation by a computer that applied the algorithm of Marquardt10 as follows: HR= P1+ P2[1+ e^-(MAP-P4)], where P1 is the lower HR plateau, P2 is the HR range, P3 is the normalized gain, P4 is the arterial pressure at the midpoint of the HR range, and MAP is mean arterial pressure. The average gain (G) or slope of the curve between the 2 inflection points is given as G= -P2×P3/4.56. In most cases, 14 points were used to construct sigmoid curves defining the MAP-HR relationship in the absence and presence of ANP.

The second baroreflex method involved rapid changes to BP induced by fast (between 4 to 6 seconds) 50-µL IV infusions of methoxamine (doses of ~100 µg/kg).11 The distinguishing feature of this method is that through rapid changes to BP, a higher proportion of nonarterial (cardiopulmonary) versus arterial afferent input to the reflex HR responses is invoked.11,12 In each experiment, 3 ramp increases in mean BP were performed during vehicle infusion followed by 3 ramps in the presence of ANP, or vice versa. The increases in mean BP of 50 to 60 mm Hg were comparable to those of the maximum response with the steady-state method. Mean BP and HR were digitized by an IBM-compatible computer via a Metrabyte data acquisition card at 300-Hz sampling rate with binning of data at 0.5 Hz. To obtain the best correlation between the HR responses to mean BP changes, linear regressions of HR versus mean BP were performed using values of mean BP ranging from 0.5 to 3.0 seconds earlier than the corresponding HR value. The time delay with the highest regression correlation was selected, usually 1 second. The slope of the regression line indicated the ramp barore-
TABLE 1. Effect of ANP on Steady-State Baroreflex Parameters in Untreated WKY and SHR and Perindopril-Treated SHR (SHR-S and SHR-L) and LV/BW Postmortem

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<th>SHR-S</th>
<th>SHR</th>
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<th>2</th>
<th>3</th>
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Baroreflex parameters of sigmoid curves calculated from steady-state responses to alternate injections of sodium nitroprusside and methoxamine in the presence of saline and ANP (150 ng/kg per minute) infusion in conscious untreated WKY (n=9), untreated SHR (n=9), and short-term (SHR-S; n=10) and long-term (SHR-L; n=9) perindopril-treated SHR. P4 (BP) indicates mean BP at 50% HR range. LV/BW (n=10 per group) with between-group comparisons as for steady-state data. Values are means. SED is an estimate of within-animal variance for comparisons between saline and ANP infusions across all groups from ANOVA.

Body weight and LV/BW data for the different groups of animals were similarly analyzed. Both the ramp and the von Bezold-Jarisch tests were analyzed by 2-factor, repeated-measure ANOVA. Orthogonal partitioning of the sums of squares was used to determine the effects of ANP on the ramp regression parameters and the dose-dependent reductions in HR and mean BP in response to bolus intravenous injections of 5-HT. Between-group comparisons were the same as for analysis of the steady-state data (see above), with an adjustment for multiple comparisons according to Bonferroni. Contrasts were considered significant, and the null hypothesis was rejected at P<0.05. By using data from all groups, linear regression was fitted to the estimates of LV/BW versus (1) steady-state HR range, (2) ramp baroreflex sensitivity, and (3) von Bezold bradycardia per microgram of 5-HT.

Evaluation of Cardiac Hypertrophy

At the completion of HR reflex assessment, the extent of cardiac hypertrophy in each animal was determined from the calculated dry left ventricle plus septum weight to body weight (LV/BW) ratio. The rats were killed with Euthatal (pentobarbitone sodium 350 mg/mL IV; Rhône Mérieux) and weighed. The chest was opened via a midline incision, and the rib cage and lungs were removed. After the heart was excised and placed in saline, it was trimmed to remove any extraneous tissue, squeezed to remove any blood, and rolled dry before weighing. Both atria were removed. The right ventricle was carefully dissected away before the left ventricle plus septum was weighed.

Statistics

Steady-state baroreflex curve parameters corresponding to the control and also the change due to ANP were analyzed by 1-way, 2-factor ANOVA with partitioning of the between-columns sums of squares to determine the within-animal effects and the between-groups effects. The between-group comparisons were WKY versus SHR, WKY versus SHR-S, WKY versus SHR-L, SHR versus SHR-S, SHR versus SHR-L, and SHR-S versus SHR-L. The Bonferroni procedure was used to adjust for multiple comparisons.

