Vasodilatory Action of Endogenous Atrial Natriuretic Factor in a Rat Model of Chronic Heart Failure as Determined by Monoclonal ANF Antibody


Elucidation of the role of (elevated) endogenous atrial natriuretic factor (ANF) in chronic heart failure has been hampered by a lack of specific inhibitors. We used a newly developed monoclonal antibody that has been shown to specifically block both exogenously and endogenously released ANF in vivo. For assessment of the vasodilatory action of ANF in chronic heart failure, either this antibody against ANF or ascites (control serum) was injected in rats with myocardial infarction and failure and in sham animals. Ascites did not alter central hemodynamics in either the sham or infarcted group. Antibody significantly increased right atrial pressure, left ventricular end-diastolic pressure, and systemic vascular resistance (SVR) in the infarction group but did not affect these variables in the sham group. Because renal blood flow, as measured by radioactive microspheres, decreased significantly in all four groups, probably due to nonspecific renal vasoconstrictor effects of the ascites, a separate group of infarcted animals was treated with purified ANF antibody (devoid of nonspecific effects) or mouse IgG as a control injection. In these animals, right atrial pressure increased from 1.1±0.7 mm Hg (p<0.001). Although SVR, renal blood flow velocity (measured by Doppler probe), and renal vascular resistance did not change in the infarcted animals after administration of purified ANF antibody, a significant correlation was found between baseline plasma ANF values and the change in SVR exerted by purified ANF antibody (r=0.758, p<0.02, n=9); that is, SVR increased in rats with high baseline plasma ANF (>350 pg/ml), but decreased in animals with plasma ANF <200 pg/ml. These results suggest that moderately elevated endogenous plasma ANF levels in chronic heart failure do affect central hemodynamics, primarily by reducing venous pressure (e.g., by decreasing intravascular volume or by venous dilation). Arterial vasodilation, however, appears to emerge when plasma ANF is greatly increased. (Circulation Research 1990;66:1371–1380)

Atrial natriuretic factor (ANF) has been shown to exert both renal and vasodilatory effects when given intravenously in animals and patients with volume overload such as congestive heart failure,1-5 although the renal effects of ANF appear to be attenuated to some degree in chronic heart failure.4,5 These findings do not establish the cardiocirculatory role of endogenous plasma levels in this setting. Since plasma ANF levels in severe heart failure are significantly elevated compared with normal levels, the question arises whether elevated endogenous ANF does in fact elicit vasodilatory and renal responses for restoration of circulatory and volume homeostasis. Plasma ANF appears to cause vascular (hemodynamic) effects in normal individuals, as suggested by low-dose ANF infusion6 or by comparison of ANF levels of venous blood samples and increase in forearm blood flow after intra-arterial ANF infusion.7 However, ANF receptors appear to be down-regulated in congestive heart failure.8 Similarly, renal ANF receptors have been shown to be down-regulated after chronic sodium load.9 Moreover, high molecular ANF forms (pro-ANF) with less vasodilatory activity may emerge during volume or pressure stimulation.10,11 Thus, the question of whether or not endogenous ANF does exert vasodilator action in heart failure remains unsettled. Elucidation of this issue has been hampered by the lack of specific ANF inhibitors.

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Supported by the Deutsche Forschungsgemeinschaft (Dr 148/3-3, 4-1).

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Received February 10, 1989; accepted December 19, 1989.
Recently a monoclonal ANF antibody was produced that specifically blocks both exogenously administered and endogenously released ANF. With the aid of this monoclonal antibody, it was shown that the strong diuresis, natriuresis, and cyclic GMP excretion as a response to acute hypervolemia is caused by a pronounced rise of ANF plasma levels. In the present study, we used this monoclonal ANF antibody to assess the cardiocirculatory role of the (elevated) endogenous ANF plasma levels in a rat model of chronic heart failure.

