Atrial Natriuretic Peptide in a Rat Model of Cardiac Failure

Atrial and Ventricular mRNA, Atrial Content, Plasma Levels, and Effect of Volume Loading

H. Drexler, MD, J. Hänge, PhD, M. Finckh, MD, W. Lu, MD, H. Just, MD, and R.E. Lang, MD

*With the technical assistance of Evi Hablawetz*

This study examined the relation between synthesis, atrial storage, and plasma levels of atrial natriuretic peptide (ANP), and it examined plasma ANP levels and hemodynamic output in response to volume expansion in a rat model of myocardial infarction and failure. Arterial ANP concentrations did not correlate linearly with infarct size, but they did show an abrupt increase when infarct size exceeded 30% of the left ventricle, similar to the abrupt increase of left ventricular end-diastolic pressure with infarct size greater than 30%. Consequently, a close relation was found between plasma ANP levels and left ventricular end-diastolic pressure (n=23, r=0.89, p < 0.001). Atrial ANP content per gram of tissue but not ANP content per pair of atria was reduced in rats with large infarcts (>40%, p < 0.05 vs. control animals). ANP mRNA level per pair of atria (related to total atrial RNA), determined by liquid hybridization (controlled by northern blot analysis), was increased by 38% in infarcted rats (p < 0.05 vs. controls), but the ratio of atrial ANP mRNA relative to atrial β-actin mRNA levels was not increased. Right and left ventricular ANP mRNA level increased by 90% and 380%, respectively, far exceeding the concomitant increase in β-actin mRNA (+26% in the left ventricle). Plasma ANP increased with volume loading in controls and rats with moderate infarcts but not in rats with large infarcts despite a similar increase in right atrial pressure (compared with control animals); thus, the relation of ΔANP/Δright atrial pressure exerted by volume loading decreased in rats with large infarcts. Similarly, the response of cardiac output and renal blood flow (determined by radioactive microspheres) to volume loading was attenuated in rats with large infarcts. Thus, in this model of chronic cardiac failure, the activation of the ANP system is closely coupled with the increase in intracardiac pressures without correlating linearly to the extent of myocardial loss. Second, in severe cardiac failure, additional stimulation such as volume loading may elicit only an attenuated ANP secretion response, for example, due to saturation of the ANP receptor sensing system or to a limited transformation rate of pro-ANP. Third, the increase in atrial ANP synthesis and the increase in atrial ANP gene expression seems limited; however, substantial specific ANP gene expression occurs in the ventricles, which, in turn, may contribute to increased plasma ANP levels in chronic heart failure. (*Circulation* 1989;79:620–633)

Previous studies showed that an atrial factor that induces natriuresis and diuresis is released from rat heart-lung preparations in response to high atrial perfusion pressure. Right atrial appendectomy has reduced the renal response to acute hypervolume in the rat, and release of immunoreactive atrial natriuretic peptide (ANP) from isolated rat hearts in response to volume loading has been shown.

Plasma ANP concentrations are elevated in patients with pressure or volume overload, such as hypertension, renal failure, or congestive heart failure and from secondary to increased atrial stretch. Conversely, recent studies indicated that cardiac atria of the Syrian hamster with cardiomyopathy or
rats with myocardial infarction are deficient in ANP when measured per gram of tissue.\textsuperscript{8,9} Furthermore, ANP messenger RNA was recently shown to increase in a rat model of circulatory failure due to aortal-caval shunt,\textsuperscript{10} indicating that atrial ANP synthesis and turnover is increased in heart failure. Additional stimuli (such as volume loading) in this situation may elicit only limited secretion of ANP into the circulation. However, significant increases in ANP content and ANP mRNA in ventricles were observed in recent studies, suggesting that the ventricle could be an important source of ANP in congestive heart failure.\textsuperscript{10,11}

To investigate biosynthesis, storage, and the mechanisms of ANP secretion in heart failure, we measured atrial and ventricular ANP mRNA levels, atrial content of ANP, plasma ANP levels, and the effects of volume loading in a rat model of myocardial infarction and failure.\textsuperscript{12,13} Specific ANP mRNA and β-actin mRNA levels in atrial and ventricular tissue were measured simultaneously to determine whether or not the changes in ANP mRNA levels are specific and not merely markers of overall protein synthesis.

**Methods**

**Experimental Preparations**

Infarction was produced by left coronary arterial ligation as described previously\textsuperscript{14} and as modified by MacLean et al.\textsuperscript{15} In male Sprague-Dawley rats, the left coronary artery was ligated approximately 2 mm from its origin with a 6-0 suture. In the sham-operated rats, the suture was tied loosely so as not to obstruct coronary flow. With this method, the 24-hour mortality rate was 40% for the infarction group and 5% for the sham-operated group. The surviving rats were maintained on standard rat chow, and subsequent experimental procedures were started 23 (range, 22–24) days after surgery. During this postsurgical period, the mortality rate in the infarction group was approximately 15%.

