Dynamics of Atrial Peptide Secretion in the Coronary Circulation of the Conscious Dog

Thomas H. Hintze, Jie Wang, Mrugesh Patel, Mary Schustek, Manuel Ochoa, Roosevelt Dean, Francisca Chiruzzo, and Guillermo Zeballos

The secretion of atrial natriuretic peptide by the heart is not simply the arterial–coronary sinus concentration difference times coronary blood flow, because only a small fraction of total coronary blood flow passes through the atria. We measured coronary sinus and arterial plasma atrial natriuretic factor (ANF) concentrations and blood flow to each part of the heart using the radioactive microsphere technique. Before acute volume expansion, the arterial–coronary sinus ANF difference was 305±23 pg/ml and rose to 1,009±220 pg/ml during volume expansion, whereas total coronary blood flow rose from 167 to 465 ml/min. Atrial blood flow rose from 2.9% to 4.6% of total coronary blood flow during volume expansion. ANF secretion rate increased from 51 to 469 ng/min. When divided by atrial weight, ANF secretion rate increased from 4.0±0.3 to 56±12 ng/min/g atrial tissue—in other words, from 0.3% to 3.7% of tissue ANF content each minute. Dividing by atrial blood flow indicated that the concentration of ANF leaving atrial tissue was 10,000 to 29,651 pg/ml, and the additional secretion of ANF was determined by the increase in coronary blood flow. Therefore, at least two mechanisms are responsible for altering coronary sinus ANF and circulating ANF: the release rate from atrial myocytes and the washout via changes in atrial blood flow. (Circulation Research 1990;67:609–614)

Secretion of atrial natriuretic factor (ANF) from the heart can be estimated by the product of the arterial-venous concentration difference across the coronary circulation and coronary blood flow. This calculation implies that ANF is produced uniformly within the heart, whereas ANF is produced primarily in the atrial appendages of normal heart.1-4 ANF is a circulating hormone rather than a local one; therefore, many physiological studies, including our own,1 related to volume or cardiovascular homeostasis, such as during acute volume expansion and hypertension, have focused on circulating atrial peptide concentrations.5 However, to determine the mechanisms of atrial peptide secretion and the alterations that might occur in diseased states, such as heart failure, in which plasma ANF rises dramatically,6,7 it is essential to understand the dynamics of atrial peptide secretion from the atria and how this is coupled to changes in atrial function and coronary blood flow regulation.

A recent study by Hirata et al8 has reported coronary sinus ANF concentrations in patients on the order of 500 pg/ml/m² and ANF secretion rates on the order of 14 ng/min/m². Because ANF, which is secreted by the normal heart, comes from the atria, and atrial blood flow is only a small fraction of blood flow through the whole heart, the ANF concentration in the coronary sinus only partially reflects the dynamic changes in ANF secretion rate that occur in the atria. This concept is important not only to understand the dynamics of atrial peptide secretion but also to understand the control of atrial peptide synthesis, because some authors have proposed that ANF secretion may exceed ANF synthesis, leading to a decrease in the content of ANF or granularity in the atria.9,10 Therefore, the goals of the current study were 1) to determine the arterial-coronary sinus ANF difference, coronary blood flow, and blood flow to each structure of the heart, particularly the atria and atrial appendages, and 2) to determine the changes in ANF secretion rate by atrial tissue that occurs during acute dramatic volume expansion in the conscious dog.

Materials and Methods

Surgical Preparation

All experiments were performed in chronically instrumented conscious dogs. The animals were pre-anesthetized with acepromazine (1 mg/kg) and anes-
thetized with pentobarbital sodium (25 mg/kg). After the dog was prepared for sterile surgery, an incision was made in the fifth left intercostal space. A catheter (Tygon, Cardiovascular Instruments, Inc., Boston) was placed in the descending aorta and in the left atrial appendage. Another catheter was placed directly into the coronary sinus through a needle puncture in the sinus and was secured with a purse-string suture. A blood sample was taken and the Po2, measured to ensure the tip of the catheter was in the coronary sinus. A Doppler ultrasonic flow transducer (Parks Instruments Inc., Eugene, Ore.) was placed around the left circumflex coronary artery. The wires and catheters were run subcutaneously across the chest to exit between the scapulae. The chest was closed in layers, the pneumothorax was reduced, and the dog was allowed to recover fully. During this recovery period, the dogs were given antibiotics and trained to lie quietly on the laboratory table. Only when the dogs were well-trained and afebrile, roughly 2–3 weeks, were experiments performed.