Materials and Methods

Experimental Preparations

Infarction was produced in male Sprague-Dawley rats by left coronary arterial ligation during anesthesia with chloral hydrate (0.3 g/kg body wt), as described previously, using a modification of the Maclean technique. The left coronary artery was ligated approximately 2 mm from its origin with a 6-0 suture. In the sham-operated animals, 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 21 days after surgery. During this postsurgical period, the mortality rate in the infarction group was approximately 15%. The selection of animals with infarction was based on an exercise duration of less than 8 minutes on a standardized exercise protocol, a left ventricular end-diastolic pressure (LVEDP) greater than 16 mm Hg, and an infarct size of greater than 24% of the left ventricle. Animals that did not meet these criteria were not included in this study.

Instrumentation

Animals were anesthetized with halothane (1% in oxygen), and catheters (PE 50) were inserted into the left ventricle via the right carotid artery, tail artery, and right jugular vein. Catheter positions in the left ventricle were determined by pressure wave detection by Statham P23ID pressure transducers (Gould, Cleveland, Ohio) and displayed on a recorder screen (Siemens Elema Mingograf, Erlangen, FRG). After closure, animals were allowed to recover for a minimum of 3 hours before experimental procedures were initiated. This recovery period has been found to be of sufficient duration to ensure a return to steady-state conditions in the rat.

Regional Blood Flow Measurements

Radioactive microspheres (141Ce, 85Sr, New England Nuclear, Dreieich, FRG), 15±5 μm in diameter, were used for measurement of regional blood flow and cardiac output according to the reference sample technique, as adapted for use in the rat. A detailed presentation of the radioactive microsphere technique as used in this study has been previously described. In brief, the microsphere suspension was thoroughly mixed by agitation and sonication (Sonifier B-30, Branson Ultrasons, Danbury, Connecticut) immediately before each injection. Approximately 250,000 microspheres (0.5 ml) were then injected into the left ventricle over a 15-second period, followed immediately by a 0.5-ml heparinized saline flush over a 15-second period. To obtain the reference sample, arterial blood was withdrawn (beginning 15 seconds before and continuing during the injection of microspheres) via the tail artery at a rate of 0.35 ml/min by use of a constant flow pump (model 906, Harvard Apparatus, South Natick, Massachusetts). The total blood withdrawal period was 1.5 minutes for each injection.

At the end of the study, animals were killed by injection of phenobarbital in the ventricle, and organs as well as tissue samples were removed. All samples were immediately blotted, weighed, and transferred to a two-channel gamma scintillation counter (model 480 AR, Kontron Instruments, Everett, Massachusetts) for determination of radioactivity levels. A Digital Equipment Corporation Professional 350 computer was used for calculation of regional blood flow as well as total cardiac output.

Hemodynamics

Tracings from the left ventricular, right atrial, and caudal catheters were recorded continuously during the experimental protocol and were used to obtain heart rate, left ventricular peak systolic pressure (LVSP), LVEDP, mean right atrial pressure (RAP), and mean arterial pressure (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. They were cut in eight transverse slices (5 mm) from apex to base, stained with van Gieson’s stain, and mounted. With a planimeter digital image analyzer (Leitz, Wetzl, FRG), the endocardial and epicardial circumferences of the infarcted and noninfarcted portions of the left ventricle were determined. The infarcted mean circumference (mean of epicardial and endocardial circumferences) of all eight slices was summed total and then expressed as the ratio of the summed circumference of the left ventricle. For all animals with infarction, myocardial infarction was transmural with a mature scar at the left anterior free wall. Area measurements of infarct size were not made because such measurements have been shown to underestimate infarct size as a result of resorption of necrotic tissue.
and subsequent wall thinning and late development of compensatory myocardial hypertrophy. Production of Monoclonal ANF Antibody

The monoclonal ANF antibody was produced according to standard methods, as previously described. Briefly, atriopeptin II was coupled to keyhole limpet hemocyanin and injected into female BALB/c mice. Fusion of spleen cells with the myeloma cell line X 63-AG 8.653 was carried out in the presence of polyethylene glycol (PEG 4000). Supernatant was taken from all wells showing cell growth after the selection procedure and screened for anti-atriopeptin antibodies by radioimmunoassay (RIA). Hybridoma cells from wells containing such antibodies were cloned twice. Cloned cells were used for production of ascitic fluid in male BALB/c mice. For differentiation of the effects of ANF antibody from nonspecific effects of the ascites, blank ascitic fluid was raised in BALB/c mice.