**Instrumentation**

Animals were anesthetized with halothane (1% in oxygen), and catheters (PE 50) were inserted into the left ventricle through the right carotid artery, tail artery, right jugular vein, and right femoral vein. Catheter positions in the left ventricle were determined by pressure wave detection by Statham P231D pressure transducers (Cleveland, Ohio) and displayed on a recorder screen (Siemens Elena Minograf, Erlangen, FRG). After closure, animals were allowed to recover for a minimum of 3 hours before experimental procedures were initiated. This recovery period is of sufficient duration to ensure a return to steady-state conditions in the rat.\textsuperscript{16}

**Regional Blood Flow Measurements**

Radioactive microspheres ($^{141}$Ce, $^{103}$Ru; New England Nuclear, Dreieich, FRG), 15±5 μm in diameter, were used to measure regional blood flow and cardiac output with the reference sample technique\textsuperscript{17} as adapted for use in the rat.\textsuperscript{18} A detailed presentation of the radioactive microsphere technique used in this study has been described.\textsuperscript{16} In brief, the microspheres suspension was thoroughly mixed by agitation and sonication (Branson Sonifier B-30, Danbury, Connecticut) immediately before each injection. Approximately 250,000 microspheres (0.5 ml) was then injected into the left ventricle during a 15-second period followed immediately by a 0.5-ml heparinized saline flush during a 15-second period. To obtain the reference sample, arterial blood was withdrawn (beginning 15 seconds before and continuing during the injection of microspheres) through the tail artery at a rate of 0.35 ml/min maintained by a Harvard constant flow pump (Model 906, South Natick, Massachusetts). The total blood withdrawal period was 1.5 minutes for each injection and was used as the blood flow reference sample. Immediately afterward, 0.5 ml blood was withdrawn for measuring plasma ANP levels.

At the end of the study, animals were killed by phenobarbital injection in the ventricle, and organs and tissue samples were removed. All samples were immediately blotted, weighed, and transferred to a two-channel Gamma scintillation counter (Kontron 480 AR, Munich, FRG) for measuring radioactivity levels. A Digital Equipment Professional 350 computer was used to calculate regional blood flow and total cardiac output.\textsuperscript{19}

**Hemodynamic Output**

Tracings from the left ventricular, right atrial, and caudal catheter were recorded continuously during the experimental protocol and were used to obtain heart rate, left ventricular peak systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), mean right atrial pressure (RAP), and mean arterial pressure (MAP) (by electronic integration). The pulsation pressure trace was displayed periodically to check for proper undamped pulse trace, particularly from the right atrial catheter. Stroke volume was calculated from cardiac output and heart rate. Total vascular resistance was determined from cardiac output and mean arterial pressure data. With the exception of cardiac output, hemodynamic data were collected immediately before the microsphere injection.

**Determination of Infarct Size**

The left ventricle and the ventricular septum were separated, weighed, and fixed in 10% formalin and subsequently cut in eight transverse slices (5 μm) from apex to base and stained with van Gieson’s stain and mounted.\textsuperscript{20} With a planimeter Digital Image Analyzer (Leitz, Wetzlar, FRG), the endocardial and epicardial circumference of the infarcted and noninfarcted portions of the left ventricle were measured. The infarcted mean circumference (mean
of epicardial and endocardial circumferences) of all eight slices was summed and then expressed as a ratio of the summed mean circumference of the left ventricle. For all animals with infarction, myocardial infarction was transmural with a mature scar located at the left anterior free wall. Area measurements of infarct size were not made because these have underestimated infarct size as a result of resolution of necrotic tissue and subsequent wall thinning and late development of compensatory myocardial hypertrophy.

**Determination of Plasma Levels and Atrial Content of Immunoreactive Atrial Natriuretic Peptide**

Blood samples were collected into plastic tubes containing disodiummethylenediaminetetraacetic acid (NaEDTA). Plasma concentrations of ANP (determined as immunoreactive atrial natriuretic factor) were determined by a direct radioimmunoassay without extraction. Fifty microliters of plasma were added to 350 μl 0.1 M Tris buffer, pH 7.4, containing 0.1% gelatin and antibody at a final dilution of 1:80,000. The characteristics and specificity of this antibody have been described. Tracer (125I)rat ANP, Amersham, Braunschweig, FRG) was added after overnight incubation (4,000 cpm/tube), and the incubation continued for another 24 hours. Separation of free from antibody bound 125I-ANP was achieved by precipitation with a second anti-rabbit immunoglobulin antibody. Synthetic rANP 99-126 was used to construct standard curves. To correct for possible interference of plasma proteins with binding, all samples of the standard curve contained 50 μl plasma from a rat plasma pool that was passed previously through SepPak cartridges for removing endogenous ANP. With this procedure, the lowest concentration of ANP yielding a binding significant from that in the absence of standard at the 95% confidence interval was 2 pg/tube. The 50% intercept was at 58 pg/tube. The interassay variation was 15%, and the intra-assay variation was 7% (n=10).

