Neurohumoral Responses to Chronic Myocardial Infarction in Rats

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In chronic cardiac failure, various neurohumoral mechanisms are activated to sustain blood volume, blood pressure, and organ perfusion. Using the coronary artery ligation model of heart failure in the rat, we have measured changes in vasoactive hormone secretion and related these changes to salt and water status during a 1-month period. When compared with controls, rats with infarction had a marked rise in plasma atrial natriuretic peptide (294 ± 59 vs. 79 ± 10 pg/ml, \( p < 0.001 \)) although there was no increase in total exchangeable body sodium. Plasma renin activity and plasma aldosterone concentrations were the same for both rats with infarction and controls. Similarly, there were no significant differences in plasma arginine vasopressin, plasma osmolality, or plasma sodium concentration in rats with infarction. Ventricular norepinephrine levels were reduced in animals with infarction (\( p < 0.01 \)). Plasma atrial natriuretic peptide levels were raised in this model of chronic left ventricular failure. However, there was no salt retention and little stimulation of the renin-angiotensin-aldosterone system or vasopressin. The results suggest that high circulating atrial natriuretic peptide levels may prevent or limit salt and water retention, either directly or indirectly, by inhibiting the renin-angiotensin-aldosterone system. (Circulation 1988;78:376–381)

Chronic cardiac failure is characterized by raised venous and intracardiac pressures and low cardiac output. These changes stimulate various neurohumoral homeostatic responses that act to maintain circulating blood volume, blood pressure, and organ perfusion, but which ultimately prove deleterious by increasing afterload and cardiac work.\(^1,2\)

Activation of the sympathetic nervous system and the renin-angiotensin system causes salt and water retention and an increase in total peripheral resistance. Moreover, there is now evidence that vasopressin contributes to increased peripheral resistance even when circulating levels are not raised.\(^3,4\) However, it has recently been reported that atrial natriuretic peptide (ANP) secretion is also increased in heart failure.\(^5–7\) This recently described hormone causes peripheral vasodilation, natriuresis, diuresis, and suppression of both renin and aldosterone secretion.\(^8,9\) It may, therefore, be an important physiological antagonist of the vasoconstrictor and fluid-retaining mechanisms activated in heart failure.

Understanding these neurohumoral mechanisms and their interrelations is important for defining the pathophysiology of heart failure and may lead to more precise therapeutic intervention. This is particularly necessary as the 5-year mortality still approaches 50% in patients with newly diagnosed congestive cardiac failure.\(^10,11\)

Because of the variability in disease and severity and the need for therapy, it is difficult to accurately measure and interpret hormonal responses in human heart failure. We have, therefore, studied the responses of plasma ANP, plasma renin activity, plasma aldosterone, plasma vasopressin, and urine and cardiac tissue catecholamines in the coronary artery ligation model of chronic heart failure in the rat. We have also measured total exchangeable body sodium as an index of extracellular fluid volume.

Materials and Methods

Induction of Heart Failure

Left ventricular free-wall myocardial infarction was induced in female Wistar rats by the method of Pfeffer et al.\(^12\) Briefly, each rat was deeply anesthetized with ether, intubated, and ventilated with a rodent respirator. A thoracotomy was performed, the heart rapidly exteriorized, and the left coronary

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artery ligated with a 6-0 Prolene suture. The heart was then returned to its normal position, the lungs reinflated with positive end-expiratory pressure, and the thorax closed by a previously placed purse-string suture. The artery cannot be visualized directly, and in some animals, the procedure does not produce a histologically detectable myocardial infarction. Mortality in these animals is insignificant, and they are used as sham-operated controls. The mortality in animals with infarction was 40–60% within the first 24 hours but was minimal during the subsequent 1-month study period. Successful occlusion of the artery results in a left ventricular free-wall infarct that after 1 month is completely replaced by a thin fibrous scar. Infarct size is quantified histologically by planimetry and is expressed as the percent ratio of infarcted to total left ventricular circumference. One month after operation, hemodynamic abnormalities include raised right and left ventricular filling pressures, reduced right and left ventricular systolic pressures, and reduced dP/dtmax.12,13 Previous studies have shown that the hemodynamic abnormalities are related to increased cardiac weight and to infarct size, both of which may be used as indexes of ventricular dysfunction.5,13