Experimental Protocols

Protocol 1. Measurements were performed in conscious animals 3 hours after anesthesia with halothane. Both sham animals and infarcted rats were divided into two groups; one group of both sham and infarcted rats received ANF antibody and the other group received ascitic fluid without antibody. Measurements of hemodynamics were performed continuously during the protocol. Regional blood flow and cardiac output were determined before and 30 minutes after injection of 0.5 ml/kg ANF antibody (titer, 1:1,600,000) or ascitic fluid without antibody over 30 seconds. The ascitic fluid was filtered sterile before injection (pore size, 0.8 µm). After each injection the jugular vein catheter was flushed with 0.3 ml of saline over 30 seconds (dead space of the catheter).

Protocol 2. For exclusion of any nonspecific action of the ascites containing the ANF antibody (and the ascitic fluid used for the control experiments), a second set of experiments was performed using the purified monoclonal ANF antibody. Purification from the mouse ascites was done by a two-step procedure. Ascites proteins were precipitated by addition of ammonium sulfate (45% final concentration). After solubilization of the pellet, the dialyzed fluid was applied to a d-methyl-amino-ethyl-cellulose column (DE-52, Whatman, Maidstone, UK). Immunoglobulins were eluted from the DE-52 column by salt gradient (0–0.3 M NaCl). According to the results of SDS gel electrophoresis, the purity was about 90%. In the control experiments, an identical volume of mouse IgG dissolved in phosphate buffered saline (PBS) was injected. Preliminary data revealed no significant differences in the plasma renin activity after the injection of mouse IgG and the purified ANF antibody (2 ml/kg body wt; antibody, 4.88±0.29 ng/ml/hr; mouse IgG, 4.08±0.35 ng/ml/hr; n=10, p=NS). Animals were instrumented with catheters in the right jugular vein and tail artery. A 0.6-mm thermistor probe, introduced via the right carotid artery, was positioned in the aorta ascendens. A miniaturized pulsed Doppler probe (Crystal Biotech, Holliston, Massachusetts) was placed around the right renal artery and secured as previously described. The lead from the Doppler probe, together with the catheters, was exteriorized at the back of the animal. Rats were allowed to recover for at least 24 hours before experiments were begun. On the day of the experiment, after stable baseline hemodynamics and renal blood flow velocity recordings were obtained, cardiac output was determined in duplicate by the thermodilution method by use of a cardiac output computer (HMV 7905, Hoyer, Bre men, FRG) and by injection of 0.25 ml ice-cold 0.9% saline in the right jugular vein. These measurements were repeated 30 minutes after administration of the purified ANF antibody.

ANF-Like Immunoreactivity in Plasma

Via the catheter in the tail artery, 0.6 ml of blood was taken during the control period and put into ice-cooled EDTA tubes. Plasma was separated by centrifugation and stored at 70°C for determination of ANF in plasma by double antibody RIA that used the monoclonal antibody directed against ANF. A 0.3-ml portion of each plasma sample was applied to a Sep-Pak C cartridge (Waters Associates, Millipore, Milford, Massachusetts). Cartridges were cleaned with 3 ml potassium phosphate buffer (0.1 M, pH 3.5) and 2 ml H2O. ANF-like immunoreactivity (ANF-IR) was eluted with 3 ml acetonitrile (0.2% trifluoro acid, 50/50), lyophilized, and redissovled in a special RIA buffer (0.1 M sodium phosphate, 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, 0.01% NaN, and 10 units/l aprotinin, pH 7.4). Ninety microliters of ascitic fluid containing the monoclonal antibody directed against atriopeptin II was added to 100 µl of either plasma samples (prepared as described below) or standard dilutions of atriopeptin II. After preincubation for 16–24 hours at 4°C, 100 µl 125-I atriopeptin III (prepared by the chloramin T method) was added, and the incubation continued for 24 hours. At the end of this period, 10 µl mouse IgG (63 µg/tube; Sigma Chemical, St. Louis) was added to each tube. Separation of free peptide from antibody-bound peptide was performed by addition of 100 µl anti-mouse IgG (33 µg/tube; Sigma Chemical) to each tube. The precipitate was allowed to form for 2 hours at room temperature, followed by addition of 1 ml polyethylene glycol (PEG 6000, 15 g plus 100 ml H2O; Serva, Heidelberg, FRG) and centrifugation for 20 minutes at 2,000g. Activity of the pellet was determined with an LKB 1271 RIA gamma counter (Turku, Finland). The sensitivity of this procedure was 4 pg/ml when defined as the least amount of atriopeptin II that could be distinguished from B based on the 95% confidence limits for each value. The monoclonal antibody reacts well with atriopeptin I, atriopeptin II, atriopeptin III, and α-ANF (1--28), while no cross-reactivity has been found to the ANF fragments (13--28) and (18--28) and to other substances.
TABLE 1. Baseline Characteristics of Control Animals and Rats With Infarction

