Ventricles as a Major Site of Atrial Natriuretic Factor Synthesis and Release in Cardiomyopathic Hamsters With Heart Failure


The aim of the present study was to correlate in cardiomyopathic hamsters with congestive heart failure the levels of atrial and ventricular atrial natriuretic factor (ANF) messenger RNA (mRNA) with immunoreactive ANF (IR-ANF) plasma levels and the relative amount of IR-ANF released by the whole heart versus isolated ventricles in the Langendorff preparation. High-performance liquid chromatography analysis of the forms of ANF present in plasma and in the Langendorff effluent of whole heart versus isolated ventricles was also performed. As previously found for cardiac IR-ANF, the levels of ANF mRNA decreased gradually in atria and increased in an analogous fashion in ventricles with the severity of congestive heart failure. Plasma IR-ANF levels (C-terminal) were more elevated in moderate than in severe congestive heart failure, as were the IR-ANF levels in the Langendorff effluent of the whole heart. On the contrary, the effluent of isolated ventricles from animals in severe heart failure yielded more IR-ANF than that from hamsters in moderate heart failure. Thus, while the isolated ventricles from controls contributed 35.8% of IR-ANF released by the whole heart, ventricles from hamsters in moderate heart failure contributed 17.5%, and those from hamsters in severe heart failure contributed 73.9%. These results indicate that atrial cardiocytes contribute more IR-ANF than their ventricular counterpart in moderate heart failure and that ventricles are a major source of plasma IR-ANF in severe heart failure. Analysis of IR-ANF from plasma and the Langendorff effluent from whole hearts and isolated ventricles revealed that the ventricles are the major source of the propeptide (and of its cleaved products) found in the circulation of cardiomyopathic hamsters. These results suggest that ANF synthesis and secretion do not increase concomitantly in atria but do increase in ventricles during congestive heart failure. (Circulation Research 1989;65:71-82)

The atria in mammals constitute an endocrine gland1-4 that, in the rat, secretes atrial natriuretic factor (ANF), a 28–amino acid peptide (Ser 99-Tyr 126),5,6 which is diuretic, natriuretic, vasodilatory, and inhibitory of aldosterone, cortisol, arginine vasopressin, and renin release. In the rat, the N-terminal moiety (Asn 1-Arg 98) of the propeptide (Asn 1-Tyr 126) of ANF (proANF) is also present in the circulation.7-9 The mechanism by which the peptide is released is not known, although an increase in atrial pressure, possibly leading to stretch of the atrial wall, may be the primary physiological regulator of ANF secretion.10-17 Angiotensin, noradrenaline, and other pressor agents have been shown to induce a marked release of ANF into the circulation both in humans and experimental animals.18-23 Because the rise of ANF in these studies was correlated with an increase in atrial pressure or other hemodynamic parameters (which in some cases were not measured), the exact role of pressor substances in the control of ANF release remains unknown. In vitro studies have suggested that acetylcholine,24-26 $\alpha$- and $\beta$-adrenoreceptors,24,26-29 and sodium ion30 enhance ANF secretion. Arginine vasopressin has also been shown to stimulate ANF release, both in vivo, where it is accompanied by increase in atrial pressure,31-33 and in vitro, from atrial tissue fragments.25,29 In one study, however,
exposure of atrial slices to arginine vasopressin did not elicit ANF secretion.  

A number of conditions are now known to produce an elevation of both immunoreactive ANF (IR-ANF) and ANF messenger RNA (mRNA) in atria and ventricles both in vivo and in vitro.  

Glucocorticoid-stimulated IR-ANF synthesis is accompanied by an increase in ANF mRNA in the rat atria.  

In primary cultures of rat atria, dexamethasone, testosterone, and triiodothyronine markedly stimulate both the synthesis and secretion of IR-ANF.  

Dexamethasone has also been shown to increase both IR-ANF and ANF mRNA in rat ventricles in vitro; the same finding has been reported in primary cultures of rat ventricular cardiocytes.  

Testosterone and triiodothyronine have comparable effects on both IR-ANF levels and secretion in cultures of rat ventricular cardiocytes.  

Sodium administration can also enhance atrial ANF release and atrial ANF content, particularly when it is associated with an increase in plasma volume.  

A pronounced elevation of ventricular ANF mRNA and IR-ANF has also been associated with hypertrophy, either in cardiomyopathic hamsters or in volume-overloaded rats.  

