Temporal changes in natriuretic and antinatriuretic systems after closure of a large arteriovenous fistula

Zaid A. Abassi, Sergey Brodsky, Tony Karram, Igor Dobkin, Joseph Winaver, Aaron Hoffman

Abstract

Objective: Surgical closure of a large arteriovenous (A-V) fistula in patients and animals is associated with prompt diuresis and natriuresis. However, the mechanisms underlying these changes remained largely unknown.

Methods: The present study evaluated the hormonal balance between major antinatriuretic systems (plasma renin activity, PRA, and arginine vasopressin, AVP) and natriuretic systems (atrial natriuretic peptide, ANP, and renal nitric oxide, NO) in Wistar rats with an A-V fistula (1.2 mm O.D., side to side) between the abdominal aorta and inferior vena cava.

Results: The placement of an A-V fistula caused progressive sodium retention (UNaV decreased from 1500 to 100 mEq/day), a significant drop in mean arterial blood pressure (MAP) from 127 ± 3 to 75 ± 2 mmHg (P < 0.01), and a significant increase in ANP (from 94 ± 12 to 389 ± 135 pg/ml, P < 0.05), PRA (from 22.1 ± 2.0 to 47 ± 14 ng angiotensin I [Ang I]/ml/h, P < 0.05), AVP (from 14.2 ± 3.6 to 37.7 ± 9.6 pg/ml, P < 0.05), norepinephrine (from 184.2 ± 40.5 to 1112.6 ± 293.2 pg/ml, P < 0.05) and epinephrine (from 667.5 ± 175.9 to 2049.8 ± 496.9 pg/ml, P < 0.05). Furthermore, these changes were associated with a 3-fold increase in the renal medullary immunoreactive levels of endothelial NO synthase (eNOS), an endogenous vasodilator that plays an important role in the regulation of medullary blood flow. After 6 days, rats with A-V fistula and maximal sodium retention underwent surgical closure of the A-V fistula. The A-V fistula closure was associated with dramatic natriuresis (UNaV = 2563 ± 78 and 1918 ± 246 mEq/day on days 3 and 6 following the closure, respectively) and restoration of MAP to normal levels (111 ± 6 mmHg); PRA decreased to 29 ± 5 ng Ang I/ml/h, AVP to 20.3 ± 7.1 pg/ml, and medullary eNOS declined to basal levels, whereas plasma ANP concentrations remained elevated (380 ± 90 pg/ml) after 3 days and returned to normal (92 ± 12 pg/ml) on day 6.

Conclusions: These results demonstrate that the creation of A-V fistula is associated with activation of both natriuretic and antinatriuretic systems. Closure of A-V fistula is characterized by shifting the balance in favor of the natriuretic substances. Moreover, the observed alterations in medullary eNOS following the creation and closure of A-V fistula suggest that this system, an important determinant of medullary blood flow, may contribute significantly to the regulation of sodium excretion in this model.

Keywords: Anti-hypertensive/diuretic agents; Heart failure; Natriuretic peptide; Renin–angiotensin system; Vasoactive agents; Blood pressure

1. Introduction

Surgical creation of a large arteriovenous (A-V) fistula usually results in the development of congestive heart failure (CHF), a clinical situation associated with increased activity of vasoconstrictor neurohormonal systems and compensatory activation of systemic and intrarenal vasodilating systems [1,2]. Among the various features of CHF are generalized vasoconstriction and marked decrease in renal blood flow [1,3,4]. The decrease in renal perfusion appears relatively early in the course of cardiac decompensation and may lead to profound alterations in renal handling of salt and water, resulting in edema formation [1,5]. Traditionally, the systemic and renal vasoconstriction has been attributed to a decline in perfusion pressure associated with compensatory activation of several vas-
Furthermore, we have shown that experimental decompen-
and a tendency to avid sodium and water retention.
[10,11,24], a marked decrease in renal blood flow [3,21], 0.5% NaCl and tap water ad libitum.

enhanced secretion of atrial natriuretic peptide (ANP) per-
temperature room, and were fed standard rat chow containing

activity of the RAS [1,2,10±16], A VP [17,18], endothelin rats of local strain weighing 280±350 g. The animals were

developed the characteristic neurohormonal and renal altera-
ations seen in patients with CHF. These include increased activity of the RAS as well as of the sympathetic nervous system and AVP may also cause profound renal hypoperfusion, thereby playing an important role in the exaggerated tubular sodium and water reabsorption. Interestingly, analysis of intrarenal blood flow measurements in CHF suggests that, while cortical blood flow is markedly diminished, medullary blood flow appears to be preserved [21,23]. The maintenance of medullary blood flow occurs despite a significant decrease in renal perfusion pressure, suggesting a decrease in medullary vascular resistance. The latter is attributed to the high abundance of endothelial nitric oxide synthase (eNOS) in the medullary tissue compared with the cortex [21].