Results

Effects of Perindopril Treatment on Weight Gain and Water Intake

Chronic perindopril treatment did not modify the normal growth of the rats, and at 14 weeks of age, when HR reflexes were tested, body weights were similar in the 4 groups (287±5 g, WKY; 290±6 g, SHR-L; 294±3 g, SHR-S; and 293±5 g, SHR). Water consumption in all groups of rats doubled over the 4-week period from 4 to 7 weeks of age (from 14.5±0.2 to 31.8±0.7 mL/d). At ~7 to 8 weeks of age, water consumption leveled off in WKY and remained at ~25 mL/d, whereas in SHR, both treated and untreated, water consumption continued to rise, although more slowly than the early stages. When perindopril was withdrawn from the drinking water in SHR-S at 9 weeks of age, water consumption in these animals returned within 2 weeks to levels similar to those in WKY.

Effects of Perindopril Treatment on Systemic BP and Heart Size

At 14 weeks of age, when HR reflexes were tested, mean BP in untreated SHR was significantly higher (by ~20 mm Hg; P<0.05) than in untreated WKY (Table 1). Treatment with

Chemosensitive Reflex Stimulation

Chemosensitive cardiac receptors were also tested using the von Bezold-Jarisch reflex, which was evoked by intravenous bolus injections of 5-hydroxytryptamine (5-HT) in the range of 5 to 60 μg/kg (serotonin, creatinine sulfate complex, Sigma Chemical Co). Stimulation of these receptors resulted in rapid (within 5 to 10 seconds) dose-dependent reflex falls in HR, often associated with a subsequent fall in BP. Changes in mean BP to 5-HT were taken at the same time as the maximum bradycardic responses. These HR responses and BP changes (measured at the same time) were determined after a low, medium, and high dose of 5-HT, administered at 5-minute intervals, in the presence of alternating infusions of vehicle and ANP. The doses of 5-HT were adjusted to accommodate the sensitivity of each rat, but always the same doses of 5-HT were given in the presence and absence of ANP. SHR generally required a greater dose of 5-HT to elicit HR responses similar to those in WKY.

Body weight and LV/BW data for the different groups of animals were similarly analyzed. Both the ramp and the von Bezold-Jarisch tests were analyzed by 2-factor, repeated-measure ANOVA. Orthogonal partitioning of the sums of squares was used to determine the effects of ANP on the ramp regression parameters and the dose-dependent reductions in HR and mean BP in response to bolus intravenous injections of 5-HT. Between-group comparisons were the same as for analysis of the steady-state data (see above), with an adjustment for multiple comparisons according to Bonferroni. Contrasts were considered significant, and the null hypothesis was rejected at P<0.05. By using data from all groups, linear regression was fitted to the estimates of LV/BW versus (1) steady-state HR range, (2) ramp baroreflex sensitivity, and (3) von Bezold bradycardia per microgram of 5-HT.

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At 14 weeks of age, when HR reflexes were tested, mean BP in untreated SHR was significantly higher (by ~20 mm Hg; P<0.05) than in untreated WKY (Table 1). Treatment with
perindopril (3 mg/kg per day) prevented this increase in mean BP in both SHR-S and SHR-L such that BPs were similar to those in WKY (Table 1). In untreated SHR, LV/BW was approximately 22% higher than in WKY (P<0.05; Table 1). With shorter-term perindopril treatment, LV/BW was lower than in untreated SHR (P<0.05), but heart size was not restored to the level of WKY (P>0.05; Table 1). Perindopril treatment for the longer 9-week period in SHR-L, however, normalized LV/BW to within 1% of the level of WKY (which was 18% less than the LV/BW of untreated SHR; Table 1, P<0.05).

