Increased thirst and vasopressin secretion after myocardial infarction in rats

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Smet, H. R. De, M. F. Menadue, J. R. Oliver, and P. A. Phillips. Increased thirst and vasopressin secretion after myocardial infarction in rats. Am J Physiol Regul Integr Comp Physiol 285: R1203–R1211, 2003; 10.1152/ajpregu.00098.2003.—Impaired regulation of salt and water balance in left ventricular dysfunction and heart failure can lead to pulmonary and peripheral edema and hyponatremia. Previous studies of disordered water regulation in heart failure have used models of low cardiac output with normal cardiac function (e.g., inferior vena cava ligation). We investigated thirst and vasopressin (AVP) secretion in a rat myocardial infarction model of chronic left ventricular dysfunction/heart failure in response to a 24-h water deprivation period. Thirst (implied from water drunk), hematocrit, plasma renin activity, and plasma AVP concentrations increased with water deprivation vs. ad libitum water access. Thirst and plasma AVP concentrations were significantly positively correlated with infarct size after 24-h water deprivation but not under ad libitum water access conditions. The mechanism by which this occurs is unclear but could involve increased osmoreceptor sensitivity, altered stimulation of baroreceptors, the renin-angiotensin system, or altered central neural control.

HEART FAILURE is a common condition with an increasing incidence and prevalence (1, 31). The most common cause of heart failure is ischemic heart disease, causing approximately more than 50% of heart failure (13, 17, 31). Ischemic heart disease can cause heart failure through ischemia, causing acute myocardial necrosis/infarction and subsequent scar formation, myocardial remodeling, and impaired left ventricular function, as well as through intermittent episodes of acute ischemia, causing myocardial stunning (13). Chronic ischemia can also aggravate myocardial infarction through causing viable myocytes to hibernate when blood flow is sufficient to maintain viability but insufficient for normal contractility (13).

Whenever left ventricular function is impaired, there is activation of neurohumoral factors such as the sympathetic nervous system, the renin-angiotensin system, and AVP secretion progressively with increasing left ventricular dysfunction (21). Packer (21) put forward the neurohumoral hypothesis, suggesting that the activation of neurohumoral factors plays a major role in the progression of heart failure. These systems are activated to maintain cardiac output, blood pressure, and tissue perfusion. However, the resulting increased cardiac loading conditions have deleterious effects on the hemodynamics and the heart (14).

Plasma AVP concentrations are increased in some patients with heart failure, despite hyponatremia and low plasma osmolality (25). Because AVP secretion would usually be inhibited in these conditions, these increased AVP levels are not a result of osmotically driven release but are probably due to the activation of high-pressure mechanoreceptors, responding to low cardiac output (14). Similarly there are anecdotal reports of excessive thirst in patients with heart failure (9). Both excessive thirst and AVP secretion might contribute to the abnormal water homeostasis seen in left ventricular dysfunction. Previously, animal models of inferior vena cava (IVC) ligation have been used to investigate the impact of low cardiac output on thirst and AVP secretion (9). However, this is not a true model of low output heart failure as cardiac function is normal. Pfeffer et al. (23) have demonstrated that acute myocardial infarction, caused by ligation of the left coronary artery, is a model of left ventricular dysfunction and low-output heart failure. As infarct size increases, left ventricular dysfunction and myocardial remodeling also increase (23).

The present study aimed to investigate the regulation of thirst and AVP secretion in the rat myocardial infarction model of left ventricular dysfunction to determine whether thirst and AVP secretion were enhanced with increasing degrees of left ventricular infarction.

METHODS

All experiments were approved by the Flinders University Animal Research Ethics Committee and were performed according to the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation.