For measuring atrial ANP content, the atrial tissue of right and left atria was minced and boiled for 15 minutes in 0.1 M HCl to avoid proteolysis. The cooled tissue was homogenized subsequently with a Polytron tissue homogenizer (Kinematika, Luzern, Switzerland) and centrifugated for 30 minutes at 3,000g. The resultant supernatant was extracted with ODS-silica cartridges as described recently. The recovery of ANP was between 85% and 90%.

**Protocol 1**

Hemodynamic output including RAP, heart rate, LVESP, LVEDP, and MAP were recorded continuously until remaining unchanged (at least 20 minutes), and 0.5 ml arterial blood was withdrawn through the catheter in the tail artery (infarcted group, n=23; sham-operated group, n=15). Nineteen of these animals (infarcted group, n=11, 401 ± 22 g body weight; sham-operated group, n=8, 389 ± 17 g body weight) successfully underwent the following volume-loading procedure. Rats were excluded if catheter problems occurred; for example, insufficient blood withdrawal from the tail artery or unreliable catheter position during volume loading. Four infarcted animals died suddenly before and during volume loading. Five milliliters 0.9% i.v. saline was administered during a 1-minute period (through the catheter in the vena femoralis), and blood was withdrawn 1, 3, and 5 minutes after the saline infusion for measuring plasma ANP concentrations. The animals were killed by an overdose of phenobarbital, and the heart was quickly removed; the right and left atria were weighed, frozen, and stored at -70°C until measuring ANP content. Tissue ANP levels were expressed per microgram of tissue and per pair of atria. The left ventricles were fixed in formalin as described above. In a separate group (infarcted, n=9; sham-operated, n=8), the animals were used solely for measuring infarct size and atrial ANP mRNA level per pair of atria.

**RNA blot hybridization and hybridization assay.** For preparing an ANP-specific complementary RNA hybridization probe, a 580-bases Pst I-restricted ANP-cDNA fragment, obtained from K. Bloch and J. Seidman, Boston, Massachusetts, was subcloned in pSP64 plasmid. The Eco RI–linearized plasmid served as a template for ANP-cRNA transcription with 100 μCi α-32P UTP (400 Ci/mmol, Amersham) and the SP 6-Polymerase-promotor systems described by Melton et al. Total RNA was prepared by the LiCl-urea technique. After quantification by absorption at 260 nm, a predetermined amount of RNA was denatured in glyoxal, loaded on a 1% agarose gel, fractionated electrophoretically, and blotted to nitrocellulose paper. Hybridization was done in 50% formamid, 50 mM Na-phosphate, pH 6.8, 1 M NaCl, 200 μg/ml herring sperm DNA, 5×Denhardt, 0.1% sodium dodecyl sulfate (SDS), and 10 mM NaEDTA for 18 hours at 65°C. Liquid hybridization was done with a modified procedure described by Durnam and Palmiter. Hybridization total RNA was dissolved in 30 μl hybridization buffer [40% formamid, 40 mM piperazine-N,N’-bis(2-ethanesulfonic acid), 0.4 M NaCl, 1 mM NaEDTA, and 1,000 cpm ANP-cRNA/μl] and incubated for 18 hours at 70°C. After RNAase treatment (dilution in a 10-fold volume of 0.3 M NaCl, 10 mM Tris buffer, pH 7.4, 5 mM NaEDTA, incubated for 1 hour at 30°C), intact cRNA-mRNA hybrid was precipitated with 10% trichloroacetic acid, the precipitate was collected in a nitrocellulose filter, and radioactivity was counted in a β-scintillation counter. The amount of hybridized mRNA was calculated from the radioactivity of the precipitate and the specific activity of the cRNA probe. Results were then expressed as picogram of mRNA per microgram of total RNA.
Protocol 2

Hemodynamic output (similar to protocol 1) was monitored during 30 minutes, and radioactive microspheres were injected at the end of this baseline period (infarcted group: $n=11$; body weight, $388\pm21$ g; sham-operated group: $n=12$; body weight, $367\pm18$ g). Arterial blood was withdrawn from the tail artery for measuring cardiac output as described above, and a second sample was obtained for measuring plasma ANP levels (approximately 2 minutes after injection of microspheres). Three minutes after starting the saline infusion (identical to protocol 1), the injection of microspheres was repeated for measuring cardiac output and regional blood flow. This short time lag between saline infusion and injection of microspheres was selected because earlier experiments showed that plasma ANP should rise to maximal levels within 5 minutes. Injection of microspheres and a 2-minute time period before blood sampling, similar to the protocol for obtaining control measurements, resulted in a maximal rise of ANP levels during the anticipated time period of 5 minutes. The animals were killed by injecting phenobarbital into the left ventricle, and the tissues including heart and kidneys were removed for $\gamma$-scintillation analysis (Kontron 480 AR). The primary objective of this second protocol (opposed to protocol 1) was to evaluate the systemic and regional vascular effects after increased plasma ANP levels induced by volume loading. Withdrawal of 0.5 ml blood for measuring ANP plasma levels and removal of blood in the catheter dead space makes up to 1 ml of blood, which may influence regional vascular blood flow by activating neurohumoral effects. Therefore, blood for measuring ANP plasma levels was withdrawn after injecting microspheres (which includes withdrawal of 0.5 ml of blood for measuring cardiac output), although it was anticipated that the volume load associated with the injection of microspheres would increase the plasma ANP concentration.