In humans, congestive heart failure is accompanied by an increase in both C- and N-terminal ANF.  

In cardiomyopathic hamsters, the plasma levels of IR-ANF are also substantially elevated, and the ratio of IR-ANF between atria and ventricles, which is 132:1 in control hamsters, becomes 4:1 in animals with severe congestive heart failure because of a decrease in the atrial levels and a concomitant increase in ventricular levels.  

The aim of the present study was assessment of whether IR-ANF levels in atria and ventricles reflect changes in gene expression or merely changes in secretory activity by measurement of ANF mRNA and evaluation of the relative contribution of atria and ventricles to plasma IR-ANF levels by measurement of ANF release in the Langendorff preparation. For comparative purposes, analogous investigations were performed with the heart of control rats. The molecular forms of IR-ANF present in plasma and released by the heart and isolated ventricles were further analyzed by high-performance liquid chromatography (HPLC).  

Materials and Methods  

Animals  

Cardiomyopathic hamsters in moderate or severe heart failure were from the UM-X7.1 cardiomyopathic line established by the crossbreeding of diseased animals from the original BIO 14.6 strain of the Bio-Research Institute (Cambridge, Massachusetts) with unrelated healthy hamsters.  

Golden Syrian hamsters (Mesocricetus auratus) (Charles River, St. Constant, Quebec, Canada) of the same age (200-300 days) were used as controls. All animals were maintained on regular pellet chow (Purina, Richmond, Indiana) and tap water ad libitum in plastic cages at 22° C with 60% humidity under a 6 AM-6 PM light regimen. Sprague-Dawley rats (190-210 g body weight) were housed in steel cages under the same conditions.  

Assessment of Severity of Congestive Heart Failure  

The clinical and histopathological evaluation of the degree of congestive heart failure was done as previously described without knowledge of the results of plasma IR-ANF or cardiac ANF mRNA determinations.  

Animals with moderate heart failure showed diffuse subcutaneous edema, liver pathology consisting of hemorrhage and necrosis around the central veins, and mild pleural effusion. Animals with severe heart failure showed generalized edema involving the subcutaneous areas of the entire body (including the facial part) together with ascites, hydrothorax, and hydropericardium. The majority of skeletal muscle groups, especially those of the hind legs and abdominal walls, were quite swollen. The mesentery of the intestine and pancreas were highly edematous, and the measurable exudates in the body cavities amounted to well over 2.5 ml. Diffuse necrotic and fibrotic lesions were present in the congested liver, and hemosiderin-laden macrophages (heart failure cells) were consistently seen in the lungs.  

Northern Blot  

The atria and ventricles of 10 control hamsters and 10 animals with moderate and severe heart failure were dissected and immediately frozen in liquid nitrogen. Total RNA was extracted from these tissues by the method of Chirgwin et al.  

Northern blots were performed in the standard manner except that 0.2-μm Nytran membranes (Schleicher & Schuell, Keene, New Hampshire) were used. The membranes were prehybridized and hybridized in an aqueous solution containing heparin. Hybridization was carried out overnight at 65° C at a probe concentration of 1×10⁶ cpm/ml. The radioactive probes were prepared by random priming and labeled to a specific activity of 0.5×10⁹ dpm/μg DNA. A 600 bp rat ANF complementary DNA (cDNA) clone was used to detect ANF mRNA. A mouse brain β-actin cDNA probe was used to monitor the amount of atrial versus ventricular RNA. Several dilutions of total RNA from cardiomyopathic animals with moderate and severe heart failure were separated by electrophoresis and hybridized successively with ANF and mouse β-actin cDNA probes. For quantitation of ANF mRNA levels, autoradiograms were scanned with a densitometer (model 620, Bio-Rad, Richmond, California) coupled with an integrator (model CD3401, Varian Associates, Palo Alto, California).
Langendorff Technique

Animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.). The thorax was opened, and the whole heart was rapidly excised and rinsed in cold Krebs solution. The aorta was cannulated, and the atria were removed surgically in half the animals. The surface of the left ventricle around the aorta was burnished with a thermocautery (heat pen, JBEM Services, Montreal, Canada) to eliminate fragments of atrial tissue; care was taken not to injure the coronary arteries that run on either side of the pulmonary conus. The whole hearts or the ventricles were then perfused according to the Langendorff technique at a constant flow of 6–19 ml/min to maintain perfusion pressure between 80 and 100 mm Hg. The flow was adjusted during the first 15 minutes of perfusion (equilibration period) to account for the fact that higher flow was needed to maintain the pressure in the perfused ventricles; the flow was kept constant during the subsequent 2-hour period of the experiments. Perfusion pressure and heart rate were monitored continuously. The perfusion solution was maintained at 37° C and oxygenated with a mixture of 95% O2 and 5% CO2. The Krebs solution used in this study contained (mM) NaCl 122, KCl 4.3, MgCl2 1.0, NaHPO4 0.8, CaCl2 2.5, NaHCO3 25.0, and dextrose 5.5. After the whole hearts or ventricles were washed for 15 minutes, the effluent was passed for successive 10-minute periods through C18 Sep-Pak cartridges (Waters Chromatography, Milford, Massachusetts) for 2 hours.

For analysis of IR-ANF by reverse-phase HPLC, the experiments were repeated, and this time, the effluent was passed through the same type of cartridges for two successive periods of 1 hour each. In all cases the cartridges were washed with 10 ml water; the adsorbed IR-ANF was eluted with 3 ml 86% ethanol, 4% acetic acid and the material was analyzed during the first or second hour of perfusion.

Immunohistochemistry

For verification of the completeness of removal of atrial tissue, perfused ventricles were fixed in Bouin’s solution for 24 hours, embedded in paraffin, and cut into serial 5-μm sections. Whole hearts were similarly prepared. They were then reacted according to the unlabeled antibody technique of Sternberger, which uses polyclonal antibodies against synthetic ANF (Arg 101-Tyr 126),43 and counterstained or not according to the periodic acid-Schiff hematoxylin technique. Controls included preimmune serum, antiserum previously adsorbed with ANF (Arg 101-Tyr 126) (solid-phase immunoadsorption),43 and omission of the primary antiserum, goat anti-rabbit IgG or peroxidase antiperoxidase. All controls gave negative results.

Radioimmunoassays

The C-terminal ANF radioimmunoassay technique has been described elsewhere43–45 and used ANF (Ser 99-Tyr 126) as both standard and iodinated tracer. The N-terminal ANF radioimmunoassay technique has also been described elsewhere.9 Rat ANF (Asn 1-Arg 98) was used as both standard and iodinated tracer. The antibodies were produced against a synthetic rat fragment ANF (Asp 11-Ala 37). The C-terminal ANF did not show any cross-reactivity. The two-site immunoradiometric assay (IRMA) for proANF (Asn 1-Tyr 126) used two different antibodies, which allowed the specific measurement of the precursor.5 The first antibody (2H2), a murine monoclonal antibody directed against the C-terminal portion of the precursor, was linked to polyacrylamide beads (Immunobeads, Bio-Rad) and used to extract any C-terminal immunoreactive material. A second antibody (GT23), which recognizes the fragment Asp 11-Ala 37 of proANF, was radiolabeled and used as a tracer. In brief, 100 μl of samples or of standard ANF (Asn 1-Tyr 126) (0–400 fmol) was incubated for 2 hours at room temperature in the presence of 50 μl (200 μg) of 2H2-Immunobeads in a total volume of 1 ml RIA buffer (0.05 M NaPO4 [pH 7.4], 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% NaN3). The beads were then centrifuged at 3,000 rpm for 15 minutes and washed twice with 2 ml RIA buffer. To the pellet was added 1 ml iodinated GT23 antibody (100,000 cpm, 2–3.6 μCi/μg). After further incubation at 4° C for 18 hours and centrifugation, the pellet was washed once with RIA buffer, and the radioactivity was counted in a γ-counter. A curvilinear relationship was established between the radioactivity and the amount of ANF (Asn 1-Tyr 126). ANF (Ser 99-Tyr 126) or ANF (Asn 1-Arg 98) up to 5 pmol/tube did not affect concentration of ANF (Asn 1-Tyr 126); however, higher amounts of ANF (Ser 99-Tyr 126) could interfere with the IRMA by saturating the 2H2-Immunobeads.