**Recording Techniques**

On the day of the experiment, the previously implanted catheters were attached to a strain-gauge manometer (P23 ID, Statham, Rahway, N.J.) for recording arterial and atrial pressures. The Doppler flow transducer was attached to a pulsed Doppler flowmeter (Triton Instruments, San Diego) for measuring coronary blood flow. All signals were recorded on a 14-channel tape recorder (3700B, Bell and Howell, Rutherford, N.J.) and played back on a direct-writing oscillograph (model 2800, Gould Instruments, Inc., Rahway, N.J.). Mean pressures were derived using 2-Hz low-pass filters.

**Experimental Protocols**

A large-bore catheter (19-gauge Intercath, Delmed Inc., Canton, Mass.) was inserted into a peripheral vein and attached to an intravenous drip line and a 1-l bag of warmed saline. The saline bag was placed in an arterial pressure bag, and the external pressure on the bag was maintained at 100–200 mm Hg. In this manner, the entire liter of saline could be given to the dog in 5–6 minutes in a controlled fashion. Using sterile techniques and local lidocaine anesthesia, another catheter was placed in a femoral artery for reference sampling during microsphere injection. This line was attached to a calibrated withdrawal pump (Harvard Instruments, Boston) set to withdraw at 10 ml/min.

After the dog was quiet for a minimum of 30 minutes, during which time hemodynamics were continuously recorded, aortic and coronary sinus blood samples were taken, the first group of microspheres was injected into the left atrium, and blood gases were checked. The saline infusion was then begun. After infusion of 500 and 1,000 ml saline, the infusion was stopped for 2–3 minutes and blood samples, microsphere injections, and hemodynamics were taken. After the experiment, the animal was anesthe-

**Hematocrit and blood gases were measured from coronary sinus and aortic** (model 170, Corning, Stamford, Conn.) blood samples. For ANF measurement, 6 ml of blood was placed in tubes containing EDTA and 10 µl aprotinin (Sigma Chemical Co., St. Louis), and centrifuged. The plasma was decanted and frozen at −40°C until used. Atrial natriuretic peptide levels in plasma were measured as previously described, using an antibody to human ANF (Peninsula, Laboratories, Inc., Los Angeles). Radioactive microspheres (<5 µm, 3M Company, Minneapolis) were used to measure intracardiac distribution of blood flow was previously described. We have used all of the recording techniques previously.

These procedures were reviewed and approved by the Institutional Animal Care Use Committee of New York Medical College and conform to the National Institutes of Health guidelines for the use and care of laboratory animals and to the principles of the American Physiological Society.

**Statistical Analysis**

Microsphere blood flows were calculated using standard methods. The blood flow in three to six tissue samples in each area of the heart was averaged and then averaged across all dogs. A t test was used to determine differences from control. Although recorded continuously, hemodynamic measurements were taken before and after 500 and 1,000 ml saline infusion. Means and SEM were calculated and significant differences determined by analysis of variance using a post hoc Scheffe’s test. To calculate average atrial blood flow, means and SEM were calculated for each region, that is, the left and right atrial appendages and body, and then the means were averaged. The average left and right ventricle and septum weights were taken from a large population of other dogs from which we had saved the hearts as a control group in another study. To calculate ANF secretion rate, we multiplied the arterial–coronary sinus ANF concentration difference by blood flow. Because of the fall in hematocrit during acute volume expansion with saline and, therefore, dilution of the plasma ANF, we multiplied blood flow by the quotient of the initial hematocrit and the hematocrit at 500 and 1,000 ml saline infusion. We refer to this as “normalized” blood flow or secretion rate.

Data are expressed as mean±SEM in the text, tables, and figures.
Results

Hemodynamic Changes During Volume Expansion

During rapid saline infusion, mean arterial pressure, mean left atrial pressure, heart rate, and coronary blood flow all increased significantly (Figure 1). Hematocrit fell from 36±1% at control to 27±1.9% and 24±1.9% after 500 and 1,000 ml volume expansion, respectively. To be certain of the position of the coronary sinus catheter, blood gases were measured in coronary sinus and arterial blood during each blood sampling for ANF. Arterial Po2, Pco2, and pH were 80±3.5 mm Hg, 32±3.5 mm Hg, and 7.43±0.01, respectively, before volume expansion and did not change during volume expansion. Coronary sinus Po2, Pco2, and pH were 17.8±0.4 mm Hg, 39±2.8 mm Hg, and 7.38±0.01, respectively, before volume expansion. Changes in these values during volume expansion were small, indicating that the heart was producing increased carbon dioxide that resulted in a lowering of pH.