Protocol 3

The hearts of seven sham-operated animals (body weight, $409\pm14$ g) and 11 infarcted animals (body weight, $424\pm17$ g) were excised 41±3 days after surgery. Atria (right and left atria together) and the right and left ventricles were separated, weighed, and frozen in liquid nitrogen. Special attention was paid to the separation of atrial and ventricular myocardium. Therefore, after weighing the tissues, the edges of the base of right and left ventricles were cut to exclude contamination of right or left ventricular myocardium with atrial tissue. The scar tissue of the left ventricle was separated, and only viable myocardium of the left ventricle (hypertrophied inferior wall and septum) was used for measuring mRNA. The infarct size of the left ventricle was estimated based on the total weight of the left ventricle and the size of the scar tissue (infarcted area). Although less accurate than histologic measurement of infarct size, this method yields infarct sizes similar to histologic planimetry (unpublished observation from this laboratory).

The ANP-specific cRNA hybridization probe was prepared as described above. For preparation of actin-specific cRNA hybridization probe, a 640-base Sal I-EcoRI fragment of $\beta$-actin–like pseudogene was subcloned into pGEM4 plasmid. The Hind III–linearized plasmid served as template for transcription with SP6 Polymerase as described for ANP. Total RNA was prepared by the LiCl-urea technique. After quantification by absorbance at 260 nm, 2.5 $\mu$g RNA was denatured in 1 M glyoxal, pH 5, 50% dimethyl sulfoxide, 0.01 M Na-phosphate, pH 7, loaded on a 1.2% agarose gel, fractionated electrophoretically, and blotted to nitran 13N membrane. Hybridization was done in 50% formalin, 50 mM Na-phosphate, pH 7, 1 M NaCl, 200 $\mu$g/ml salmon sperm DNA, 200 $\mu$g/ml yeast RNA, 5x Denhardt, 0.2% SDS, and 10 mM NEdTA for 3 hours at 70°C. Then, 2x10 cpm/ml $\beta$-actin and ANP probe, respectively, were added to the hybridization mix, and incubation was continued for a further 18 hours. The membranes were washed in 0.1x15 mM NaCl and 1.5 mM Na-citrate, 0.1% SDS at room temperature and at 70°C and then exposed to radiographic film with intensifying screens at −70°C. The specific bands of $\beta$-actin and ANP were cut, and radioactivity was measured in a $\beta$-scintillation counter. In the case of the atria blots, ANP and $\beta$-actin hybridization was performed separately because of a very strong ANP signal compared with the $\beta$-actin signal.

Statistical Analysis

Data are reported as mean±SEM. Student’s $t$ test and analysis of variance followed by Student-Newman-Keuls test were used to determine statistical significance of differences.

Results

Weights of right ventricle, lungs, and atria of three infarcted rats used for microsphere studies were measured and were significantly elevated compared with sham-operated control rats (atria: sham-operated group/infarcted group, 0.25±0.03/0.61±0.05 g/kg body weight; right ventricle, 0.57±0.04/0.81±0.05 g/kg body weight; lungs, 3.43±0.3/6.22±0.9 g/kg body weight; for all three organ weights, $p<0.02$). Infarct size of these animals averaged 38.4±2% of the left ventricle. Cardiac output and renal blood flow were significantly decreased in infarcted animals ($p<0.05$, Table 1), whereas LVEDP (infarcted group, 25±2 mm Hg; sham-operated group, 8±1 mm Hg; $p<0.01$) and RAP ($p<0.05$, Table 1) were significantly elevated. Thus, depressed cardiac performance indicating chronic heart failure is documented by hemodynamic output and confirmatory signs of heart failure such as atrial and right ventricular hypertrophy or increased lung weights.
TABLE 1. Cardiac Output, Right Atrial Pressure, Plasma ANP, and Regional Blood Flow Before and After Volume Loading

<table>
<thead>
<tr>
<th>Hemodynamic value</th>
<th>Sham-operated (n=12)</th>
<th>Infarcted (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Volume loading</td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>443±22</td>
<td>557±35*</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>0.4±0.3</td>
<td>3.5±0.6*</td>
</tr>
<tr>
<td>Plasma ANP (pg/ml)</td>
<td>1259±109</td>
<td>2009±198*</td>
</tr>
<tr>
<td>Regional blood flow (ml/min/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>4.6±0.3</td>
<td>5.8±0.4*</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>6.9±0.6</td>
<td>9.3±1.2*</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>5.7±0.5</td>
<td>8.6±1.3</td>
</tr>
<tr>
<td>Intestines</td>
<td>3.2±0.2</td>
<td>4.1±0.4*</td>
</tr>
<tr>
<td>Brain</td>
<td>1.1±0.1</td>
<td>1.4±0.1*</td>
</tr>
<tr>
<td>Skin</td>
<td>0.07±0.004</td>
<td>0.009±0.009*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.27±0.05</td>
<td>0.44±0.06</td>
</tr>
</tbody>
</table>

Data are mean±SEM. ANP, atrial natriuretic peptide.