Surgical Procedures

Induction of myocardial infarction was based on the method of Pfeffer et al. (23). This model produces a range of infarct sizes, with not all animals exhibiting overt clinical

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Coronary Snares Implantation

Sprague-Dawley rats (250–300 g, n = 330) were anesthetized with 2% isoflurane (Forthane; Abbott, Kurnell, Australia), intubated, and artificially ventilated with a rodent ventilator (Harvard, model 683, Holliston, MA) with 3.5% enflurane (Ethrane, Abbott). A left thoracotomy was performed to expose the heart. A prolene suture, size 6-0 with C-1 taper needle (Ethicon, Somerville, NJ), was passed around the left coronary artery between the left atrial appendage and the pulmonary root. The free end of the suture was passed through the lumen of a piece of PVC tubing (OD 0.80 mm × ID 0.50 mm, Critchley Electrical, Auburn, Australia), which was tunneled subcutaneously to emerge in the interscapular space. The heart was returned to its original position, and the thoracic cavity was closed. Analgesics [buprenorphine (Temgesic; Reckitt and Colman Products, West Ryde, Australia) 0.01 mg/kg body wt iv and bupivacaine (Marcain; Astra Pharmaceuticals, North Ryde, Australia) 0.2 ml of 0.25% locally] and antibiotics [ampicillin (Ampycyn, Fisons Pharmaceuticals, Sydney, Australia) 0.15 g/kg body wt] were administered once postoperatively. Animals were given paracetamol (Panadol; SmithKline Beecham, Ermington, Australia) 0.2 ml/kg ip and bupivacaine (Marcain; Astra Pharmaceuticals, North Ryde, Australia) 0.01 mg/kg body wt ip and oxygen through a facial mask.

Induction of Myocardial Infarction

One week after implantation of the coronary snare line, rats were anesthetized as above, a PVC catheter (OD 0.96 mm × ID 0.58 mm; Critchley Electrical) was inserted into the femoral artery and tunneled subcutaneously to emerge in the interscapular space. Animals were allowed to recover overnight.

The next day, rats were anesthetized with isoflurane (2.5%) and then continuously ventilated with 3% enflurane and oxygen through a facial mask. Buprenorphine (0.01 mg/kg body wt) was given intraperitoneally as above. Baseline measurements of mean arterial pressure (MAP) and heart rate (HR) were taken through the arterial cannula attached to a Maclab system (version 3.5.6; AD Instruments, Castle Hill, Australia) for 3 min before induction of infarction. Infarction was confirmed by a decrease in MAP. To ensure the suture line stayed tight around the coronary artery for the duration of the experiments, a metal screw was tightened over the tubing, and the free ends of the tubing and prolene were melted together. The immediate mortality rate of myocardial infarction was 56%. Rats were then allocated randomly to either the thirst or AVP study (see below).

Thirst Study

In this study, thirst was inferred from water intake. Animals (n = 70) were studied at 1, 2, 4, 5, 8, and 9 wk after myocardial infarction. In each of weeks 1, 4, and 8, animals were placed in metabolism cages for measurement of body weight, food intake, water intake, and urine output for an acclimatization period of 3 days. On day 4 of each of these weeks, rats were randomized to receive water ad libitum or water deprivation for 24 h. On day 5, after 24 h of ad libitum water access or water deprivation, water intake was measured hourly for 6 h. The other treatment (water ad libitum or water deprivation) was performed the following week. The difference between the volume drunk in the 6 h after 24 h of water deprivation and after water ad libitum water access for each rat was taken as the water deprivation-induced water intake for each of weeks 1–2, 4–5, and 8–9 and is expressed as milliliters per kilogram body weight. Rats were killed 24 h after the end of the water deprivation period by stunning and decapitation. Trunk blood was collected for measurement of plasma variables. The heart was dissected into chambers and weighed. The left ventricle was preserved for infarct size determination (see below).

The lungs were removed and weighed wet, dried, and weighed again.

AVP Study

Animals (n = 66) were allocated randomly to one of four treatment groups: 1) water ad libitum at 1 wk after infarct induction; 2) water deprivation at 1 wk; 3) water ad libitum at 8 wk; and 4) water deprivation at 8 wk. Rats were placed in metabolism cages for the same measurements as above. A 3-day acclimatization period was allowed before the 24-h treatment. Rats were killed at the end of the 24-h treatment period as described above.

Measurement of Plasma Variables

After decapitation, blood was collected into tubes containing lithium heparin, and the hematocrit (Ht) was measured. The blood was centrifuged at 4°C, and the plasma was removed and stored frozen at −70°C until assayed for osmolality, renin activity, and sodium, potassium, AVP, and atrial natriuretic peptide (ANP) concentrations. Osmolality was determined using a Fiske one-ten osmometer (Fisc Associates, Norwood, MA). Plasma sodium and potassium were measured using a Hitachi 917 Automatic Analyzer (Roche Diagnostics, Castle Hill, Australia).