**Effects of Perindopril Treatment on HR Reflexes**

**Steady-State Baroreflex**

Mean sigmoidal-shaped baroreflex curves with clearly defined upper and lower HR plateaus from the 4 groups of rats are illustrated in Figure 1, with the corresponding steady-state baroreflex parameters summarized in Table 2. Similar to findings of our previous report, untreated SHR exhibited a rightward shift of the curve in line with their elevated resting MAP (P<0.05; Table 1, Figure 1) and a reduced HR range (P<0.05, Table 1, top panels in Figures 1 and 2), which was predominantly due to a lesser bradycardic capacity (P<0.05, Table 1, Figure 1) compared with normotensive WKY rats. Baroreflex curves of both groups of perindopril-treated SHR were shifted back to the left, in line with their normalized MAP, compared with untreated SHR (BPso values, P<0.05, Table 1, Figure 1). In addition, perindopril treatment improved the HR range in SHR toward the level in WKY (by 12% in SHR-S and by 25% in SHR-L; top panels in Figures 1 and 2, Table 1) such that in SHR-L, the HR range was not different from that in WKY (Table 1, top panel in Figure 2). There was a significant positive correlation between the HR range and LV/BW across all groups (r=0.38, P<0.05). There was no difference in steady-state baroreflex gain between the groups (Table 1).

**Rapid Ramp Baroreflex**

Ramp baroreflex sensitivity in untreated WKY of 2 to 2.5 bpm per mm Hg was approximately 30% lower than the average gain of the arterial baroreflex measured with the steady-state method (Tables 1 and 2). In untreated SHR compared with WKY, ramp baroreflex sensitivity was markedly reduced (by ≈57%) (middle panel of Figure 2, Table 2), confirming our previous findings. Ramp baroreflex sensitivity tended to increase with the duration of perindopril therapy (to ≈62% in SHR-S and ≈76% in SHR-L of the WKY ramp sensitivity; middle panel of Figure 2, Table 2). The ramp baroreflex responses in the different groups were determined over a similar range of change in mean BP. The rate of rise in BP produced by rapid injection of methoxamine, however, was faster in WKY and SHR-S compared with untreated SHR rats (≈22% and ≈19%, respectively; P<0.05; Table 2). A positive association between ramp sensitivity and LV/BW was not demonstrated (P=0.126, r=0.246).

**Cardiac Chemosensitive Reflex (von Bezold-Jarisch Reflex)**

Serotonin administration evoked dose-dependent reductions in HR in untreated WKY of 98±14, 184±16, and 231±13 bpm in response to low, medium, and high doses of 5-HT, respectively (refer to Table 3 for average bradycardic response). Doses of 5-HT used to achieve these changes in HR were 6:2:1, 13±1, and 28±2 μg/kg, respectively (average dose in Table 3). As we previously reported, untreated SHR were less sensitive to 5-HT than WKY (bottom panel of Figure 2, Table 3). To ensure similar von Bezold-Jarisch vagal bradycardic responses across groups, 75% higher doses of 5-HT were administered to untreated SHR compared with WKY (Table 3), resulting in falls in HR of 60±12, 170±8, and 213±7 bpm with low (12±1 μg/kg), medium (26±2 μg/kg), and high (44±5 μg/kg) doses of 5-HT, respectively.

Unlike the improvement in baroreflex sensitivity with perindopril treatment, the von Bezold-Jarisch reflex was not altered by perindopril (bottom panel of Figure 2). Similar to untreated SHR, perindopril-treated rats required higher doses of 5-HT compared with WKY to achieve comparable bradycardic responses (Table 3). In SHR-S, mean HR fell by 90±24, 161±23, and 221±14 bpm in response to low (13±1 μg/kg), medium (27±3 μg/kg), and high (47±4 μg/kg) doses of 5-HT, respectively (see Table 3 for average 5-HT dose and bradycardic response). In SHR-L, HR fell dose dependently by 60±16, 165±21, and 201±19 bpm with low (12±1 μg/kg), medium (29±3 μg/kg), and high (45±3 μg/kg) doses of 5-HT, respectively (see Table 3 for average 5-HT dose and bradycardic response). Mean bradycardic...
TABLE 2. Effect of ANP on Ramp Baroreflex Parameters in Untreated WKY and SHR and Perindopril-Treated SHR