*p<0.05 vs. corresponding control; †p<0.05 vs. sham-operated control; ‡p<0.01 vs. sham-operated control.

Figure 1 (top panel) shows the relation of LVEDP to infarct size, indicating that LVEDP is consistently increased when infarct size exceeds the 30% level. Arterial ANP concentrations did not correlate linearly with infarct size but showed the same abrupt increase (compared with LVEDP) when the infarct size exceeded 30% (Figure 1, middle panel). However, the arterial plasma ANP levels were closely correlated with LVEDP (Figure 1, bottom panel). In 12 infarcted rats, in which reliable measurements of right atrial pressure were obtained, the correlation between arterial ANP levels and RAP yielded r=0.612, p<0.05. Atrial immunoreactive ANP concentration per gram of tissue was decreased in animals with large infarcts (>40%), whereas no difference was found between control rats and rats with infarcts less than 30% (Figure 2). However, when atrial ANP content was expressed per total weight of both atria, no differences were found between rats with large infarcts and sham-operated rats. In Figure 3, the atrial ANP mRNA per total RNA (determined by liquid hybridization) is plotted against infarct size. The validity of the liquid hybridization data was controlled by northern blotting in a smaller number of hearts, yielding similar results. The atrial ANP mRNA content was increased (by more than 2 SD of the mean of the control group) only in rats with large infarcts (>40%), which corresponds to the reduced atrial ANP content per pair of atria in these animals (Figure 2). In a separate group of animals, atrial ANP mRNA per total RNA was increased in infarcted rats compared with sham-operated rats (+38%, p<0.05) (Figure 4). Infarct size of the latter group averaged 33±4% (range, 25–40%) of the left ventricle, and the weights of atria and right ventricle were significantly higher than in the corresponding sham-operated animals (atria, 0.29±0.04 vs. 0.45±0.07 g; right ventricle, 0.62±0.05 vs. 0.84±0.06 g; p<0.05 sham-operated vs. infarcted). The atrial β-actin mRNA per total RNA of sham-operated and infarction groups did not differ significantly, although a slight increase occurred in the infarction group (+13%) (Figure 4).

However, the ratio of atrial ANP mRNA to β-actin mRNA was not significantly elevated in the infarction group (10.1 vs. 8.9), indicating that the increase in ANP mRNA relative to β-actin mRNA may not be specific in this group of animals (Figure 5). In contrast, the ratio of ANP mRNA to β-actin mRNA was substantially increased in both ventricles in the infarction group compared with the sham-operated group (Figure 5).

The right and left ventricular ANP mRNA levels were 1.9 and 3.8 times higher (+90% and 380%) in the infarction groups than in sham-operated animals (Figures 4 and 6). In contrast, right and left ventricular β-actin mRNA levels of infarction and sham-operated groups did not differ significantly (Figures 4 and 6).

Thus, despite significant ventricular hypertrophy and increased overall protein synthesis in this setting, a marked specific increase in ANP mRNA was found in both ventricles in this model of myocardial infarction and failure.

The plasma ANP levels of animals with moderate infarcts (<30%) were higher than in control animals; however, the difference was not significant because of the wide range of the ANP concentrations of this infarcted group. However, the plasma levels in animals with large infarcts were substantially and significantly higher than in control animals and in animals with moderate infarcts (Figure 7).

After volume loading, the plasma concentrations of ANP did not increase in animals with large infarcts. In rats with moderate infarcts (<30%) and in the control group, a significant increase in plasma
ANP concentrations occurred. Of note, the increase in right atrial pressure during volume loading was similar in all three groups (Table 2). Baseline atrial pressure was higher in the group with large infarcts, although the difference was not significant, perhaps because of the small sample size (type II error; note, that in two sham-operated animals and in animals with moderate infarcts no reliable measurements of RAP were obtained and, therefore, were excluded from RAP analysis). However, RAP was significantly increased in a group of animals that underwent blood flow studies (see below and Table 1). Considering the time course after volume loading, plasma ANP concentrations decreased faster (5 minutes after volume loading, Figure 7) in the control group than in the infarcted group with moderate infarcts. The decrease in plasma ANP levels between 1 and 5 minutes after volume loading was significant in the sham-operated group only ($p<0.05$ by ANOVA). The difference between the sham-operated and infarcted group with moderate infarcts for the plasma ANP values 5 minutes after volume loading was not significant ($p=0.1-0.05$ by nonpaired $t$ test).