Plasma renin activity. Plasma renin activity was measured using a commercially available RIA kit (GammaCoat Plasma Renin Activity 125I-RIA kit; Diasorin, Stillwater, MN).

AVP RIA. Plasma AVP was quantified in rat blood using a commercial RIA kit (Euro-Diagnostica, Malmo, Sweden). A modification of the kit methodology involved direct measurement of AVP in plasma (200 μl) without prior extraction. A prolonged incubation period (60 h) at 4°C with antibody (diluted 2:3) before the addition of radiolabeled AVP and a further incubation period of 24 h were used. Extraction of plasma was found unnecessary following studies that demonstrated parallelism between dilutional curves for plasma AVP and synthetic [Arg³]AVP standards. Furthermore, dilution of plasma AVP showed a linear relationship between observed and expected values for AVP with a correlation coefficient of r = 0.999. Sensitivity of the assay under these conditions was 0.5 pmol/l with intra- and interassay coefficient of variation of <8.5%. One plasma AVP sample was excluded (from the AVP study, week 8, rat with ad libitum water access) because of technical problems.

α-ANP assay. ANP was measured in rat plasma using a rabbit antibody against rat α-ANP 99–126 and 125I-radiolabeled human α-ANP 99–126 (Amersham Pharmacia Biotech). The antiserum showed 100% crossreactivity with rat and human α-ANP 99–126, atriopeptin II and III, <1.5% with
atriopeptin fragments 13–28 and 18–28, and <0.002% with unrelated peptides including [Arg8]vasopressin, oxytocin, and ANG II. The assay buffer consisted of PBS, pH 7.4, containing 1% bovine serum albumin (RIA grade), 0.01 M EDTA, 0.01 M 6-aminohexanoic acid, 3 mM L-cystine, and 5 mM thimerosal. This assay buffer was used to dilute all reagents.

Briefly the assay involved incubation of 100 μl of rat plasma with antiserum at a final dilution of 1:48,000 for 48 h at 4°C. Approximately 10,000 cpm of radiolabeled ANP (specific activity = 74 TBU/mmol) was added, and the incubation continued for a further 24 h at 4°C. Bound and free hormone were separated using a second antibody and polyethylene glycol precipitation (3.5%). The pellet representing the bound fraction was counted in a gamma counter (Packard RIA-STAR).

A standard curve was constructed using synthetic human α-ANP99–126 (Auspep). Sensitivity of the assay was 1.8 ± 0.8 pg, and the inter- and intra-assay coefficients of variation were ±11% and less than ±7%, respectively.

Histological determination of infarct size. At the completion of the study, each rat was killed by stunning and decapitation, and the heart was removed and dissected into chambers to determine chamber weights. After weighing, the left ventricle was placed into 4% formaldehyde solution for fixation. The fixed left ventricle had the apex and base removed, and the remainder was cut in four transverse slices, which were processed for routine histology in paraffin wax. Sections 5 μm thick were cut and stained with Van Gieson stain. Digital photographs were taken of the slices, and infarct size was determined using Scionimage (Scion, www.scioncorp.com). The entire left ventricle and scarred endocardial and epicardial circumferences for all slices were measured to obtain endocardial and epicardial circumference for both the left ventricle and myocardial scar. The scar circumferences were compared with the total left ventricle circumferences to give an endocardial and epicardial infarct size as a percentage of the total circumference. The epicardial and endocardial infarcts sizes were averaged to give the final infarct size for each rat. Infarct sizes are therefore expressed as a percentage of left ventricle size. This method is different from that described by Pfeffer et al. (23), which only measured endocardial circumferences infarcted. Because the coronary artery was not directly visible at snare implantation, the suture did not always incorporate the artery, and ligation did not always produce a histologically detectable myocardial infarction. These animals were used as controls. For further analysis, rats were divided into three groups according to their infarct size: 1) control; 2) 15–40%; 3) >40%. Determination of infarct size was performed without knowledge of the experiment or treatment group.

Statistical Analyses

Data are expressed as means ± SE. Statistical significance was accepted at P < 0.05.

For animals in both studies, body weights, heart chamber weights, and infarct sizes were compared for infarct size groups, while plasma variables were compared for treatment groups, by one-way ANOVA followed by the Bonferroni post hoc test for multiple comparisons.