<table>
<thead>
<tr>
<th></th>
<th>Sham Ascites</th>
<th>Sham Antibody</th>
<th>Infarct Ascites</th>
<th>Infarct Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>352±18</td>
<td>369±14</td>
<td>368±22</td>
<td>345±24</td>
</tr>
<tr>
<td>RV weight (g/kg body wt)</td>
<td>0.50±0.03</td>
<td>0.42±0.03</td>
<td>0.78±0.06*</td>
<td>0.79±0.06*</td>
</tr>
<tr>
<td>Infarct size (% of LV)</td>
<td>. . .</td>
<td>. . .</td>
<td>31±2.5</td>
<td>33±3.1</td>
</tr>
<tr>
<td>Plasma ANF-IR (pg/ml)</td>
<td>105±40</td>
<td>61±20</td>
<td>314±87†</td>
<td>441±134†</td>
</tr>
</tbody>
</table>

Data are mean±SEM. RV, right ventricle; LV, left ventricle; ANF-IR, atrial natriuretic factor immunoreactivity.  
*p<0.01 vs. sham groups.  
†p<0.05 vs. corresponding sham group.

(e.g., aldosterone, ACTH, angiotensin II, leucine enkephalin, [Arg⁸]-vasopressin, γ-melanocyte stimulating hormone, substance P, bradykinin, renin, insulin, and cyclic GMP).12

Statistical Analysis

Data are given as mean±SEM. Differences between groups were analyzed by analysis of variance (ANOVA). The difference in treatment effects (ANF antibody versus unspecified ascitic fluid effect) was evaluated by ANOVA of the increase or decrease of each variable measured (comparison of the four groups). Individual comparisons were made by the Student-Newman-Keuls test.23 Regression lines were fitted by the method of least squares and calculated by the RS/1 program (Bott Beranek and Newman, Cambridge, Massachusetts) for the DEC Professional 350. The pooled baseline hemodynamics (all infarcted animals versus all sham animals) were compared by two-tailed nonpaired t test. The comparative effects of purified ANF antibody and mouse IgG on hemodynamics (protocol 2) were assessed by Fisher’s exact test.

Results

Table 1 depicts the baseline characteristics of the four experimental groups (protocol 1). Plasma ANF-IR was significantly increased in both infarction groups compared with the sham animals. ANF-IR in plasma was measured with a double-antibody RIA using a monoclonal antibody directed against ANF. Although the baseline plasma ANF-IR was somewhat higher in the myocardial infarct group receiving ANF antibody compared with the myocardial infarct group receiving ascites, the difference was not statistically significant; when one animal with extremely high ANF-IR (1,198 pg/ml) was excluded, the mean of the ANF-IR for the infarction group receiving antibody averaged 333 pg/ml, similar to the infarction group receiving ascites (Table 1).

With respect to the baseline hemodynamics, the two infarction groups were similar (Tables 2 and 3, Figures 1–3), but differed significantly from the sham group receiving ANF antibody and the sham group receiving ascites. LVEDP was significantly elevated, and LVSP, cardiac output, and cutaneous blood flow were reduced. Blood flow to kidney tended to be lower, and systemic vascular resistance (SVR) and RAP tended to be higher in both infarction groups compared with the sham groups.

When the hemodynamic baseline data of all infarcted animals (those receiving either ascites or ANF antibody) were pooled, the difference compared with the pooled data of all sham animals was statistically significant (p<0.05) for SVR (0.29 vs. 0.22 mm Hg·kg·min/ml), RAP (2.1 vs. 0.6 mm Hg), and renal flow (3.9 vs. 4.8 ml/min/g). Moreover, right ventricular hypertrophy emerged in both infarction groups, as indicated by a significant increase of the weight of the right ventricle (Table 1).