Measurement of IR-ANF in Plasma

Five controls, five cardiomyopathic hamsters with moderate heart failure, and five cardiomyopathic hamsters with severe heart failure were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Blood was withdrawn from the jugular vein into tubes cooled in crushed ice and containing 10−3 M EDTA, 10−3 M phenylmethylsulfonyl fluoride, and 5×10−3 M pepstatin. The blood was centrifuged immediately at 4° C, and the plasma was used as previously described for measurement of the C-terminal43 and the N-terminal8 moieties of ANF.
Measurement of IR-ANF in Ventricles

To find out if the release of IR-ANF from isolated ventricles could be correlated with changes of IR-ANF content in the ventricles themselves, we measured IR-ANF as previously described in the whole ventricles of control rats and control hamsters either before or after 2-hour perfusion in the Langendorff preparation.

HPLC Pattern of Plasma IR-ANF of Rats and Hamsters

Each series of studies used five rats, five control hamsters, five cardiomyopathic hamsters with moderate heart failure, and five cardiomyopathic hamsters with severe heart failure. Blood was withdrawn and treated as above. Plasma was extracted with Sep-Pak cartridges. The cartridges were washed with 10 ml water, the adsorbed IR-ANF was eluted with 3 ml 86% ethanol in 4% acetic acid, and the solution was evaporated in a Savant Speed Vac. The material was analyzed on C4 Vydac columns (Separations Group, Hesperin, California) eluted with a 0.3%/min linear gradient (25-50%) of acetonitrile in 0.13% heptafluorobutyric acid and a flow of 1 ml/min.

HPLC Pattern of the Effluent of the Whole Heart and Ventricular IR-ANF

The lyophilized material was analyzed by HPLC as above. In all cases, the C-terminal, the N-terminal, and the propeptide of ANF were measured in aliquots of HPLC fractions (1 ml) by radioimmunoassay as described above.

Results

Northern Blot Analysis

As depicted in Figure 1, ANF mRNAs of similar size (~1,000 nt) are detected in both atria and ventricles of normal and cardiomyopathic hamsters. In control hamsters, as has been shown for the rat IR-ANF levels, ANF mRNA was twice as abundant in the right atrium as in the left atrium. In cardiomyopathic hamsters, atrial ANF mRNA levels were slightly decreased (~20% in moderate and ~40% in severe heart failure) as compared with control animals. In contrast, a striking increase in ANF mRNA levels was observed in ventricles of cardiomyopathic hamsters. The specificity of the large increase of ventricular ANF mRNA levels was assessed by simultaneous measurement of myosin light-chain mRNA concentration using a mouse probe. This probe, which cross-hybridizes with both atrial (~925 nt) and ventricular (~1,000 nt) mRNA, does not show any quantitative or qualitative changes in ventricular myosin light-chain gene expression with the progression of the cardiomyopathy (data not shown). In these conditions, analysis of serial dilutions of total RNA from atria and ventricles of cardiomyopathic hamsters clearly shows that ANF mRNA concentrations are only twofold higher in atria than in ventricles, an indication that ventricular ANF mRNA concentration is increased about 100-fold in cardiomyopathic hamsters. Since the total ventricular mass is about 20 times larger than the atrial mass, the ventricles of cardiomyopathic hamsters with congestive heart failure may contain up to 10 times more ANF mRNA than the atria.

Immunohistochemistry

In the rat, atrial cardiocytes, as previously described, were reactive, with the greatest reactivity in the auricles. Examination of serial sections of isolated ventricles after surgery and thermocautery revealed that all atrial tissue had been removed. The small remnants of atrial tissue located at the root of the aorta were destroyed by heat. The ventricular cardiocytes remained unreactive.

In hamsters, as in rats, in whole hearts atrial cardiocytes were reactive, with the most intensely granulated cells localized in the auricles. In control and cardiomyopathic hamster hearts, serial sections also showed that all remnants of atrial tissue had been removed. While reactive cells were not present in control ventricles, there was an increasing gradient of reactivity from the subendocardial to the subepicardial region of the ventricles from hamsters in moderate or severe heart failure, as previously described.

Plasma Levels of IR-ANF

As previously demonstrated, the plasma levels of the C-terminal moiety of ANF were higher in hamsters with moderate heart failure than in controls (Table 1). In severe congestive heart failure, the levels were lower than in moderate heart failure but higher than in controls. The levels of the N-terminal moiety of ANF were higher in moderate heart failure than in controls and still higher in severe heart failure.
TABLE 1. Plasma Immunoreactive Atrial Natriuretic Factor (C-Terminal and N-Terminal) Levels in Control and Cardiomyopathic Hamsters