Thirst study. Cumulative hourly water intakes for infarct size groups were compared by one-way ANOVA with the Bonferroni post hoc test. A linear regression analysis was performed to examine the relationship between any infarct size and water intake. Group means for controls (0% infarct size) and rats with 15–40% and >40% infarct sizes were compared using one-way ANOVA with the Bonferroni post hoc test.

AVP study. Separate regression analyses of plasma AVP concentration vs. any infarct size were performed for water deprivation and ad libitum treatments. A two-factor ANOVA with infarct size and treatment as factors was performed to compare groups [for controls (0% infarct size) and rats with 15–40% and >40% infarct sizes], followed by the Bonferroni post hoc test for multiple comparisons. A one-way ANOVA was performed to check differences between treatments. Analyses were performed on week 1 and week 8 data. Data from week 1 and week 8 were then combined for the same analyses.

For the analysis of the relationship between plasma osmolality and plasma AVP concentration, the infarct groups of 15–40% and >40% were combined into one group of infarcted animals, and a regression analysis was performed on both control and infarcted animals separately.

Regression analysis of wet lung weight vs. infarct size was performed for both treatment groups separately.

RESULTS

Body weights, heart chamber weights, and infarct sizes for animals in each study are given in Table 1. A consistent increase in body weight throughout the experiment was seen in both infarcted and control animals, indicating animals remained in good health.

Thirst Study

Of the 70 animals surviving myocardial infarction, three animals developed infective pericarditis and were excluded from the analysis. Of the remaining 67 animals, 69% had a histologically detectable infarct. Four animals died before the end point of the experiment due to myocardial failure and had only limited data available.

As shown in previous studies using similar models (23), there was remodeling of cardiac chamber sizes with increasing infarct size (Table 1).

The cumulative hourly water deprivation-induced water intakes for 6 h are shown in Fig. 1. There was a statistically significant difference between infarct size groups at all hours at both 1 and 8 wk but only at the 1st h at 4–5 wk.

At 1–2 wk after myocardial infarction, a linear positive dependent relationship (P = 0.019) of water deprivation-induced water intake with infarct size was seen (Fig. 2A). As shown in Fig. 2B, water intake varied significantly (P = 0.01) depending on infarct size. A linear trend was seen at 4–5 wk (Fig. 2C), but this did not reach statistical significance. The same linear relationship (P = 0.002) was seen at 8–9 wk after myocardial infarction (Fig. 2E), with again a significant difference (P = 0.015) between groups (Fig. 2F).

Plasma data obtained from all rats in the thirst experiments at 24 h after the end of ad libitum water access or water deprivation are shown in Table 2. None of the measured plasma variables differed significantly between groups, indicating that the usual access to water for the 24 h after water deprivation had allowed the plasma variables to return to normal.
Table 1. Body weights, heart chamber weights, and infarct size for rates in thirst and AVP studies

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>15–40%</th>
<th>&gt;40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thirst study</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 13</td>
</tr>
<tr>
<td>BW, g</td>
<td>440 ± 9</td>
<td>475 ± 8</td>
<td>455 ± 8</td>
</tr>
<tr>
<td>LV/BW, g/100 g</td>
<td>0.202 ± 0.003</td>
<td>0.204 ± 0.005</td>
<td>0.213 ± 0.003</td>
</tr>
<tr>
<td>RV/BW, g/100 g</td>
<td>0.056 ± 0.001</td>
<td>0.057 ± 0.001</td>
<td>0.075 ± 0.008*</td>
</tr>
<tr>
<td>LA/BW, g/100 g</td>
<td>0.009 ± 0.0004</td>
<td>0.013 ± 0.001b</td>
<td>0.020 ± 0.002b</td>
</tr>
<tr>
<td>RA/BW, g/100 g</td>
<td>0.020 ± 0.0006</td>
<td>0.021 ± 0.0008a</td>
<td>0.029 ± 0.002a</td>
</tr>
<tr>
<td>Mean infarct size, %</td>
<td>≤ 0</td>
<td>29.6 ± 2a</td>
<td>46.7 ± 1.4a</td>
</tr>
<tr>
<td>AVP study</td>
<td>n = 19</td>
<td>n = 20</td>
<td>n = 17</td>
</tr>
<tr>
<td>BW, g</td>
<td>371 ± 15</td>
<td>370 ± 15</td>
<td>359 ± 18</td>
</tr>
<tr>
<td>LV/BW, g/100 g</td>
<td>0.216 ± 0.003</td>
<td>0.222 ± 0.006</td>
<td>0.233 ± 0.005*</td>
</tr>
<tr>
<td>RV/BW, g/100 g</td>
<td>0.063 ± 0.002</td>
<td>0.062 ± 0.004</td>
<td>0.073 ± 0.004</td>
</tr>
<tr>
<td>LA/BW, g/100 g</td>
<td>0.009 ± 0.0003</td>
<td>0.014 ± 0.0009f</td>
<td>0.02 ± 0.002f</td>
</tr>
<tr>
<td>RA/BW, g/100 g</td>
<td>0.021 ± 0.001</td>
<td>0.026 ± 0.001</td>
<td>0.032 ± 0.002f</td>
</tr>
<tr>
<td>Mean infarct size, %</td>
<td>≤ 0</td>
<td>29.3 ± 1.8</td>
<td>45.3 ± 1.1b</td>
</tr>
</tbody>
</table>