Infarcted animals (infarct size, $38.4\pm2\%$) did not increase significantly cardiac output after volume loading (Table 1), which is in contrast to the control group. Similarly, renal blood flow was increased after volume loading in the control group but not in the infarction group. Plasma ANP concentrations did rise significantly with volume loading only in the control group, not in the infarction group, although the increase in RAP achieved with volume loading was similar for both groups (Table 1). When a
subset of five infarcted animals that had an increase in cardiac output was analyzed, no consistent increase in renal flow was observed. The baseline values of RAP (1.7±0.9 mm Hg) of the latter five animals were lower compared with the group as a whole, but these latter animals had experienced similar increases in RAP after volume loading (5.8±1.3 mm Hg). Plasma ANP increased in this subgroup, but this increase was not statistically significant (1,690±130 vs. 2,142±182 pg/ml).

Blood flows (other than to the renal bed) before and after volume loading are given in Table 1, indicating increases in blood flow to most circulatory beds in the sham-operated group and to the heart in the infarction group. Please note that blood flow data for the left ventricle represent the composite of values for flows to normal tissue, infarcted tissue, and intermediate regions of residual ischemic tissue because the left ventricle of these animals was used for measuring infarct sizes (transverse slices) after measuring blood flow.

Figure 8 depicts the relation between infarct size and ΔANP/ΔRAP elicited by volume loading. The increase in plasma ANP related to the increase of RAP (exerted by volume loading) varies widely in sham-operated animals; nevertheless, the ability to increase plasma ANP levels for a given increase in RAP seems attenuated in animals with large infarcts. This is evident also from Table 2 where RAP and ANP values of sham-operated rats and of rats with moderate and large infarcts are given separately.

The plasma ANP values in sham-operated rats in Table 1 were considerably higher than in the groups in Figures 1 and 7 because these blood samples were obtained after injecting microspheres, which, in itself, is associated with a moderate volume load.

**Discussion**

This animal model of myocardial infarction and failure yielded a wide range of left and right ventricular dysfunctions in proportion to infarct size, similar to recent reports from Pfeffer et al.\(^\text{12}\) and Tsunoda et al.\(^\text{9}\) However, a threshold phenomenon rather than a close linear relation was observed between LVEDP and infarct size. Pfeffer et al. reported that LVEDP was consistently increased when infarct size exceeded 46% of the left ventricle.\(^\text{12}\) Although the threshold for infarct size was lower in the present study, perhaps because of the different method of measuring infarct size or a different rat strain or both, we observed a similar abrupt rise in LVEDP, when infarct size approached 30–35% of the left ventricle (Figure 1). A similar threshold phenomenon was observed when infarct size was plotted against plasma ANP concentrations; consequently, ANP plasma levels showed a close linear relation to the LVEDP as shown in Figure 1. A similar relation was observed between plasma ANP levels and RAP; also, this correlation was less close. RAP may correlate more closely to right ventricular or pulmonary artery ANP levels than in arterial blood samples measured in the present study.

The threshold phenomenon between infarct size and atrial ANP levels may indicate that the regional wall motion abnormality represented by the moderate infarcts are compensated by cardiac mechanisms, which do not include the (atrial) ANP system. If atrial natriuretic peptide was activated under these circumstances, one would expect a stimulated synthesis and turnover, possibly combined with a
FIGURE 4. Bar graphs of messenger RNA of β-actin and atrial natriuretic peptide (ANP) (cpm/2.5 mg total RNA) of atria—pair of atria, right and left ventricle in sham-operated (n=7) and infarcted (n=11) rats. Note that the atrial β-actin mRNA levels are not comparable to the ventricular β-actin mRNA values (see “Methods”). *p<0.05 vs. corresponding sham-operated value; **p<0.001 vs. corresponding sham-operated value.
Figure 5. Bar graph of ratio of atrial natriuretic peptide (ANP) messenger RNA to β-actin mRNA in atria and right and left ventricles (RV, LV) of sham-operated (n=7) and infarcted (n=11) rats. The ratio increased by 2.5 times for RV and LV. *p<0.01 vs. corresponding sham-operated value; **p<0.005 vs. corresponding sham-operated value.

Reduced atrial content of atrial natriuretic peptide. However, no significant difference in atrial ANP content nor in plasma ANP concentrations was found between rats with moderate infarctions and sham-operated animals.

In accord with recent studies, however, atrial content of ANP per gram of tissue was reduced in animals with severe left ventricular dysfunction. However, total atrial ANP content per pair of atria was not reduced even in rats with large infarcts due to substantial atrial hypertrophy. This finding is in keeping with a recent study investigating atrial ANP content (per gram and pair of atria) of the Syrian hamster. The atrial ANP mRNA related to total atrial RNA was increased by 38% in infarcted rats compared with controls, which is consistent with recent observations. Total atrial RNA itself, increases with elevated intracardiac pressures possibly with proportional increases in ANP mRNA. This would indicate that the increases in atrial ANP mRNA may not be specific for the ANP gene as suggested by Mendez et al but is merely a marker for increased total mRNA and protein synthesis. These investigators reported similar relative atrial ANP mRNA levels (atrial ANP mRNA to 18S RNA) in rats with infarct size greater than 30% compared with controls. To address this issue of whether or not these changes in ANP mRNA are specific, both atrial ANP mRNA and β-actin mRNA levels were simultaneously measured. In contrast to atrial ANP mRNA, there was only a minor and insignificant increase in atrial β-actin mRNA level in this model. However, the ratio of ANP mRNA to β-actin mRNA, although slightly increased (10 vs. 8.9), did not differ significantly for the sham-operated and infarcted groups. Thus, the increase in atrial ANP mRNA may not be specific in this group with moderate infarct size (33%); however, with very severe cardiac failure, ANP mRNA may increase out of proportion as suggested by the substantially increased ANP mRNA levels observed in rats with infarct sizes greater than 40%. Nevertheless, the increases in atrial ANP mRNA levels are moderate, at best, compared with the markedly increased ventricular ANP gene expression. As suggested by Franch et al, the ANP gene may be expressed in normal atria near its maximal rate.