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<thead>
<tr>
<th>Parameter</th>
<th>WKY (Effect of ANP)</th>
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<th>SHR-S (Effect of ANP)</th>
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<td>Saline</td>
<td>ANP</td>
<td>1 5 6</td>
</tr>
<tr>
<td></td>
<td>295±7</td>
<td>15±6*</td>
<td>303±14</td>
<td>19±4*</td>
<td>††</td>
</tr>
<tr>
<td></td>
<td>312±7</td>
<td>3±4</td>
<td>312±7</td>
<td>3±4</td>
<td>††</td>
</tr>
</tbody>
</table>

Baroreflex parameters from rapid ramp infusions of methoxamine in conscious untreated WKY (n=10), untreated SHR (n=10), and long-term (SHR-L; n=10) and short-term (SHR-S; n=10) perindopril-treated SHR during saline infusion. Values are mean±SEM (between animals). Effect of ANP is the mean change in baroreflex parameter during ANP infusion compared with vehicle (saline) infusion±SED (within animal estimate). Measurements in each animal were the averages of 3 replicates. R² indicates regression correlation coefficient; BP range, range over which BP changed during ramp measurements; and rate of change in BP, change in BP over time during ramp measurements.

*Significant effects of ANP (P<0.05). Between-group comparisons with vehicle infusion represented by †P<0.05, as per Table 1.

Responses to all the doses of 5-HT during saline infusion were not significantly different between groups (Table 3, P=0.531). The sensitivity to von Bezold-Jarisch reflex was not related to LV/BW (P=0.706, r=0.063).

In addition to bradycardia, the von Bezold-Jarisch reflex is characterized by its hypotensive effects. BP falls in response to 5-HT were measured at the point of maximal bradycardic effect (within 5 to 10 seconds), since as we have recently reported,³ the BP response to 5-HT in WKY and SHR is variable. The mean BP changes evoked by 5-HT were not significantly different among the 4 groups of rats (−20±4 mm Hg, WKY; −23±2 mm Hg, SHR; −25±2 mm Hg, SHR-S; and −21±2 mm Hg, SHR-L; P=0.263).

Actions of ANP on HR Reflexes in Perindopril-Treated Rats

Steady-State Baroreflex

ANP infusion had no effect on any steady-state baroreflex parameter in SHR-L or WKY. There was also no effect of ANP administration in untreated SHR, other than a small but significant reduction in resting MAP by ≈7% (P<0.05; Table 1, Figure 1). In the SHR-S, ANP increased the

TABLE 3. Effect of ANP on von Bezold-Jarisch Reflex

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of 5-HT, μg/kg</th>
<th>Change in HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>16±2</td>
<td>-171</td>
</tr>
<tr>
<td>SHR-L</td>
<td>29±3</td>
<td>-142</td>
</tr>
<tr>
<td>SHR-S</td>
<td>29±3</td>
<td>-158</td>
</tr>
<tr>
<td>SHR</td>
<td>28±3</td>
<td>-148</td>
</tr>
</tbody>
</table>

Average changes in HR to 3 doses of 5-HT (average dose indicated in second column) in groups of 14-week-old rats (abbreviations given in Figure 1). Because the sensitivity to 5-HT varied between groups (also see Figure 2, bottom), the concentration of 5-HT was adjusted according to the sensitivity of each animal to achieve comparable HR responses across groups. The same doses of 5-HT were used in each animal to test the von Bezold-Jarisch reflex during saline and ANP infusion. Values of doses are mean±SEM (between-animal estimate). Changes in HR values are mean±SED (within-animal variance for comparisons between saline and ANP infusions).

*Significant effect of ANP (P<0.05).
Significantly enhanced reflex bradycardic responses to 5-HT (to contrast, ANP caused a 28% increase in sensitivity in SHR-L on rapid ramp sensitivity in SHR-S (Figure 3). By contrast, in SHR-L, ANP evoked a substantial increase in reflex bradycardic responses to 5-HT. In these animals, the magnitude of the HR fall in response to 5-HT increased by 36±8% (P<0.05; Table 3, Figure 3), an effect that was approximately 2-fold greater than that of ANP on the HR response in WKY animals. ANP did not alter the hypertensive responses to 5-HT in any of the groups of rats (P=0.444, WKY; P=0.312, SHR; P=0.911, SHR-S; and P=0.634, SHR-L).