Values are means ± SE. BW, body wt; LV/BW, left ventricle wt/body wt; RV/BW, right ventricle wt/body wt; LA/BW, left atrium wt/body wt; RA/BW, right atrium wt/body wt. *RV: P = 0.001, 40 vs. control; P = 0.003, 40 vs. 15–40. bLA: P = 0.000, 40 vs. control; P = 0.002, 40 vs. 15–40; 0.005, 15–40 vs. control. RA: P = 0.000, 40 vs. control; P = 0.009, 40 vs. 15–40; 0.000, 40 vs. 15–40. Infarct size: P = 0.000, 40 vs. control; P = 0.000, 40 vs. 15–40; P = 0.009, 15–40 vs. control. bRA: P = 0.000, 40 vs. control; P = 0.005, 40 vs. 15–40. Infarct size: P = 0.000, 40 vs. control; P = 0.000, 40 vs. 15–40; P = 0.004, 40 vs. 15–40; P = 0.009, 15–40 vs. control. 4RA: P = 0.000, 40 vs. control; P = 0.005, 40 vs. 15–40. Infarct size: P = 0.000, 40 vs. control; P = 0.000, 40 vs. 15–40; P = 0.004, 40 vs. 15–40; P = 0.009, 15–40 vs. control.

AVP Study

Of the 66 animals surviving myocardial infarction, 3 animals developed infective pericarditis and were excluded from the analysis. Of the remaining 63 animals, 70% had a histologically detectable infarct. Again, there was the expected remodeling of cardiac chamber sizes with increasing infarct size (Table 1).

Plasma data obtained at the end of ad libitum water access or water deprivation are shown in Table 2. At 1 wk postinfarction, plasma sodium concentration, plasma osmolality, hematocrit, plasma AVP concentration, and plasma renin activity were all significantly higher in water-deprived rats compared with rats with ad libitum water access, while plasma potassium concentration was lower. Plasma ANP concentrations were lower in water-deprived rats (794 ± 100 vs. 1,001 ± 97 pmol/L), but this was not statistically significant. At 8 wk postinfarction, plasma osmolality, hematocrit, plasma AVP concentration, and plasma renin activity were significantly higher, while plasma ANP was significantly lower, in water-deprived rats compared with rats with ad libitum water access.

Plasma AVP concentrations were significantly higher and plasma renin activity significantly lower at week 8 compared with week 1 for rats with ad libitum water access. Hematocrit was significantly higher at week 8 vs. week 1 for water-deprived animals.

At 1 wk after myocardial infarction, the plasma AVP concentrations of water-deprived animals were positively linearly related to infarct sizes (Fig. 3A, P = 0.033). This was not the case for animals that had been allowed water ad libitum during the same time, with plasma AVP levels constant and not dependent on infarct size. There was a statistically significant difference in plasma AVP concentrations between water-deprived and ad libitum water access animals for all infarct size groups (Fig. 3B) consistent with the known effects of water deprivation to raise plasma AVP concentrations. However, there was no significant difference for infarct size groups by two-way ANOVA (P = 0.297).