<table>
<thead>
<tr>
<th></th>
<th>IR-ANF (C-terminal)</th>
<th>IR-ANF (N-terminal)</th>
</tr>
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<tbody>
<tr>
<td>Control hamsters (n=5)</td>
<td>8.9±0.7</td>
<td>151.8±9.5</td>
</tr>
<tr>
<td>Cardiomyopathic hamsters (Moderate heart failure) (n=5)</td>
<td>114.8±2.3*</td>
<td>1,309.3±208.7*</td>
</tr>
<tr>
<td>Cardiomyopathic hamsters (Severe heart failure) (n=5)</td>
<td>71.4±16.1†</td>
<td>1,907.0±70.2†</td>
</tr>
</tbody>
</table>

Statistical analysis of data was performed by one-way analysis of variance and the Bonferroni test. IR-ANF, immunoreactive atrial natriuretic factor.

* p<0.05 as compared with control and cardiomyopathic hamsters with severe heart failure.
† p<0.05 as compared with control and cardiomyopathic hamsters with moderate heart failure.

Release of IR-ANF in the Langendorff Preparation

In rats the levels of IR-ANF expressed per milliliter of effluent for the first 10-minute period after equilibration was in the nanogram range in the case of the whole heart (490.0±83 fmol/ml), 50 times higher than the level found in the coronary effluent of isolated ventricles (9.8±2.9 fmol/ml) (Figure 2). These values, in each case, tended to decrease with time but not in a significant manner. The ratio of the total amount secreted within two hours by whole hearts and isolated ventricles is thus 28:1 (Figure 3), which corresponds to a participation of 3.4% by the ventricles. Cardiac frequency, which varied between 221.4±14.2 and 172.4±8.5 beats/min in whole hearts during the entire perfusion period, decreased to substantially lower values ranging between 112.6±10.3 and 91.0±10.2 beats/min in isolated ventricles.

In control hamsters (Figure 4) the level of IR-ANF released by the whole heart during the first 10 minutes of the perfusion period after equilibration was significantly smaller (26.2±2.5 fmol/ml) than that released by the rat heart. The amount released under the same conditions by the isolated ventricles of control animals was 7.1±0.4 fmol/ml, significantly less than that for the whole hearts but about equal to the amount released by the control rat isolated ventricles. The amount released by the whole hearts of cardiomyopathic hamsters in moderate heart failure during the first 10 minutes of perfusion (134.0±9.2 fmol/ml) is significantly higher than the amount found in the effluent of control hamsters for the same period. The amount released by the isolated ventricles, when compared with the whole heart, revealed a significant drop (16.6±4.0 fmol/ml), which is nevertheless significantly higher than the amount originating from control hamster and rat ventricles. Here again, the levels of IR-ANF released by whole hearts or by isolated ventricles tended to decrease with time but not to a significant degree. In severe heart failure, the amount released by the whole heart (40.4±7.1 fmol/ml) during the same time period was significantly less than in moderate heart failure, but the isolated ventricles of hamsters with severe heart failure released significantly more (41.2±5.1 fmol/ml) IR-ANF than did the ventricles of hamsters with moderate heart failure. All these values tended to decrease with perfusion time, particularly those of the ventricular effluent of hamsters with severe heart failure, but
not in a significant manner. The ratio of the total amount of IR-ANF released by the whole heart as compared with the ventricles (3:1 in control hamsters) became 6:1 in moderate heart failure and 13:1 in severe heart failure (Figure 3). Thus, while the ventricle contributed 35.8% of the total amount of released IR-ANF in control hamsters, the amount released by the ventricles in moderate heart failure decreased to 17.5% but reached 73.9% in severe heart failure. The frequency of contraction of the whole heart of control hamsters varied from 205.0±32.9 to 116.8±23.4 beats/min, while that of isolated control ventricles varied between 127.6±23.0 and 89.2±11.9 beats/min. In moderate heart failure, the whole heart contracted at frequencies varying between 159.6±22.9 and 102.2±12.6 beats/min while the isolated ventricle had a frequency of contraction varying between 127.6±23.6 and 89.2±11.9 beats/min. In severe heart failure the frequency of contraction varied between 159.7±26 and 114.4±22.0 beats/min in the whole heart, while in the isolated ventricles, the frequency varied between 150.0±24.3 and 122.1±22.3 beats/min.

In all these cases the frequency, with many variations, tended to decrease with the length of perfusion.