Intracoronary Blood Flow Changes

Blood flow increased in all areas of the heart during volume expansion, as shown in Table 1. Control blood flows in all areas of the atria were similar and changed to a surprisingly similar degree during volume expansion. Using the average of these values times the average tissue weight, we calculated total coronary blood flow to each area of the heart. As shown in Table 2, blood flow to each area of the heart and to the total heart increased dramatically.

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![Graphs showing changes in AP, LAP, HR, and CBF with volume expansion](image)

**Figure 1.** Change in mean arterial pressure (AP), mean left atrial pressure (LAP), heart rate (HR), and mean left circumflex coronary blood flow (CBF) during volume expansion of 500 and 1,000 ml from control (C). Arterial pressure and heart rate increase showing evidence of the Bainbridge reflex, and coronary blood flow rises secondary to the increase in myocardial metabolic demand and hemodilution. *p<0.05 from control.

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**Table 1. Effects of Volume Expansion on Distribution of Blood Flow in Heart**

<table>
<thead>
<tr>
<th>Area</th>
<th>Control</th>
<th>500 ml</th>
<th>1,000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appendage</td>
<td>0.41±0.06</td>
<td>0.99±0.21*</td>
<td>1.65±0.25*</td>
</tr>
<tr>
<td>Body</td>
<td>0.43±0.08</td>
<td>1.09±0.24*</td>
<td>1.77±0.15*</td>
</tr>
<tr>
<td>Left atrium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appendage</td>
<td>0.46±0.09</td>
<td>0.86±0.23*</td>
<td>1.75±0.53*</td>
</tr>
<tr>
<td>Body</td>
<td>0.29±0.04</td>
<td>0.79±0.27*</td>
<td>1.70±0.52*</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.04±0.19</td>
<td>2.24±0.65*</td>
<td>3.05±0.63*</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.92±0.13</td>
<td>1.73±0.31*</td>
<td>2.24±0.44*</td>
</tr>
<tr>
<td>Septum</td>
<td>1.27±0.22</td>
<td>2.67±0.66</td>
<td>3.34±0.56*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Blood flow in the heart is in milliliters per minute per gram of tissue. Average atrial blood flow was taken as the average of the four atrial blood flows in each column and were 0.40, 0.94, and 1.72 ml/min/g. *p<0.05 from control.
TABLE 612

Circulation Research Vol Atrial Natriuretic Factor

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>500 ml</th>
<th>1,000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atria</td>
<td>4.99</td>
<td>11.70</td>
<td>21.48</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>82</td>
<td>176</td>
<td>239</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>38</td>
<td>72</td>
<td>93</td>
</tr>
<tr>
<td>Septum</td>
<td>42</td>
<td>89</td>
<td>111</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>348</td>
<td>465</td>
</tr>
<tr>
<td>Normal total</td>
<td>167</td>
<td>464</td>
<td>698</td>
</tr>
</tbody>
</table>

Values are calculated by multiplying average microsphere-measured blood flow times average weight of the chamber or structure. Average atrial weight was 12.49±1.0 g; left ventricle weight, 78.5±3.1 g; right ventricle weight, 41.6±1.9 g; septum weight, 33.3±1.9 g. Total blood flow is the sum of all components including the atria; Normal total is total multiplied by the ratio of the hematocrit changes during volume expansion.