Figure 1 shows the effects of ANF antibody versus control injection with ascites lacking ANF antibody on right atrial pressure. There was a slight increase of RAP in the sham group receiving antibody, but it was not significant; however, there was a substantial, statistically significant increase of RAP in the infarction group receiving antibody. The control ascites injection did not affect RAP. Similarly, ANF antibody increased LVEDP only in the infarction group, and the control ascites injection did not exert changes in LVEDP (Figure 2). In both the sham and infarct groups, ascites tended to increase SVR. ANF antibody also elevated SVR in both the infarction and sham groups, an effect that was statistically
TABLE 3. Effect of Ascites and ANF Antibody on Selected Regional Blood Flow (ml/min/g of Tissue)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ascites</th>
<th>Control</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>3.5±0.4</td>
<td>3.0±0.2</td>
<td>3.3±0.8</td>
<td>3.3±0.7</td>
</tr>
<tr>
<td>MI</td>
<td>2.7±0.4</td>
<td>2.1±0.2*</td>
<td>2.5±0.3</td>
<td>2.2±0.3</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.14±0.04</td>
<td>0.12±0.05</td>
<td>0.17±0.03</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>MI</td>
<td>0.16±0.04</td>
<td>0.18±0.05</td>
<td>0.16±0.03</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td>Right ventricle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6.6±0.8</td>
<td>5.9±0.7</td>
<td>5.7±0.9</td>
<td>6.0±0.7</td>
</tr>
<tr>
<td>MI</td>
<td>5.7±0.6</td>
<td>5.7±0.6</td>
<td>5.1±0.6</td>
<td>5.1±0.5</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>0.12±0.04</td>
<td>0.10±0.05</td>
<td>0.14±0.05</td>
<td>0.11±0.03</td>
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<tr>
<td>MI</td>
<td>0.11±0.03</td>
<td>0.10±0.02</td>
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<td>0.07±0.02</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.095±0.01</td>
<td>0.08±0.01</td>
<td>0.105±0.01</td>
<td>0.098±0.01</td>
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<tr>
<td>MI</td>
<td>0.065±0.01†</td>
<td>0.049±0.02*</td>
<td>0.064±0.03†</td>
<td>0.040±0.02*</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>5.1±0.6</td>
<td>3.5±0.4*</td>
<td>4.5±0.5</td>
<td>3.2±0.3*</td>
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<tr>
<td>MI</td>
<td>4.3±0.4</td>
<td>3.1±0.3*</td>
<td>3.7±0.4</td>
<td>2.1±0.3*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. MI, myocardial infarction.

*p<0.05 vs. corresponding control value.
†p<0.05 vs. sham control value.

significant for the infarction group only. The Δ increase in SVR elicited by ANF antibody was significantly greater than the Δ increase exerted by ascites (Table 2).

When the infarcted rat with extremely high plasma ANF-IR (1,198 pg/ml) was excluded, a significant correlation between baseline plasma ANF values and the change in SVR was found (r=0.687, p<0.01, n=14), as shown in Figure 3. The excluded animal had a very large infarction (45% of the left ventricle) with severely depressed hemodynamics and appeared to be in severe heart failure. Hemodynamics including SVR and regional blood flow did not change with injection of ANF antibody in this animal.

Cardiac output and stroke volume decreased with both ascites and ANF antibody in all four groups; the effect was statistically significant for the infarction group receiving antibody (Table 2). The decrease in cardiac output of the infarction group receiving the antibody averaged 83 ml/min/kg compared with 46 ml/min/kg for the infarction group receiving ascites. The group difference between these changes (Δ change of antibody versus Δ change of ascites) was not statistically significant. No significant changes

![RIGHT ATRIAL PRESSURE](image-url)

**FIGURE 1.** Effect of control ascites and atrial natriuretic factor antibody on right atrial pressure in sham-operated and infarcted rats. Data are mean±SEM. Open bars, control values before ascites; hatched bars, values after ascites; black bars, control values before antibody; crossed bars, values after antibody. *p<0.05 vs. corresponding control value of same group.
were observed in mean arterial pressure and heart rate in any of the groups. Renal blood flow decreased with both ascites and antibody in both sham and infarction groups (Table 3). Similarly, blood flow decreased to the intestines and skin with both ascites and antibody in infarcted animals (Table 3), which indicates a nonspecific response to the ascites (the antibody was raised in ascites). No group differences in the response to antibody versus ascites were present in any of these vascular beds.