Hormone Assays

Plasma atrial natriuretic peptide. Plasma immunoreactive ANP was measured by a previously reported method.14 Briefly, blood was taken into chilled tubes containing ethylenediaminetetraacetic acid (1 mg/ml) and aprotinin (500 kIU/ml). The plasma was immediately separated and stored at −70°C until extraction with Vycor glass beads. Adsorbed ANP was eluted from the beads with an acetone-water (60:40) mixture that was then evaporated to dryness under an air stream. The samples were reconstituted in assay buffer and then radioimmunoassay performed with synthetic rat ANP as standards. Separation was performed with goat anti-rabbit second antibody. The sensitivity of the assay was 11 pg rat α-ANP/ml plasma. Intra-assay and interassay variances were 5.3% and 16.3%, respectively. Recovery of added rat α-ANP from plasma averaged 98% throughout the range of 5–2,000 pg/ml.

Plasma arginine vasopressin. Plasma arginine vasopressin was measured by radioimmunoassay.15 Blood was taken into chilled tubes containing lithium heparin. Plasma was immediately separated and stored at −20°C until assayed. Arginine vasopressin was extracted with an acetone-ether mixture, and radioimmunoassay was performed with synthetic arginine vasopressin standard and rabbit antibody. Separation was performed with goat anti-rabbit second antibody. Intra-assay and interassay variances were 8.4% and 11.6%, respectively. Assay sensitivity was 0.7 pg arginine vasopressin/ml plasma.

Plasma renin activity and plasma aldosterone. Plasma renin activity was measured by the enzyme kinetic generation of angiotensin I, which was measured by radioimmunoassay.16 Plasma aldosterone was measured by radioimmunoassay with a commercial kit (Diagnostic Products, Los Angeles, California).

Urine and cardiac tissue catecholamines. The ventricles were individually dissected, weighed, snap frozen, and stored at −80°C until assayed. Samples were homogenized in 0.1 M perchloric acid containing 100 ng/ml dihydroxybenzylamine (DHBA) as an internal standard. The homogenate was then centrifuged, and the supernatant was extracted on an alumina column. The catecholamines were eluted with 250 μl 0.1 M perchloric acid, and aliquots were assayed by high-performance liquid chromatography with electrochemical detection.17 The between-day coefficient of variation for the assay of each catecholamine was 8%, and the limit of detection was 20 pg. Urine catecholamines were extracted by a combined cation exchange column technique.18 Samples were assayed by high-performance liquid chromatography with electrochemical detection with DHBA as an internal standard.

Measurement of total exchangeable body sodium. Total exchangeable body sodium (NaE) was measured by isotope dilution with a modification of the method of Dusting et al.19 This method is simple, accurate, and reproducible, and the results correlate closely with measurement of total body sodium made by ashying or acid digestion.20 Briefly, animals were fed a salt-free solid diet (<50 mg/kg) supplemented by a sodium chloride drinking solution (75 mM) containing 22Na (4 nCi/mm Na, Amersham, Arlington Heights, Illinois). The radioisotope equilibrates with endogenous isotope in all body tissues and fluids except bone. As most sodium is extracellular, NaE is proportional to extracellular fluid volume and also to plasma volume in most pathophysiological conditions. Equilibration of the isotopes takes 4–14 days and is known to be complete when the specific activity of the urine is equal to that of the drinking solution. NaE may then be measured sequentially by counting the total radioactivity of each animal in a Hewlett-Packard Armac Gamma Counter (Palo Alto, California). The count is corrected for body weight and compared with that of a counting standard of known volume and specific activity. NaE, expressed as mmol/kg, is then calculated by applying the following formula:

\[
NaE = \frac{\text{counts (rat)} \times \text{volume (standard)} \times \text{concentration (standard)}}{\text{counts (standard)} \times \text{weight (rat)}}
\]