Atrial Natriuretic Factor Secretion

Plasma arterial and coronary sinus ANF increased significantly during volume expansion, as shown in Figure 2. Using measurements of total cardiac and atrial blood flow, we calculated the ANF secretion rate from the atria (Table 3), assuming that the atria are the only sources of ANF in the normal dog heart. Arterial ANF levels were 65 pg/ml, whereas coronary sinus ANF levels were 387±26 pg/ml and the arterial—coronary sinus difference is on the order of 300 pg/ml. Multiplying the arterial—coronary sinus difference by average atrial blood flow (as measured by the radioactive microsphere technique) as an index of atrial secretion rate indicates that the secretion rate of atrial tissue was on the order was 122±9.1 pg/min/g and rose to 644±127 and 1,735±378 pg/min/g after 500 and 1,000 ml saline infusion, respectively. When this is multiplied by the average weight of all the atrial tissue (12.49 g), ANF secretion per atria increases from 1.5±0.1 to 7.7±1.7 and 21.7±6.1 (p<0.05) ng/min after 500 and 1,000 ml saline infusion, respectively. ANF secretion also increases during volume expansion almost 22 times (Figure 2).

The underlying assumptions here are not correct, however, because the coronary sinus ANF already represents the effect of ANF dilution in the total blood flow of the heart. For instance, based on blood flows in Table 2 before volume loading, atrial blood flow is 2.9% of total coronary blood flow, whereas during volume expansion, this value rises to only 3.4% and 4.6% at 500 and 1,000 ml saline, respectively. Multiplying total coronary blood flow times the arterial—coronary sinus, ANF difference gives the total ANF secreted, which is 51 ng/min and rises to 469 ng/min during volume expansion (Table 3). Because hematocrit falls during volume loading with saline, plasma flow increases out of proportion with blood flow. This can be corrected by multiplying the total coronary blood flow by the original hematocrit divided by the measured hematocrit at 500 and 1,000 ml volume expansion and using that flow to calculate total ANF secretion (Table 3, Normalized secretion), which then results in an increase in secretion rate to more than 703 ng/min during volume expansion. Dividing by the total weight of the atria (12.49 g) results in the ANF secretion rate for each gram of atrial tissue (Table 3, Secretion). ANF secretion rate therefore increases from 4 to more than 50 ng/min/g of tissue in the atrium. Dividing ANF secretion rate (Table 3, Secretion) by average microsphere-measured blood flow per gram of tissue (see Table 1) results in atrial venous ANF concentrations of 10, 26.6, and 29.7 ng/ml, or for direct comparison to arterial and coronary sinus levels, 10,000, 26,695, and 29,651 pg/ml, approximately 25,000 pg/ml for 500 and 1,000 ml volume expansion. In a previous study, we found that most ANF is localized in the atrial appendage. It is also well established that removal of the atrial appendage eliminates ANF release during volume expansion in the rat and monkey2,4 and that little ANF comes from the ventricle in the normal adult animal.18 If we assume that most of the atrial peptide (to make the calculation simple, 100%) is in the appendage and that only this contributes to ANF release, then the concentration of ANF leaving the

FIGURE 2. Percent changes in atrial natriuretic factor (ANF) levels in the aorta (ART) and the coronary sinus (CS), and secretion of the whole heart (HT) and atria (ATRIA) after 500 (upper panel) and 1,000 ml (lower panel) saline infusion. There were significant (*p<0.05 from control) increases in all of these values during volume expansion. Because ANF concentration progressively falls as ANF leaves the heart, the percent changes reflect alterations in coronary blood flow rate and progressive dilution of ANF.
atrial appendages is twice as high as we calculated. Furthermore, if one divides normalized ANF secretion (Table 3) by estimated cardiac outputs (i.e., 100 ml/min/kg body weight at rest and an additional 500 and 1,000 ml during volume loading) of 2,500, 3,000, and 3,500 ml/min, the calculated values of 20, 103, and 234 pg/ml are remarkably close to our measured arterial ANF levels (Table 3).

**Discussion**

The most striking findings of the current investigation were the extremely high concentration of ANF in the atrial venous blood and the fact that even calculations of ANF secretion across the whole heart are not indicative of the magnitude of ANF secretion rate from the atria. This stems largely from the small fraction (2–5%) of total coronary sinus blood flow that comes from the atria. Using the radioactive microsphere technique and some assumptions, we were able to calculate not just the myocardial ANF secretion rate but also total atrial and atrial per unit weight ANF secretion. Furthermore, given previous experiments that have shown that surgical removal of the atrial appendages essentially eliminates the rise in arterial plasma ANF during atrial distention,2–4 we can predict the ANF concentration leaving the atrial appendages. Because we did not remove one atrial appendage or the other and a number of studies suggest that volume expansion leads to ANF release from both the right and left atrium,17,18 we cannot make inferences on the relative contribution of the right and left atrial appendages to the rise in coronary sinus ANF during acute volume expansion.