For exclusion of any nonspecific effect of the ascites, a purified monoclonal ANF antibody was used in a second set of experiments. In contrast to a mouse IgG (used for control injection), purified ANF antibody significantly increased RAP in rats with infarction (Figure 4). The group difference between the Δ effects of purified ANF antibody versus IgG was significant (p<0.01). Neither mouse IgG nor purified ANF antibody exerted significant effects on mean arterial pressure, heart rate, cardiac output, SVR, or renal blood flow velocity in these animals with infarction. However, cardiac output decreased in the three rats with high plasma ANF values at baseline (>350 pg/ml), whereas a moderate increase in cardiac output was observed in the animals with plasma ANF values between 144 and 240 pg/ml. Plasma ANF values averaged 67±5 pg/ml in a group of sham-operated animals (Table 4). Moreover, a significant correlation was found between the change in the SVR and the plasma ANF concentration at baseline (Figure 5). An increase in SVR was observed primarily in rats with high plasma ANF values. It should be noted that in the group receiving the mouse IgG, no relation between baseline plasma ANF and the change in SVR was found; in addition,

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**Figure 2.** Effect of control ascites and atrial natriuretic factor antibody on left ventricular end-diastolic pressure (LVEDP) in sham-operated and infarcted rats. Data are mean±SEM. Open bars, control values before ascites; hatched bars, values after ascites; black bars, control values before antibody; crossed bars, values after antibody. *p<0.05 vs. corresponding control value of same group; †p<0.01 compared with corresponding sham baseline values.

**Figure 3.** Relation between basal plasma atrial natriuretic factor immunoreactivity (ANF-IR) and change in systemic vascular resistance (SVR) in animals receiving monoclonal antibody (protocol 1). Correlation coefficient r is given for the group excluding one animal with extremely high plasma ANF-IR (1,198 pg/ml). See text for further explanations.
there was no increase in RAP in the infarction group receiving the mouse IgG (Figure 4).

Discussion

Although it is generally assumed that ANF is involved in the regulation of renal and cardiovascular homeostasis, definite conclusions regarding the physiological role of ANF are difficult to draw from infusion studies that apply exogenous ANF. Circulating concentrations of ANF achieved by infusion varied widely in reported studies and were usually greater than those produced by endogenous ANF release. Interpretation of infusion studies is further complicated by the fact that ANF antagonizes the effects of several vasoconstrictors. Therefore, the degree of activation of these vasoconstrictors would substantially influence the response to ANF infusion. Nevertheless, recent studies using antisera or monoclonal antibodies against ANF indicate that endogenous ANF may exert both vasodilatory and renal effects in anesthetized rats.

Although plasma ANF levels are significantly elevated in heart failure and appear to correlate with right and left ventricular filling pressures, the cardiovascular role of endogenous ANF in chronic heart failure may be questioned. Several studies have demonstrated attenuated hemodynamic responses even with high doses of intravenous ANF in animals or patients with chronic heart failure. Down-regulation of ANF receptors may emerge in chronic heart failure, as suggested by Schiffrin. Moreover, a reduction in renal ANF binding sites in the rat model of myocardial infarction and failure has been demonstrated recently.

To evaluate the pathophysiological role of endogenous ANF in regulation of the systemic and regional hemodynamics in chronic heart failure, we used a monoclonal antibody that specifically blocks circulating plasma ANF in vivo. It has been shown previously that this monoclonal antibody antagonizes the renal (excretory) effects of exogenous ANF in vivo. Thus, differences in effects between ascites alone (control injection) and ascites containing ANF antibody should be due to removal of action of endogenous ANF. To exclude the interference of anesthesia and its circulatory, neurohumoral, and reflex-mediated effects, measurements were performed in conscious animals, in contrast to recent studies in anesthetized rats.