**Discussion**

The major finding of the present study was a link between vagal reflex actions of ANP and hypertension-induced LVH. Although ANP was shown to be an effective agent to enhance reflex bradycardia in normotensive rats, whether induced by cardiac baroreceptor or cardiac chemoreceptor activation, ANP was unable to improve vagal reflex function in SHR unless their LVH was completely prevented. Perindopril treatment for 9 weeks in young SHR effectively prevented LVH and was associated with enhanced vagal HR reflex responses to ANP. Even a small degree of LVH in the SHR treated with perindopril for 6 weeks was sufficient to prevent the reflex bradycardic actions of the ANP. Restoration of reflex function in the SHR was not linked with the elevated BP per se, since normalization of BP alone was not associated with ANP sensitization of vagal HR reflexes. Furthermore, because reflex responsiveness to ANP in SHR was reversible (provided heart size was normal), an intractable inherited factor in SHR can be ruled out.

These findings confirm and extend observations made previously by our group that ANP enhanced reflex bradycardia in conscious, normotensive rats but was ineffective in SHR.9,10 In most forms of hypertension, the deficit in baroreflex control of HR is observed after the development of hypertension. However, several lines of evidence suggest that cardiac sensory nerve function is altered in association with cardiac hypertrophy, rather than attributable to the elevated BP alone. Head and coworkers20 reported that the vagal deficit in SHR coincides with the onset of cardiac hypertrophy. Subsequently, Minami and Head17 demonstrated that regression of LVH was important in reversing the vagal deficit in these animals. The correlation between heart size and reduced HR range which that study found, and which we confirmed in the present study, adds further evidence for an anatomic link between diminished baroreflex function with LVH. A progressive reversal of cardiac hypertrophy and concomitant normalization of baroreflex gain has also been reported in humans.21 Rats that were made hypertensive by acute or chronic nitric oxide synthase blockade did not have cardiac enlargement, but baroreflex activity may22 or may not23 have been altered. To our knowledge, no one has examined the effect of ANP on baroreflex responses in this new model of hypertension.

The components of the vagal HR-reflex pathways on which ANP may act are (1) the cardiac sensory afferents, including the region of the myocardium that is in contact with
these nerve endings, (2) the central sites of cardiovascular reflex processing, and (3) the cholinergic efferent nerves supplying the sinoatrial node and myocardial conducting tissue. A sensitizing action of ANP on efferent projections is unlikely because ANP did not enhance reflex bradycardia after activation of arterial baroreceptors (present findings and References 8 and 9), even when the sympathetic and parasympathetic limbs of the reflex were pharmacologically separated. A central action is also unlikely because administration of ANP into the cerebral ventricles of SHR had opposite effects on baroreflexes \(^{24}\) compared with those we observed with peripheral administration. We previously proposed that ANP acts selectively on nonarterial afferents, \(^{8,9}\) and the present finding of a link between ANP reflex actions and heart size implicates a direct action of the hormone on a myocardial structure, although a central region may also be modified by prolonged LVH. Likely targets for ANP action are the cardiac sensory afferents or the nearby myocytes. Genes for the 3 natriuretic peptide receptor subtypes (NP\(_A\), NP\(_B\), and NP\(_C\)) are expressed in the rat heart. \(^{25}\) Myocytes in the cardiac ventricle, where the cardiac mechanosensitive and cardiac chemosensitive afferents predominantly originate, produce predominantly NP\(_A\) receptors. \(^{25}\) Messenger RNAs for all 3 natriuretic peptides have also been identified in cultures of nonmyocytic fibroblast cells. \(^{25}\) The question of which natriuretic peptide receptor, or whether the sensory nerves themselves or a factor associated with their function, such as nitric oxide or a prostaglandin, may be involved awaits further investigation.

In the present study, we used the previous observations of Minami and Head \(^{17}\) that the length of ACE inhibitor treatment (perindopril), in addition to normalizing BP, was closely associated with the development of LVH. Confirming their results, we showed that perindopril treatment prevented hypertension regardless of whether the duration was 6 or 9 weeks, but only the longer term treatment effectively prevented the development or possible redevelopment of LVH. Associated with the reduced cardiac size in the treated SHR was also an improvement in the HR range, measured by the steady-state baroreflex method, and in the ramp range (see Figure 2). Recovery of baroreflex function with ACE inhibitor treatment has been observed by others, but the improvement in both arterial and nonarterial baroreflex function in the same animals has not been demonstrated before. Surprisingly, the improved baroreflex activity with ACE inhibitor treatment was not accompanied by a recovery of von Bezold-Jarisch reflex function. Even with normal BP and, in the case of SHR-L, normal cardiac size, the HR responsiveness to 5-HT remained substantially lower than that in the WKY. Despite this attenuated von Bezold-Jarisch reflex in the chronically treated SHR, the sensitizing actions of ANP on this reflex were completely restored. Thus, the reflex bradycardic effects of ANP do not require “normal” cardiac chemosensory function.