At 8 wk, although there was a positive linear relationship between plasma AVP concentration and infarct size, this was not statistically significant possibly due to the numbers of animals used at this timepoint (Fig. 3C). As shown in Fig. 3D, there was still a significant increase in plasma AVP due to water deprivation.

When weeks 1 and 8 data were combined, as shown in Fig. 3, E and F, there was again a significant positive linear relationship between plasma AVP concentration and infarct size (P = 0.009) for water-deprived animals but not for animals with ad libitum water access. Plasma AVP concentration was significantly increased with water deprivation (P = 0.000). There was a tendency for plasma AVP concentration to be increased with infarct size (Fig. 3F, P = 0.095 by ANOVA).

For Fig. 4, the plasma AVP concentrations for ad libitum/water deprivation rats in the 15–40% and >40% infarct size groups were combined into one group and plotted vs. plasma osmolality for ad libitum/water deprivation rats in the noninfarcted control group. There was a significant positive linear relationship between plasma osmolality and plasma AVP concentration for the infarcted animals (P = 0.018) with a tendency for a similar relationship in the control noninfarcted rats (P = 0.106). For a given plasma osmolality, plasma AVP concentrations tended to be higher in infarcted rats vs. noninfarcted control rats.

Wet lung weight was significantly positively correlated (n = 32, P = 0.01, r = 0.448) with infarct size when animals had ad libitum water access consistent with the effects of left ventricular dysfunction to increase extracellular fluid volume, left heart filling pressures, and pulmonary capillary venous pressures (23).
However, there was no such significant correlation in the water-deprived animals ($n = 31$, $P = 0.3$, $r = 0.192$), consistent with a reduction in extracellular fluid volume (and so left heart filling pressures and pulmonary capillary venous pressures) after 24-h water deprivation.

**DISCUSSION**

These studies demonstrate that rats with increasing degrees of left ventricular infarction have increased thirst and plasma AVP concentrations when these homeostatic mechanisms are stimulated by 24-h water deprivation. Under baseline ad libitum conditions, left ventricular infarction had no impact on thirst or plasma AVP concentrations except that as rats survived longer, plasma AVP concentrations increased, rising from $4.5 \pm 0.2$ pmol/l at week 1 to $8.7 \pm 2.9$ pmol/l at week 8 (Table 2).

There have been no previous studies of the impact of myocardial infarction on thirst and only limited studies on stimulated AVP secretion. Previous studies of AVP secretion in the rat myocardial infarction model of left ventricular dysfunction have relied on baseline levels during periods of ad libitum water access. Increased plasma AVP concentrations and increased hypothalamic AVP gene expression have been demonstrated (15). In our studies, baseline plasma AVP concentrations under ad libitum water access were higher at 8 wk than at 1 wk postinfarction consistent with activation of the hypothalamic controls for AVP synthesis and secretion with left ventricular dysfunction (15). Furthermore, stimulation of thirst and AVP secretion with a period of water deprivation showed that stimulated thirst and plasma AVP concentrations increased further, depending on the degree of left ventricular infarction.

The mechanism underlying the increased thirst and vasopressin secretion (implied from the increased plasma AVP concentrations) is unclear and possibly multifactorial. As shown in Fig. 4 there was a tendency for a higher plasma AVP concentration for a given plasma osmolality, implying possible increased sensitivity for the osmotic control of AVP release. We were unable to perform a similar analysis for thirst; however, a similar mechanism might be present for the increased thirst. Such increased osmoreceptor sensitivity might occur through a variety of mechanisms.

Potentially, reduced cardiac output secondary to left ventricular dysfunction caused by the myocardial infarction, acting through high pressure baroreceptors, might be one mechanism for the increased thirst and plasma AVP concentrations. Altered osmotic control with higher or lower stimulation of baroreceptors is a well accepted concept. However, the interaction between low- and high-pressure baroreceptors might also be important in the results seen. Increased atrial pressures are well documented in this rat model (23) and, combined with increased pulmonary venous pressure, would tend to inhibit thirst and AVP secretion (5, 6, 18, 24). In contrast, activation of high-pressure baroreceptors through reduced cardiac output might tend to increase these (24). Under resting ad libitum water access, these influences might balance; however, after 24-h water deprivation there would be a decrease in extracellular fluid volume, possibly reducing atrial and pulmonary venous pressures, allowing the effects of reduced cardiac output to become predominant, thus producing increased osmotic thirst and vasopressin secretion. This is supported by the finding that there was no correlation between infarct size and wet lung weight after 24-h water deprivation, suggesting that extracellular fluid volume and so left heart filling pressures and pulmonary capillary venous pressures had reduced.