HPLC Pattern of Plasma and Langendorff Effluent

The HPLC pattern of rat plasma IR-ANF revealed two main peaks of immunoreactivity corresponding to the C- and N-terminal moieties (Figure 5). In addition, a small peak corresponding to the whole propeptide as visualized by the IRMA technique was also recognized by the C- and N-terminal antibodies. The HPLC pattern of the Langendorff effluent from the whole heart was almost identical to that of plasma, while that of ventricular effluent was very different: the greater part of the released form was the propeptide with smaller peaks of the C- and N-terminal peptides.

The HPLC pattern of IR-ANF in control hamsters was slightly different from that of rats (Figure 6). Most of the plasma peptide eluted as the N-terminal with a smaller peak of C-terminal ANF. No high molecular weight corresponding to the propeptide was found in plasma. In the Langendorff effluent of the whole heart, both C- and N-terminal forms were found, again with a predominance of the N-terminal and a very small peak of the propeptide. In the effluent from ventricles, the amount of propeptide was slightly higher than that of the C-terminal with a predominance, again, of the N-terminal.

The HPLC pattern of plasma IR-ANF in cardiomyopathic hamsters with moderate heart failure was similar to that of control animals except for the presence of a small peak of the propeptide recognized by the IRMA technique and the two other antibodies (Figure 7). This small peak was also present in the effluent of the whole heart as in
control animals and increased markedly in the ventricular effluent.

In cardiomyopathic hamsters with severe heart failure, plasma contained a small amount of the propeptide (Figure 8). The amount of the propeptide was considerably increased in the effluent from the whole heart and particularly from ventricles.

**IR-ANF in Ventricles of Control Animals**

The total amount of IR-ANF (C-terminal) in ventricles of control animals before and after 2 hours of perfusion in the Langendorff preparation is shown in Table 2. The approximate percentage of ANF released by the ventricles after 2-hours of perfusion was greater in the rat (34%) than in the hamster (8%).

**Discussion**

Early in development ANF mRNA concentrations are similar in atria and ventricles, but these concentrations decrease precipitously in ventricles after birth to reach levels in the adult heart that are about 100 times lower than in atria and become set for life at these levels in control animals. Since ANF is controlled by a single gene, the decrease of ANF mRNA and peptide...
from ventricular muscle may reflect a tissue-specific inactivation of the ANF gene after cessation of cardiac cell growth. The presence of low amounts of ANF mRNA in adult ventricles and their modulation by hemodynamic and hormonal manipulation36-37 suggest that the inactivation is not irreversible. In fact, ventricular growth in cardiomyopathic hamsters is accompanied by increases in ANF mRNA and peptide when compensatory cardiac hypertrophy develops before the hemodynamic changes observed in heart failure (M. Nemer et al, unpublished data). In cardiomyopathic hamsters of 106 days at the onset of hypertrophy (at a stage where left and right ventricular pressures are not increased), ANF mRNA levels are already increased tenfold in the ventricles (M. Nemer et al, unpublished data). Ventricular ANF levels further increase with the progression of hypertrophy (217 days of age) and reach levels only tenfold lower than in atria (M. Nemer et al, unpublished data). In contrast, the levels of ANF mRNA in the atria of cardiomyopathic ham-
Table 2. Total Amount of Immunoreactive Atrial Natriuretic Factor (C-Terminal) in Ventricles of Control Animals Before and After 2 Hours of Perfusion in Langendorff Preparation

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<th>Body weight (g)</th>
<th>Ventricular weight (mg) (n=6)</th>
<th>Total IR-ANF in ventricles before perfusion (pmol) (n=3)</th>
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<td>Rat</td>
<td>200.1±2.1</td>
<td>370.0±4.5</td>
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<td>8.7±3.7</td>
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<td>34</td>
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<td>Hamster</td>
<td>198.3±15.8</td>
<td>450.0±18.6</td>
<td>8.2±1.2</td>
<td>6.9±2.4</td>
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IR-ANF, immunoreactive atrial natriuretic factor.

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IR-ANF, immunoreactive atrial natriuretic factor.