If we compare whether the 3 reflexes can be influenced by cardiac hypertrophy and whether ANP can influence reflex activity in a manner that is dependent on the level of cardiac hypertrophy, we see an interesting pattern emerge. The steady-state baroreflex response was markedly influenced by cardiac hypertrophy in the present study but was not influenced at all by ANP. Thus, some cardiac pressure-sensitive fibers may not respond to ANP. The rapid ramp-induced bradycardia and the enhancement of the bradycardia produced by ANP, on the other hand, were both influenced negatively by cardiac hypertrophy, suggesting that some cardiac baroreceptor fibers are sensitive to ANP. The von Bezold-Jarisch reflex, which is evoked by activation of cardiac chemosensitive fibers, was not at all influenced by cardiac hypertrophy, but the effect of ANP on this reflex was. This suggests that chemosensitive fibers are also sensitive to ANP. Taken together, these results support the view that the chemosensitive and pressure-sensitive cardiac afferent pathways are likely to be quite separate entities. \(^{11}\) Moreover, it suggests that the pressure-sensitive pathways are differentially affected by ANP. Those that are activated by very rapid rises in blood pressure may possess ANP receptors or local factors that are responsive to ANP such as nitric oxide, kinin, or a prostaglandin element, while those activated by more modest rates of pressure increase may not. In this way, the heart could provide itself quite specific protection in response to rapid increases in afterload when ANP levels are high (such as during exercise \(^{26}\) or cardiac failure \(^{27}\)). This amplifier property of ANP on reflex pathways may not be needed when the heart is exposed to more gentle changes in pressure.

One possible mechanism for the recovery of reflex function of ANP after long-term perindopril treatment is that of residual effects of cardiac ACE inhibition. The 2-week washout period between ceasing the perindopril treatment and the reflex testing was long enough to ensure that components of the circulating renin-angiotensin system had returned to normal. \(^{28}\) On the other hand, the local cardiac production of angiotensin II may have still been inhibited. The presence of ACE inhibitor enhanced the sensitivity of the arterial baroreflex in SHR \(^{29}\) but not to a ramp baroreflex in humans. \(^{30}\) In the latter study, the ability of ANP to enhance reflex bradycardic responses to rapid increases in afterload with phenylephrine was lost after 5 days of enalapril treatment. Thus, in the present study, the presence of residual ACE inhibitor activity is not likely to have mediated the recovery we observed in ANP responsiveness in the chronically treated SHR.

We found that perindopril treatment stimulated drinking in SHR, an observation made previously by Minami and Head. \(^{17}\) Among the multiple effects of angiotensin II is the promotion of thirst. \(^{31}\) A possible explanation for the dipsogenic response may be that angiotensin I, present in high concentrations in the brain, may have promoted thirst. Among these concentrations in the periphery as a consequence of perindopril treatment, crossed the blood-brain barrier and was subsequently converted locally to angiotensin II. Thus, elevated angiotensin II levels in the brain may have promoted thirst.

In conclusion, we demonstrated that the presence of even a modest degree of cardiac hypertrophy, despite normalized BP, was sufficient to prevent the reflex bradycardic actions of ANP activated through either nonarterial mechanosensory or cardiac chemosensory afferent pathways. Thus, it appears that the inability of infused ANP to enhance bradycardic reflexes in SHR was related to structural alterations in the myocardium associated with cardiac hypertrophy, rather than...
the stimulus of increased BP alone or an irreversible inherited component. If a major role of ANP is to protect the myocar-
dium through its interactions with HR reflexes from rapid
rises in afterload or from chemical insult, then one of the
functional consequences of hypertension-induced LVH is the
loss of this cardioprotective action of ANP.

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