The results of the present study demonstrate that the removal of (unbound) endogenous ANF from the circulation causes an elevation of right and left ventricular filling pressures in animals with chronic heart failure. Moreover, ANF antibody appears to elicit an increase in SVR, at least in animals with greatly elevated plasma ANF levels.

Table 4. Baseline Characteristics of Infarcted Rats Treated With Purified ANF Antibody or Mouse IgG

<table>
<thead>
<tr>
<th></th>
<th>IgG group (n=9)</th>
<th>AB group (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>SVR</td>
</tr>
<tr>
<td>Control</td>
<td>109±6</td>
<td>450±12</td>
</tr>
<tr>
<td>IgG</td>
<td>110±5</td>
<td>452±12</td>
</tr>
<tr>
<td>AB group</td>
<td>112±5</td>
<td>427±21</td>
</tr>
<tr>
<td>ANF antibody</td>
<td>117±6</td>
<td>428±23</td>
</tr>
</tbody>
</table>

Data are mean±SEM. MAP, mean arterial pressure; HR, heart rate; SVR, systemic vascular resistance; RBFV, renal blood flow velocity (arbitrary dimensions); CO, cardiac output; IgG group, animals receiving mouse IgG; AB group, animals receiving purified ANF antibody.

*Parameter not measured in one animal for technical reasons; data are for n=10.
RAP increased substantially after injection of ANF antibody in the infarction group only, indicating that endogenous ANF is involved in the regulation of central venous pressure, that is, endogenous ANF appears to reduce (high) RAP in chronic heart failure. The present study did not address the mechanisms responsible for this effect; however, several studies have indicated that ANF diminishes venous return by increasing the efflux of fluid from capillaries.\textsuperscript{32,33} Thus, removal of free circulating ANF is likely to increase RAP by shifting fluid from the extravascular to the intravascular space, which, in turn, would facilitate venous return. In sham-operated animals no significant changes were observed in right or left ventricular filling pressures or SVR. It should be noted, however, that plasma ANF levels were low in these animals and within the normal range of the RIA, indicating that ANF may not affect right and left ventricular filling pressures in normal animals under resting conditions.

Renal blood flow decreased significantly in all four groups. Although the percent change was greater in the infarction group receiving ANF antibody compared with the three other groups, the difference of treatment effects was not statistically significant. Similarly, intestinal and cutaneous blood flow was reduced to some extent in all groups, although these reductions were generally less pronounced compared with the effects observed in the renal circulation. Therefore, it appears that the ascitic fluid itself produced nonspecific regional (vasoconstrictor) cardiovascular effects. In a second set of experiments using a highly purified ANF monoclonal antibody, no significant changes in renal blood flow velocity were observed in animals with either low or high baseline plasma ANF values, indicating that the purified ANF antibody was devoid of nonspecific effects. Indeed, the failure of the purified ANF antibody to exert renal vascular effects suggests that the renal vascular effects of endogenous ANF are blunted in chronic heart failure or, possibly, totally counteracted by vasoconstrictor forces. Recent studies have shown that the renal vascular effects are attenuated in this model of myocardial infarction and failure\textsuperscript{4} (e.g., due to reduced renal ANF binding sites, as reported recently).\textsuperscript{31)

SVR increased after injection of ANF antibody in the infarction group. Nonspecific vasoconstricting effects of the ascitic fluid containing the ANF antibody may have contributed to the increased SVR in the first set of experiments; however, SVR increased even with purified ANF antibody in those rats with greatly elevated endogenous baseline ANF values. Moreover, the change in SVR elicited by purified ANF antibody was significantly related to baseline ANF values in these infarcted rats (Figure 5). Thus, high endogenous plasma ANF values appear to exert arterial vasodilatory action in rats with myocardial infarction and failure. It should be noted that the failure to demonstrate arterial vasodilation in infarcted rats with moderately elevated plasma ANF levels does not exclude a vasodilatory action of endogenous ANF in these animals. RAP increased in all infarcted animals after administration of purified ANF antibody, very likely due to increased venous return. This increase in right and, possibly, left ventricular filling pressure may have resulted in an augmented cardiac output, which, in turn, would be accompanied by (a reflex) decrease in SVR. Thus, systemic vasoconstriction, elicited by removal of endogenous ANF by purified ANF antibody, may be offset or even overridden by this mechanism. Conversely, the decrease in cardiac output after administration has been shown to be secondary to a reduction in preload.\textsuperscript{6,34,35}