Experimental Protocol

Three groups of rats underwent coronary artery ligation and were decapitated 4 weeks later. In the
first group (n=70), aortic blood was collected for measurement of plasma renin activity, and the remaining blood was collected for measurement of plasma ANP. In this group, the animals were allowed to equilibrate with the radiolabeled drinking solution for 3 weeks before operation. NaE was measured on the day after operation and at weekly intervals until decapitation after 4 weeks. In the second group (n=25), blood was collected for measurement of plasma arginine vasopressin, plasma osmolality, and plasma sodium. In both groups, the left ventricles (including intraventricular septum) were dissected free and fixed in 10% buffered formalin for histological study and measurement of infarct size.

In the third group (n=30), surviving rats were allowed to equilibrate in metabolic cages for 24 hours, and then urine was collected under oil for an additional 24 hours before decapitation. The hearts were removed and weighed, and the free right ventricular wall and the noninfarcted septum were individually dissected from the left ventricle and atria. Infarct size could not be quantified because both right and left ventricles were used for measurement of tissue catecholamines. However, the presence of infarction was confirmed visually, and increased cardiac weight was used as further evidence of chronic infarction6 (see "Results").

Statistical Analysis
Data were analyzed by analysis of variance or Student's unpaired t test as indicated. Regression lines were fitted by the method of least squares. Data are presented as mean±SEM with p<0.05 considered significant.

Experiments were approved by the Austin Hospital Ethics Committee, Heidelberg, Australia.

Results

Group 1
Plasma ANP and exchangeable body sodium. Myocardial infarction (mean infarct size, 35±2%) was confirmed in 37 of 70 surviving animals. Rats with infarction did not retain sodium. NaE in sham-operated control rats was 44.9±0.5 mmol/kg on the day after operation and was 44.7±0.4 mmol/kg after 1 month. NaE in rats with infarction was 45.6±0.5 mmol/kg on the day after operation and was 45.1±0.5 mmol/kg after 1 month (Figure 1). Corrected hepatic weight in rats with infarction (34.2±1.2 mg/g) was similar to that of controls (34.0±1.5 mg/g). Plasma ANP was markedly elevated in animals with infarction when compared with sham-operated controls (p<0.001, Table 1). Plasma ANP levels were correlated with cardiac weight (r=0.66, y=0.003x+3.6, p<0.05) that in turn was correlated with infarct size (r=0.60, y=9.4x−1.65, p<0.05). However, there was no relation between plasma ANP and NaE.

![Figure 1. Plot of total exchangeable body sodium (NaE) in sham-operated control rats (○, n=33) and rats with chronic myocardial infarction (●, n=37). Rats with infarction did not retain sodium.](http://circ.ahajournals.org/)

**Plasma renin activity and plasma aldosterone.** Plasma renin activity was similar in both rats with infarction and in the controls (Table 1). There was considerable overlap between the two groups, and the minor difference was not statistically significant. Plasma renin activity in 10 normal nonoperated Wistar rats was 6.5±1.1 ng/ml/hr. Plasma aldosterone was similar in controls and rats with infarction (Table 1). There was no relation between plasma renin activity and cardiac weight, infarct size, plasma ANP, or NaE.

Group 2
Plasma arginine vasopressin, sodium, and osmolality. Myocardial infarction (mean infarct size, 42±4%) was confirmed histologically in 15 of 25 surviving animals. Mean plasma arginine vasopressin, plasma sodium, and plasma osmolality were similar in animals with infarction compared with controls (Figure 2). There was no relation between infarct size and either plasma arginine vasopressin, plasma sodium, or plasma osmolality.