...ters are highest in young (100-day-old animals) and decrease slightly but constantly throughout the course of the disease to reach their lowest levels in severe congestive heart failure. As presently shown, the changes in ANF mRNA observed in the atria and ventricles of cardiomyopathic hamsters with moderate or severe heart failure are in general agreement with the values of IR-ANF measured in these cardiac chambers; both IR-ANF and ANF mRNA decrease in the atria and increase concomitantly in the ventricles. In control hamsters the ratio of atrial to ventricular IR-ANF (100:1) is analogous to the ANF mRNA ratio (100:1). In cardiomyopathic hamsters with severe congestive heart failure, atrial ANF levels are decreased by 50% 43 while ANF mRNA is decreased by 40%. At the ultrastructural level, the atrial cardiocytes of these animals show a pattern of intense secretory activity. These findings are compatible with the hypothesis of maximally stimulated cells, where low levels of IR-ANF are found possibly due to rapid generation and release. The initial increase in plasma ANF levels, contributed predominantly by the atria as shown in this study, may be derived from the previously abundant stores in the secretory granules. The subsequent decline of atrial ANF secretion and total ANF plasma levels in severe congestive heart failure would reflect the eventual decrease in atrial mRNA levels and atrial IR-ANF amount in secretory granules. Thus, the apparent uncoupling between low ANF mRNA levels and intense secretory activity may be due to a lag phase between ANF mRNA/secretory granule ANF content and secretion because of the abundant ANF stored pool in secretory granules originally present in atria.

These findings are also compatible with the hypothesis of maximally stimulated cells whose gene expression is not increased to a level commensurate with demand and suggest that gene expression is uncoupled from secretory activity. Whether the decrease in gene expression is due to a negative feedback effect of increased plasma ANF levels or to a different response of atrial and ventricular cardiocytes to overload remains to be determined.

In severe heart failure, IR-ANF levels in ventricles are increased 32-fold 43 while ANF mRNA is increased 100-fold. Even if ~20% of cardiocytes harbor secretory granules in congestive heart failure, the bulk of IR-ANF is localized in the inner half of the ventricles whereas granulated cells are dispersed, seemingly haphazardly, in the ventricular wall. 43 As revealed by HPLC analysis, the molecular form of ANF in atria and ventricles is not changed in congestive heart failure. 35 These results tend to indicate that ventricular cardiocytes in severe congestive heart failure are maximally stimulated and that the discrepancy between the 32-fold increase in IR-ANF and 100-fold increase in ANF mRNA may be due to lower hormone accumulation in ventricular cardiocytes because of the overall constitutive nature of their ANF secretion.

The levels of plasma IR-ANF C-terminal presently observed in control and cardiomyopathic hamsters are in general agreement with previous results: they reach a maximum in moderate heart failure and are decreased in severe congestive heart failure, although still significantly higher than in controls. As in the rat, 9 the N-terminal moiety of the peptide is also circulating in the hamster, and its values are substantially increased in both moderate and severe heart failure. The absence of correspondence between the plasma levels of the two peptides that are released in equimolar amounts by the heart may be due, as in the rat, 9 to the longer half-life of the N-terminal moiety in the circulation. The significant increment of this peptide in severe congestive heart failure may be due to renal impairment. In renal insufficiency in man, the plasma levels of N-terminal ANF are markedly elevated. 44 Bilateral nephrectomy in the rat has the same effect.

The results obtained with the perfused rat heart are in general agreement with already reported IR-ANF values in the effluent of the Langendorff preparation but yield higher values, probably because of the use of Sep-Pak cartridges to collect IR-ANF. As already noted, attempts to measure IR-ANF directly (i.e., without Sep-Pak extraction) in the effluent of isolated rat ventricles failed because the levels were below the detection limit of the radioimmunoassay (1.3 fmol) (data not shown). Preliminary experiments also revealed the impossibility of removal of all atrial tissue by surgery alone. While this can be done by removal of a thin rim of ventricular tissue up to the pulmonary valve, the same type of approach obviously cannot be used at the aortic level. Thermocautery permitted destruction of small remnants of atrial tissue in the posterior and posterolateral aspects of the aorta...
while sparing the coronary arteries that run on either side of the pulmonary conus.

In isolated ventricles, the amount of perfusion fluid had to be increased to keep perfusion pressure equal to that of the whole heart. This was done during the equilibration period preceding each experiment and, once established, was kept at the same level during the whole 2-hour period of observation. Examination of serial sections of the heart and isolated ventricles according to the unlabeled antibody technique of Sternberger permitted the rejection of 10% of the ventricles in which atrial remnants were not completely eliminated and verified that perfusion was adequate in all cases since no necrotic foci were found.