Increased demands due to elevated pressure and stretch and stimulated secretion then would lead to reduced atrial ANP storage (atrial ANP per milligram of tissue) as observed in animals with large infarcts in the present study as well as in several previous studies.

The present finding of substantial increases in ventricular ANP mRNA is consistent with recent reports, although the magnitude of changes in ANP mRNA seems different in the hamster and in the spontaneously hypertensive rat (perhaps because of a different time course of the disease). Franch et al reported that ventricular ANP mRNA levels were seven and 13 times higher in hamsters with moderate and severe heart failure, respectively, than in control animals. ANP mRNA levels in ventricles of spontaneously hypertensive rats were seven times higher than in those of control Wistar-Kyoto rats. Day et al reported a 2.5–3-fold increase in relative ANP mRNA levels of the left ventricle after aortic banding and left ventricular hypertrophy. The latter
is close to the 3.8 times increase in left ventricular ANP mRNA levels observed in the present study.

Similar to atrial ANP mRNA, this increase in ventricular ANP mRNA level could reflect an overall increase of protein synthesis during right and left ventricular hypertrophy. In fact, substantial right ventricular hypertrophy is documented by significantly increased weight of the right ventricle in infarcted animals. Compensatory left ventricular hypertrophy in this model has been reported by several investigators.\textsuperscript{21,32,33} In contrast to the slight increase in left ventricular \( \beta \)-actin mRNA, left ventricular ANP mRNA levels were markedly elevated in the infarction group compared with the sham-operated group. Consequently, the ratio of ventricular ANP mRNA to \( \beta \)-actin mRNA in infarcted rats was 2.5 times that of sham-operated animals. Thus, the increase in ventricular ANP gene expression is specific for the ANP gene beyond an increase in protein and mRNA synthesis in response to a stimulus to hypertrophy. Nevertheless, this increased expression of ventricular ANP gene may be related (to some extent) to ventricular hypertrophy; for example, both the development of hypertrophy and ANP gene expression may be secondary to increased wall stress. In fact, this augmented expression of the ANP gene may operate as a self-compensatory mechanism of the heart as suggested by Arai et al.\textsuperscript{311} Considering the close relation between LVEDP and arterial plasma ANP levels in the present study, it is tempting to speculate that the left ventricle serves as an important source of ANP, which, in turn, may contribute to reduction of afterload and left ventricular wall stress.

The combined measurements of ANP mRNA, atrial ANP content, and plasma ANP levels show that increased intracardiac pressures cause long-term stimulation of ANP synthesis and secretion of ANP into the circulation. If this long-term stimulation is pronounced, atrial ANP content (per gram of tissue) eventually decreases. The plasma concentrations before and after volume loading are consistent
with this hypothesis. If left ventricular dysfunction is only moderate, the response to volume loading would be similar to the response in control animals. Similarly, preliminary results were reported recently from patients with moderate cardiac failure when secretion of ANP was stimulated by volume expansion.\textsuperscript{34} The relatively slow decrease in ANP plasma levels after volume loading (in animals with moderate infarcts, see Figure 7) may be explained by the time course of atrial pressure in these animals. Compared with the control animals, RAP required a longer time period to reach the baseline values. In animals with large infarcts, plasma ANP concentrations were markedly elevated; however, volume loading appears to elicit no significant further increase (i.e., attenuated response) under these circumstances. Similarly, a reduced response to acute volume loading was observed in spontaneously hypertensive rats.\textsuperscript{35} In contrast, a recent report by Chien et al\textsuperscript{36} suggested that increased ANP secretion after volume loading is preserved in rats with heart failure. However, this conclusion was based on six rats with infarcts less than 20% and four animals with infarcts greater than 20%. In the present study, an attenuated secretion of ANP after volume loading was noted only in rats with large infarcts (\textgreater{}40\%, see Figure 7), whereas the response of rats with moderate infarcts (\textless{}30\%) did not differ from control animals, which is similar to the observations of Chien et al. Furthermore, and of note, our infusion rate was twice as fast as the one used by those investigators. Our results obtained in animals with large infarcts suggest that activation of ANP was near maximal at baseline, and therefore, additional stimulation such as volume loading may exert only an attenuated ANP secretion response, for example, one that is due to saturation of the ANP receptor sensing system or to a limited transformation rate of pro-ANP. In support of the latter hypothesis, Garcia et al\textsuperscript{37} recently observed that most of the released ANP during blood volume expansion in the rat corresponded to a high molecular weight peptide. This suggests that the processing enzymes are not able to transform pro-ANP into the shorter peptide during volume challenge. Although some dysfunction of the sensing mecha-