Changes in hypothalamic function in heart failure are now becoming recognized (7, 11, 12). Anteroventral
third ventricle (AV3V) lesions attenuate increases in plasma renin activity in the rat infarction model of heart failure and improve survival (11), while activation of hypothalamic mineralocorticoid receptors might play a role in the fluid and electrolyte homeostasis, sympathetic drive, and impaired baroreflex function in this model (12). Therefore, changes in intrinsic hypothalamic osmoreceptor function might also play a role in the changes in water homeostasis demonstrated here through altered hemodynamic and central cardiovascular neural control input, or intracerebral or circulating ANG II production. This could be assessed in rats with myocardial infarction-induced left ventricular dysfunction via studies investigating the effects of intracerebroventricular cholinergic osmoreceptor stimulation or blockade (8) as the osmoreceptors for thirst and AVP secretion seem to be located close to the third cerebral ventricle, possibly in the organum vasculosum of the lamina terminalis (9).

Increased ANG II production with water deprivation might also be a factor in the increased thirst and AVP secretion seen here. At both week 1 and week 8, plasma renin activity increased significantly with water deprivation. ANG II has been shown to be a potent stimulus for thirst and AVP secretion and to be an important mechanism for the increased thirst (9) seen in the IVC ligation model of low cardiac output. Interestingly, ANG II might play a lesser role in the AVP secretion stimulated by IVC ligation (26). The site of the action of ANG II is most probably the subfornical organ (9) adjacent to the third cerebral ventricle. Further experiments investigating this possibility might involve the

Fig. 2. Water deprivation induced water intake vs. any infarct size at 1–2 wk (A and B), 4–5 wk (C and D), and 8–9 wk (E and F) after myocardial infarction, for all individual rats (A, C, and E) and in groups (B, D, and F), rats with infarct between 1 and 14% not included. P value shown is from ANOVA. *Significantly different from control by post hoc comparison.
use of intracerebroventricular ANG II receptor blockade. This would block any effects of intracerebral or circulating ANG II without affecting hemodynamic parameters and baroreceptors. It is important to understand that although IVC ligation certainly produces low cardiac output, left ventricular function is normal. In our study, the myocardial infarction model of left ventricular dysfunction was used in which greater left ventricular dysfunction occurs with increasing infarct size (23). This most closely resembles a common condition leading to left ventricular dysfunction and heart failure in humans, i.e., ischemic heart disease.

Another mechanism by which thirst and AVP secretion could have increased more in rats with larger infarcts and potentially greater degrees of left ventricular dysfunction is through a loss of inhibition of thirst and AVP secretion (3, 28) by the high ANP levels seen with heart failure. With water deprivation, plasma ANP concentrations fell. Potentially, this would reduce the inhibitory effect of ANP on thirst and AVP secretion (3, 28).

Plasma AVP concentrations have also been shown to be elevated in other animal models of left ventricular dysfunction or heart failure. These include the sheep or dog pacing model in which the heart is electronically paced over a long period to produce high cardiac filling pressures and reduced cardiac output (19). Over long periods myocardial function is also compromised. AVP V1 and V2 receptor antagonism improves hemodynamic parameters and causes aquarexis, respectively, in this model, indicating a role for AVP in the altered hemodynamics and water homeostasis present (19). However, there have been no studies of thirst in these models.

Although there are only anecdotal reports of increased thirst in people with impaired left ventricular function (8, 9), increased plasma AVP concentrations that correlate with the degree of ventricular dysfunction are well documented in humans (21). It is important to realize that although clinical heart failure is the worst manifestation of the most severely impaired cardiac function, neuroendocrine activation has been shown to occur in patients with left ventricular dysfunction, even without the presence of heart failure symptoms (10). Hence the disturbances in homeostatic mechanisms seen here, including thirst and vasopressin secretion, can be seen as graded responses to the varying degrees of impaired cardiac function and not as “all or nothing” phenomena. In fact Pfeffer et al. (23) originally showed that the hemodynamic manifestations of smaller myocardial infarcts may only become manifest under conditions of hemodynamic stress. Thus the abnormalities in other homeostatic mechanisms, such as thirst and AVP secretion, may only become overtly manifest under conditions that stress these also, such as water deprivation.