The lower levels of total IR-ANF released in the Langendorff preparation by the whole heart of control hamsters as compared with control rats may be related to the lower total amount of IR-ANF present in these hearts. In the adult rat, the atria contain ~6.2 nmol IR-ANF35,43 while the ventricles contain 25.8 pmol.45 Thus, the ratio between atria and ventricles is 240:1. In control hamsters, the total amount of IR-ANF is 1,079 pmol in the atria35,43 and 8.2 pmol in the ventricles,45 with a ratio of 132:1 between atria and ventricles. The amount of C-terminal IR-ANF released by the whole heart of hamsters in moderate and severe congestive heart failure is in agreement with the plasma levels of IR-ANF observed in these animals: in both cases, they are higher in moderate than in severe congestive heart failure.35,43 This suggests that the results of perfusion in the Langendorff preparation may serve as a useful estimate of the in vivo situation. The results must be interpreted with caution, however, since only variable amounts of IR-ANF are released by the isolated ventricles during a 2-hour perfusion period. The relative proportion of C-terminal IR-ANF released by the isolated ventricle versus the whole heart is very small (3.5%) in the rat but much higher (35.8%) in the control hamster. In moderate heart failure, the amount released by the ventricles is relatively smaller (17.5%) but reaches 73.9% in severe congestive heart failure. Thus, the contribution of atria versus ventricles to plasma ANF levels may be more important in moderate heart failure than in controls; the reverse may be true in the severe stage of the disease. These results are also in general agreement with the total amount of IR-ANF present in the atria and ventricles of cardiomyopathic hamsters, where the ratio becomes 7.1:1 in moderate heart failure and 3.9:1 in severe heart failure because of concomitant decreases in the amount of atrial IR-ANF (18% in moderate and 50% in severe heart failure) and increases in ventricular IR-ANF (2,600% in moderate heart failure and 3,300% in severe heart failure).35,43

HPLC analysis of plasma and Langendorff effluent from whole hearts of control rats by radioimmunoassay indicates that the C-terminal and N-terminal moieties of ANF are properly cleaved in vitro as they are in vivo. A small amount of the propeptide is found in rat plasma and in the effluent from the whole heart. The cleavage of ANF by the isolated rat ventricles is different since the major portion is recognized as the propeptide by the IRMA technique and by both C- and N-terminal antibodies. Almost identical patterns emerge from the analysis of control and cardiomyopathic hamster hearts, thus indicating that the relative absence of maturation of the propeptide by ventricular cardiocytes is not increased in heart failure. Thus, it is likely that the small amount of the propeptide in plasma originates from the ventricles. The absence of the propeptide in control hamster plasma may be due to cleavage in the circulation, as is possibly the case for the small fragments surrounding the C- or N-terminal peaks. In control rat plasma, for instance, ~10% of ANF is in the form of Ser 103-Tyr 126.2 In the rat plasma, analysis by HPLC at different time intervals after prohormone injection revealed non-specific hydrolysis of the proANF molecule.9 Analysis of cardiomyopathic hamster plasma by a different HPLC method has also shown the presence of a small amount of high molecular weight ANF.35 These results indicate that ventricular cardiocytes from both control and cardiomyopathic animals cleave only a variable portion of the secretory product. This is at variance with other cell types in which constitutively secreted products like albumin from hepatocytes66,67 or α-mating factor from yeast68 are properly cleaved. The same is true for many viral membrane glycoproteins that are constitutively exported and yet undergo proper proteolytic processing.69,70 These observations raise the possibility that the ANF released by atrial cardiocytes in culture, which is predominantly in the propeptide form,71,72 is constitutively secreted.70

Taken together, all these results suggest that, in experimental congestive heart failure, secretion from atrial and ventricular cardiocytes is maximally stimulated. While this is accompanied by decreased gene expression in atrial cardiocytes, there is markedly increased gene expression in ventricular cardiocytes. Thus, gene expression and secretory activity do not increase conjointly in atrial cardiocytes but do increase in ventricular cardiocytes. The increase in plasma ANF observed in moderate congestive heart failure is mainly due to atrial hypersecretion, whereas ventricular secretion is much more important in severe heart failure. While the propeptide is properly cleaved by atria, a relatively large portion of proANF is released as such by the ventricles, whether from control or cardiomyopathic animals. This leads to the presence of a small quantity of the propeptide in the circulation, while the remainder is nonspecifically hydrolyzed.

References


Key Words • atrial natriuretic factor • congestive heart failure • gene expression • Langendorff preparation
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