\begin{table}
\centering
\caption{Increase in Right Atrial Pressure and Atrial Natriuretic Pressure With Volume Loading in Rats With Moderate or Large Infarcts or Without Infarcts}
\begin{tabular}{lccc}
\hline
 & Sham-operated \hspace{0.5cm} & Myocardial infarction \hspace{0.5cm} & Myocardial infarction \\
 & \hspace{0.5cm} (n=6) & \hspace{0.5cm} <30\% \hspace{0.5cm} (n=4) & \hspace{0.5cm} >40\% \hspace{0.5cm} (n=5) \\
\hline
Right atrial pressure (mm Hg) & & & \\
Before volume loading & & & \\
\hspace{0.5cm} -1 min & -0.33±0.42 & 0.66±0.19 & 1.13±0.52 \\
After volume loading & & & \\
\hspace{0.5cm} 1 min & 4.20±1.1 & 5.61±0.85 & 6.20±0.82 \\
\hspace{0.5cm} 3 min & -0.10±0.55 & 2.53±0.42* & 3.31±0.63* \\
\hspace{0.5cm} 5 min & 0.21±0.87 & 0.78±0.58 & 1.70±0.47 \\
\Delta Right atrial pressure (mm Hg) & 4.53 & 4.95 & 5.07 \\
\Delta Atrial natriuretic peptide (pg/ml) & 1,274 & 870 & 145 \\
\hline
\end{tabular}
\end{table}

Data are mean±SEM.
*p<0.05 vs. corresponding data of the sham-operated group at 3 minutes.
nism for the release of ANP cannot be totally excluded from the present data, this interpretation seems unlikely from our analysis of the relation between $\Delta$ANP and $\Delta$RAP in these animals (Figure 8, Table 2).

Taken together, synthesis, atrial content, and plasma ANP concentrations appear to respond appropriately to a wide range of cardiac dysfunction (according to the increases in intracardiac pressures). During continued stimulation of ANP secretion (due to markedly elevated LVEDP in the presence of large infarction), the storage of ANP in the atria is decreased; for example, the storage of ANP may equilibrate at a lower level during increased synthesis and turnover. Previous histologic studies on atria have indeed shown that granules in atria were not restored under long-term stimulation. Nevertheless, plasma ANP concentrations at baseline do correlate with the degree of cardiac dysfunction (as estimated by LVEDP). This indicates that despite reduced atrial ANP storage, the capability for ANP secretion into the circulation is not impaired. However, ANP secretion in response to further stimulation by volume loading may be limited in severe cardiac failure and markedly elevated LVEDP.

We have shown recently that the ability of ANP to reduce cardiac filling pressures and to improve renal perfusion is attenuated in this rat model of cardiac failure, which is consistent with findings in the dog and in patients with congestive heart failure. One may speculate, therefore, that the acute regulatory role of ANP is limited under these circumstances. This does not preclude ANP from having a role in the early phase of heart failure or from exerting sustained humoral long-term effects or both. ANP has inhibited renin release and aldosterone production. Increased plasma ANP levels during the development of cardiac failure may counterbalance the vasopressor systems, such as the renin-angiotensin and the sympathetic nervous systems. Indeed, an increase in plasma ANP was observed in the early phase of cardiac failure in a canine model without stimulation of the renin-angiotensin-system. Moreover, a sustained hypotensive response was observed in saralasin-sensitive two-kidney, one-clip rats during long-term ANP infusion, which may be attributed to an inhibitory effect on renin release. Furthermore, endogenous ANP has modulated sympathetic activity by inhibiting epinephrine release and baroreceptor reflexes. Whether or not these actions of ANP persist in chronic heart failure remains to be seen. Recent studies from this laboratory using monoclonal ANP antibodies suggest that the elevated endogenous ANP plasma levels do exert vasodilatory action indicating a cardiocirculatory role of ANP in this rat model of cardiac failure.

In summary, the present studies suggest that activation of the ANP system is directly coupled with the increase of intracardiac pressures without correlating linearly to the extent of myocardial loss (as estimated by infarct size). In severe cardiac failure, the ability of ANP secretion in response to further stimulation seems limited, which is similar to the attenuated response of target organs to ANP under these circumstances. Whereas the increase in atrial ANP synthesis and the increase of atrial ANP gene expression may be limited in severe chronic heart failure, substantial specific ANP gene expression emerges in the ventricles. This ventricular biosynthesis of ANP, in turn, may contribute to increased plasma ANP levels in chronic heart failure and—by reducing afterload and left ventricular wall stress—may constitute a self-compensatory mechanism in this setting.

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