Plotting of the baseline plasma ANF levels against changes in SVR elicited by the (nonpurified) ANF antibody produced a significant correlation only if one infarcted animal with extremely high plasma ANF was excluded from the analysis. Endogenous ANF apparently did not elicit arterial vasodilatory action in this infarcted rat (e.g., due to receptor down-regulation\textsuperscript{49} in the face of extremely high circulating plasma ANF and/or secretion of less vasoactive forms of ANF\textsuperscript{31} due to chronic stimulation of ANF secretion by high atrial pressure). In this animal, right atrial pressure was 5 mm Hg versus a mean of 2.6 mm Hg for the infarction group. LVEDP was 33 mm Hg versus a mean of 24 mm Hg for the infarction group, and right ventricular weight was 1.05 g/kg body wt versus a mean of 0.79 g/kg body wt for the whole group. This observation raises the possibility that the relation between plasma ANF levels and the change in SVR may not be a linear one. Therefore, the relation as depicted in Figure 5 may apply only for moderate to severe but not for advanced severe chronic heart failure. The responsiveness to greatly elevated ANF levels may be completely lost in very severe chronic heart failure when vasoconstrictor systems may dominate.

Renal vascular resistance (unchanged renal blood flow velocity and arterial pressure) did not change with purified ANF antibody. Thus, the increase in

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Relation between baseline plasma atrial natriuretic factor (ANF) levels and change in systemic vascular resistance (SVR) in animals receiving purified ANF antibody (protocol 2). ANF values and SVR data were available for nine infarcted animals. ANF-IR, ANF-like immunoreactivity.}
\end{figure}
SVR after ANF antibody in infarcted animals with high baseline ANF values cannot be explained by the elevation of vascular resistance in the renal circulation. Therefore, it appears that other organs and tissues contributed to the increased SVR, indicating a more systemic vasoconstriction. Several studies have demonstrated that the direct vasodilatory effect of ANF preferentially occurs in the systemic vasoconstriction. Indeed, it has been shown that ANF reduces angiotensin and/or inhibits sympathoadrenal activity. Thus, one consequence of endogenous ANF on vasoconstrictor-induced vasoconstriction in the peripheral circulation. Indeed, it has been shown that ANF reduces the systemic effect of endogenous ANF uncovered in the present study may be due, in part, to a modulating effect of endogenous ANF on vasoconstrictor forces. This could explain the relation between the change in SVR and the baseline values of endogenous plasma ANF (Figure 5). Consistent with this hypothesis, Hodson et al reported that plasma renin activity, aldosterone, and vasopressin are not increased in the face of greatly elevated plasma ANF values in this model of myocardial infarction and failure, suggesting that the increased ANF levels act to suppress the renin-angiotensin system.

In summary, the present study demonstrates that blocking of elevated endogenous plasma ANF by a monoclonal ANF antibody results in elevated right and left ventricular filling pressures. Moreover, when plasma ANF values are considerably increased, SVR increases in chronic heart failure after blocking the plasma ANF. This suggests that circulating endogenous ANF plays an important role in reduction of elevated right atrial and left ventricular filling pressures in chronic heart failure. Moreover, greatly elevated endogenous plasma ANF appears to elicit arterial vasodilatory action (either directly or by modulation of neurohumoral vasoconstriction) in this setting, thereby opposing vasoconstrictor forces such as the renin-angiotensin system.

Acknowledgment

The authors would like to thank Evi Hablawetz for technical assistance.

References


KEY WORDS • atrial natriuretic factor • monoclonal ANF antibody • chronic heart failure • vasodilation
Vasodilatory action of endogenous atrial natriuretic factor in a rat model of chronic heart failure as determined by monoclonal ANF antibody.
H Drexler, C Hirth, H P Stasch, W Lu, D Neuser and H Just

doi: 10.1161/01.RES.66.5.1371

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/66/5/1371

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