Group 3
Urine and cardiac catecholamines. Cardiac tissue catecholamines were measured in 15 animals with infarction and in 15 controls. Right ventricular catecholamine concentrations were reduced in rats with infarction although this reduction was significant only for norepinephrine (Table 2). When the results were expressed as norepinephrine level per

<table>
<thead>
<tr>
<th>Table 1. Plasma Hormone Activities and Concentrations</th>
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<tr>
<td>Plasma hormones</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
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</tbody>
</table>

Values are mean±SEM. Numbers in parentheses are numbers in each group.

Plasma hormone concentrations in rats with infarction compared with sham-operated controls.
PRA, plasma renin activity; ANP, atrial natriuretic peptide.
*p<0.001.
ventricle, the differences between the two groups were no longer statistically significant although the levels were still lower. Similar trends were observed in left ventricular (septal) catecholamines, but the changes were not statistically significant (data not shown).

Urine norepinephrine, epinephrine, and dopamine measurements varied widely. Mean values in rats with infarction (1,399 ± 250, 214 ± 48, and 1,665 ± 381 ng/24 hr, respectively) were not different from those of controls (1,946 ± 547, 321 ± 108, and 1,981 ± 721 ng/24 hr, respectively).

Cardiac weight in the rats with infarction was 1,377 ± 85 mg compared with 1,060 ± 42 mg in controls (p<0.005). No relation between cardiac catecholamine content and infarct size could be determined because infarct size could not be measured in these animals.

Discussion

The pathophysiological importance of the vasoconstrictor and fluid-retaining mechanisms operating in human heart failure is now well established, although our understanding is incomplete in several areas. The importance of increased mineralocorticoid activity and increased renin secretion in the genesis of fluid retention in heart failure has been known for many years.21 However, plasma renin and aldosterone levels are extremely variable in both human22 and animal heart failure.23,24 This variability has not been satisfactorily explained. The role of vasopressin in heart failure is also unclear,2,3 and in some respects, these uncertainties parallel those associated with the renin-angiotensin system. Raised plasma vasopressin levels, usually with hypo-osmolality and hyponatremia, are sometimes, but not always, present in patients with severe congestive heart failure.25,26

Circulating catecholamines are almost invariably raised in human27–29 and animal heart failure.30 It has been suggested that increased circulating catecholamines are a marker of disease severity and have little direct pathophysiological role in heart failure.1 However, total peripheral resistance is largely sustained by sympathetic nervous system tone, and in human heart failure, more than 50% of the increased release occurs in vascular beds other than the heart.28 Elevated sympathetic activity in cardiac failure, while helping to preserve cardiac output, probably contributes to the deleterious rise in peripheral resistance and may contribute to the increased incidence of fatal cardiac arrhythmias.

Coronary artery ligation in the rat is an excellent model of chronic left ventricular failure that closely mimics the human condition. Rats with infarction have reduced cardiac output with a rise in peripheral resistance.12,13 Animals with infarction develop cardiomegaly and lung congestion. However, they do not have increased liver or body weight when measured 3–4 weeks after infarction.6 The lack of change in NaE in the present study strongly supports the pathological evidence that right ventricular failure does not occur, at least early in the pathological process.

The hemodynamic abnormalities in this model have been studied extensively and with great precision. However, with the recent exception of ANP, we are unaware of published reports of vasoactive hormone concentrations or body sodium status. These must be known to correctly interpret past and present experimental data in this model. In the present study, we have measured the responses of the most important vasoactive hormones operative in heart failure.

Mean plasma renin levels were slightly increased in animals with infarction although the difference was not statistically significant. Moreover there was no increase in plasma aldosterone, which is in keeping with the absence of sodium retention. These findings suggest that either the renin-angiotensin system is not stimulated in this model or, alterna-

![Figure 2](http://circ.ahajournals.org/)

**FIGURE 2.** Bar charts of plasma arginine vasopressin (AVP), plasma osmolality, and plasma sodium in sham-operated control rats (open bars, n=9) and rats with infarction (closed bars, n=16). In rats with infarction, AVP was inappropriately raised in relation to plasma osmolality and sodium, but these differences were small and not statistically significant.