In a previous study,1 we found that acute volume expansion increased atrial diameter, pressure, and wall stress and that 66% of the ANF in dog heart was localized in the atrial appendages. From that study we also postulated that atrial wall stress was the driving force or the in vivo stretch of the atrial appendage that results in ANF release. In the ventricle, myocardial metabolism is determined in part by changes in wall stress19; therefore, coronary blood flow should be proportional to changes in wall stress. The increase in atrial blood flow, from 0.40 to 1.70 ml/min/g, is greater (363±111%) than the increase in blood flow to the left ventricle (200±45%) and septum (200±51%) because the atrial wall is more easily deformed, is thinner with less muscle mass, and wall stress increases to a greater degree. Additionally, the percent of atrial blood flow with respect to total coronary blood flow increases from 2.9% to 4.6%, supporting the conclusion that atrial blood flow increases more than ventricular blood flow.

Most previous studies that have determined the effects of physical distortion, temperature, and contraction frequency on ANF release have been conducted in isolated atrial strips under some initial tension.20,21 These studies have measured the amount of ANF in the bathing fluid after a number of minutes of stimulation22,23 and have attributed this to leakage from the atrial tissue. This leakage must occur from the atrial tissue, through the capillary wall, then down a concentration gradient into the bathing fluid. Alternatively, ANF secretion can come from the cut edge of these tissues and move into the bathing fluid. These mechanisms of ANF extrusion also hold true for studies using atrial minces that are not under any basal tension.

In the intact animal24 and in the Langendorff heart preparation,25,26 the contribution of changes in coronary blood flow to ANF secretion can be determined, although it has not been studied because of the lack of measures of atrial blood flow. For instance, in our study after 500 ml volume expansion, the concentration of ANF in blood leaving the atria increases very little, and it is primarily the increase in coronary blood flow that is responsible for the continued elevation in plasma arterial and coronary sinus ANF. Thus, we believe it is essential to understand the secretion of ANF at both the cellular and organ levels, because mechanisms inherent to both systems are responsible for the measured changes in arterial ANF and to its potential action as a hormone. If, for instance, coronary blood flow regulation is markedly altered—such as in hypertension, valvular disease, or heart failure—the washout of ANF from the atria may be curtailed and become a limiting factor for the increases in plasma ANF. Finally, our studies set the

<table>
<thead>
<tr>
<th>TABLE 3. Effects of Volume Expansion on the Dynamics of Atrial Natriuretic Factor in Systemic and Coronary Circulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>CBF (ml/min)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
</tr>
<tr>
<td>Arterial ANF (pg/ml)</td>
</tr>
<tr>
<td>Coronary sinus ANF (pg/ml)</td>
</tr>
<tr>
<td>A-V ANF (pg/ml)</td>
</tr>
<tr>
<td>Total secretion (ng/min)</td>
</tr>
<tr>
<td>Normalized secretion (ng/min)</td>
</tr>
<tr>
<td>Secretion (ng/min/g)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CBF, mean left circumflex coronary blood flow; ANF, atrial natriuretic factor; A-V, arterial–coronary sinus difference; Total secretion, total cardiac blood flow times A-V ANF; Normalized secretion, total secretion times normalized hematocrit change; Secretion, normalized secretion divided by atrial weight (12.49 g).

*p<0.05 from control.
groundwork for the calculation of ANF secretion from the hypertrophied, failing, or diseased heart, in which important questions still remain concerning the balance between ANF synthesis and secretion and the contribution of the ventricles to the rise in plasma ANF in heart failure. In our study, ANF secretion rate from the atria was initially 4 ng/min/g of tissue and rose to 56 ng/min/g, whereas ANF concentration in the dog atrium is approximately 1.5 μg/g or 1.500 ng/g of tissues. Thus, initially 0.3% of the stored ANF is secreted each minute, and during volume loading, this rises to 3.7%. To prevent depletion of stored ANF, synthesis must equal up to 4% each minute of the total stored during volume expansion, which is, perhaps, the most physiological stimulus for ANF secretion.

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References


KEY WORDS • microspheres • atrial natriuretic factor • atrial secretion • secretion • coronary sinus ANF • ANF secretion rate • atrial blood flow • total cardiac blood flow
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