The combination of possibly increased thirst and increased plasma AVP concentrations has been hypothesized as the mechanism for the hyponatremia seen in some patients with impaired cardiac function (25). The correlation of increased plasma AVP levels with increasing degrees of left ventricular dysfunction in humans, and the demonstration here of increased stimulated thirst and plasma AVP concentrations with increasing infarct size, would also explain why hyponatremia is an adverse prognostic indicator (21). Those with the greatest infarct-induced damage and worst

Table 2. Plasma variables for rats in thirst and AVP studies

<table>
<thead>
<tr>
<th></th>
<th>Ad Libitum</th>
<th>Deprivation</th>
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<tbody>
<tr>
<td><strong>Thirst study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>143 ± 0.6</td>
<td>145 ± 0.7</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>6.55 ± 0.11</td>
<td>6.69 ± 0.11</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg H2O</td>
<td>294 ± 0.9</td>
<td>294 ± 1.2</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>46.6 ± 0.4</td>
<td>46.3 ± 0.6</td>
</tr>
<tr>
<td>AVP, pmol/l</td>
<td>5.4 ± 0.7</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>Renin activity, ng·ml⁻¹·h⁻¹</td>
<td>3.0 ± 0.4</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>ANP, pg/ml</td>
<td>1050 ± 95</td>
<td>825 ± 61a</td>
</tr>
</tbody>
</table>

| **AVP study**        |            |             |
| n                    | 22         | 19          |
| Sodium, mmol/l       | 143 ± 0.6  | 145 ± 0.6b  |
| Potassium, mmol/l    | 6.34 ± 0.15| 5.99 ± 0.09c|
| Osmolality, mosmol/kg H2O | 292 ± 1   | 296 ± 1.4f |
| Hematocrit, %        | 43.9 ± 0.6 | 46.5 ± 0.6g |
| AVP, pmol/l          | 4.5 ± 0.2  | 18.5 ± 1.7f |
| Renin activity, ng·ml⁻¹·h⁻¹ | 4.9 ± 0.6  | 12.3 ± 1.7g |
| ANP, pg/ml           | 1,001 ± 97 | 794 ± 100   |
| Week 1               | 178 ± 651  | 1,324 ± 178 |
| Week 8               | 178 ± 651  | 1,324 ± 178 |

Values are means ± SE. Data in thirst study are from all rats at 24 h after end of water ad libitum or water deprivation at week 9. *P = 0.052, deprivation vs. ad libitum. †P = 0.029; ‡P = 0.052; ‡P = 0.002; §P = 0.003; ‡P = 0.000; ‡P = 0.064; ‡P = 0.000; ‡P = 0.055; ‡P = 0.003; ‡P = 0.008, deprivation vs. ad libitum; ‡P = 0.001; ‡P = 0.028; ‡P = 0.003, week 8 vs. week 1. *One plasma AVP sample was excluded due to technical difficulties with the assay.
cardiac function have the worst disturbances in thirst and AVP control.

The aquaretic effect of AVP V2 receptor antagonists has been demonstrated in patients with heart failure (16, 27) as well as in animal models of heart failure (4, 19, 20, 30). These agents may prove especially useful in the treatment of hyponatremia in heart failure (29) but also may improve adverse hemodynamics (19, 22, 27). Their aquaretic effect might allow free water access, and even increased water intake, if AVP V2 receptor blockade allowed the excretion of the excess water ingested.

In conclusion, we have demonstrated that in rats with myocardial infarction, stimulated thirst and plasma AVP concentrations are increased in proportion to the extent of infarction but are no different from rats without infarction under ad libitum water access.
These findings might help to explain the abnormal water homeostasis seen in humans with impaired cardiac function. Further studies are needed to elucidate the mechanism of the increased stimulated thirst and AVP secretion; however, modulation of osmoreceptor function per se, or through increased high pressure baroreceptor input centrally because of low cardiac output, and/or increased water deprivation-induced ANG II production, are all possibilities.

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DISCLOSURES

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REFERENCES