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**TABLE 2. Cardiac Catecholamine Measurements**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,118 ± 151</td>
<td>26.0 ± 6.7</td>
<td>19.4 ± 3.5</td>
</tr>
<tr>
<td>Infarct</td>
<td>602 ± 112*</td>
<td>17.1 ± 4.1</td>
<td>15.1 ± 4.2</td>
</tr>
<tr>
<td>Content</td>
<td>231 ± 32</td>
<td>5.4 ± 1.4</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Infarct</td>
<td>165 ± 23</td>
<td>4.9 ± 1.1</td>
<td>4.5 ± 1.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Right ventricular catecholamines in controls (n=15) and rats with infarction (n=15). Ventricular catecholamine concentrations in nanograms per gram of body weight are compared with ventricular content in micrograms per ventricle.

*p = 0.01.
tively, that active suppression of the renin-angiotensin-aldosterone system is mediated by the increased circulating levels of ANP. Plasma renin activity is increased in anesthetized dogs with myocardial infarction. However, in contrast to the present study, these measurements were made acutely within 6 hours of infarction. It is possible that the renin-angiotensin system is stimulated acutely, but not chronically, in response to myocardial infarction, although we have not examined this question in the rat model.

Plasma ANP was markedly elevated in animals with infarction, confirming our previously reported findings. Although plasma ANP was again found to be correlated with cardiac hypertrophy, there was no relation between plasma ANP and body sodium. This strongly suggests that plasma ANP is raised in response to increased intracardiac pressures and that this may occur independently of salt and water retention. It is possible that increased ANP release may maintain salt and water homeostasis either directly or indirectly by suppressing the renin-angiotensin-aldosterone system. However, if this hypothesis is correct, it is not clear why this should occur in this model of left ventricular failure and not in right ventricular or congestive cardiac failure in which edema is often marked.

Plasma vasopressin levels were minimally increased with reciprocal changes in plasma sodium and osmolality in animals with infarction. However, this inappropriately raised plasma arginine vasopres-

sin was not statistically significant nor likely to be of physiological significance. Thus, in this model, there is no evidence for increased arginine vasopressin release, although increased arginine vasopressin levels have been reported in other models of heart failure including rats with arteriovenous fistulas and cardiomyopathic hamsters.

Cardiac tissue norepinephrine depletion associated with increased coronary sinus plasma norepinephrine concentrations appears to be a reproducible finding in chronic heart failure. This has previously been attributed to increased cardiac sympathetic activity, which by increasing norepinephrine turnover leads to a reduction in the norepinephrine concentration of sympathetic nerves. However, as total ventricular norepinephrine content was not significantly reduced in the present study, the reduction in cardiac norepinephrine concentration may have been due partly to sympathetic nerve dilution by cardiac hypertrophy. Urine catecholamine levels were extremely variable with no significant differences between control animals and animals with cardiac failure. Further studies are required to define the role of catecholamines in this model.

In summary, in the coronary artery ligation model of chronic left ventricular failure in the rat, several vasoactive hormone systems have been studied. Plasma renin activity and aldosterone are not increased, but plasma ANP levels are markedly and consistently activated and possibly act to suppress the renin-angiotensin system. It is clear that the neurohumoral responses to heart failure may vary with the model tested. In particular, it appears that the neurohumoral responses of the present model of left ventricular failure appear different from those found in right ventricular or congestive cardiac failure. This may explain the wide variability in humoral responses noted in previous studies of both human and animal heart failure. This variability in neurohumoral responsiveness may reflect important differences in pathophysiological mechanisms and may have important implications in the future drug treatment of heart failure in humans. Further studies are required to refine our understanding of the vasoactive hormones. In particular, the pathophysiological role of ANP in heart failure may not be known until effective ANP antagonists become available.

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KEY WORDS • heart failure • atrial natriuretic peptide • total exchangeable body sodium • renin-angiotensin system
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