Previously, we have demonstrated that rats with an A±V fistula, an experimental model of CHF [3,11,12,24], develop the characteristic neurohormonal and renal alterations seen in patients with CHF. These include increased activity of the RAS [1,2,10±16], AVP [17,18], endothelin [25] and the sympathetic nervous system [1,6±9], enhanced secretion of atrial natriuretic peptide (ANP) [10,11,24], a marked decrease in renal blood flow [3,21], and a tendency to avid sodium and water retention. Furthermore, we have shown that experimental decompensated CHF is associated with an adaptive increase in the expression of eNOS in the kidney [21]. In view of the importance of nitric oxide (NO) in regulating renal blood flow and sodium excretion, it is possible that the obtained alterations in eNOS may contribute to the changes in renal hemodynamics and tubular salt reabsorption in CHF [21,26]. Moreover, based upon their daily sodium excretion, rats with A±V fistula may be further subdivided into salt-retaining (decompensated) and compensated subgroups [10,11].

Previous data from our laboratory [10,11,24] and from other groups [14,15] suggest that urinary sodium excretion in this model of CHF is largely determined by the balance between two antagonistic hormonal systems: the vasoconstrictor–sodium-retaining factors such as RAS, endothelin, the sympathetic nervous system and AVP, and vasodilator–natriuretic substances such as ANP and NO. Through a variety of tubular and hemodynamic mechanisms, the kidney integrates the signals from several opposing as well as synergistic systems to yield the final excretion of sodium and water. In decompensated CHF, enhanced activities of sodium-retaining systems overwhelm the effects of vasodilatory/natriuretic systems, leading to a net reduction in sodium/water excretion [10,11,24]. For compensation to occur, the effects of natriuretic factors must prevail over those of the opposing systems, resulting in renal sodium/water excretion [10,11,24]. This notion is supported by clinical and experimental studies where pharmacological intervention corrected the imbalance present in CHF [11,24]. Thus, a shift in the balance in favor of natriuresis may be achieved by either increasing the activity of natriuretic factors or reducing the influence of the antinatriuretic systems [24]. Moreover, closure of an A±V fistula induces both characteristic diuretic and natriuretic responses in experimental animals [27] and reversal of the clinical signs of CHF in patients [28]. The mechanisms of these responses remain largely unknown, although the involvement of the RAS and sympathetic nervous system has been proposed [27–29]. The present study was designed to examine the renal and endocrine effects of closure of a surgically placed A±V fistula in rats with decompensated CHF.

2. Methods

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Studies were conducted on 30 male Wistar rats of local strain weighing 280–350 g. The animals were kept in individual metabolic cages, in a controlled temperature room, and were fed standard rat chow containing 0.5% NaCl and tap water ad libitum.

2.1. Experimental model

An aortocaval [A±V] fistula was surgically created between the abdominal aorta and inferior vena cava, as adapted in our laboratory [10,11] from the method originally described by Stumpe et al. [30]. Briefly, the rats were anesthetized with a mixture of halothane (Fluothane, ICI Pharmaceuticals, Macclesfield, Cheshire, UK) and oxygen. A midline abdominal incision was performed to expose the vena cava and abdominal aorta distal to the origin of the renal arteries. Miniature surgical clamps were placed around both vessels, 10–15 mm apart, and a longitudinal incision was made in the outer wall of the vena cava. The common wall between the aorta and vena cava was grasped through the incision under binocular magnification, and a fistula (1.0–1.2 mm O.D.) was created between two vessels. The opening of the outer wall of the vena cava was then closed with a continuous suture (7-0 Prolene non-absorbable polypropylene suture, Ethicon, Edinburgh, UK). Following the surgical procedure, the animals were allowed to recover and then returned to the metabolic cages for daily monitoring of urine output and sodium excretion. A matched group of normal rats served as controls. At 5–7 days after the operation, rats with A±V
fistula were divided into two subgroups, according to their daily absolute rate of sodium excretion (UNaV): rats with UNaV < 200 μequiv./24 h (decompensated) and rats with UNaV > 1200 μequiv./24 h (compensated).

2.2. Closure of aortocaval fistula

Either 3 or 6 days after placement of the A–V fistula, closure of the fistula was performed only in rats that developed overt signs of severe CHF, especially avid sodium retention (n = 6–12 for each closure period). Because of the small size of the vessels, simple repair of the fistula was not feasible; therefore, closure of the fistula was accomplished under halothane anesthesia by quadruple ligation of both aorta and vena cava around the fistula. This procedure was well tolerated by most of the rats, and animals that developed signs of hindquarter ischemia were excluded from the study. Rats with closed fistula were returned to metabolic cages for follow-up of daily urinary sodium excretion.

2.3. Effects of closure on selected endocrine systems

The effects of fistula closure on ANP, plasma renin activity (PRA), catecholamines, and AVP were determined in separate groups of rats, i.e. sham-operated controls (n = 7) and decompensated CHF before and after closure (3 and 6 days) (n = 5–9). After placement of the fistula, animals were placed in metabolic cages for daily measurements of their sodium excretion. Six days later, rats with sodium retention (UNaV < 200 μequiv./24 h) either were sacrificed or underwent fistula closure, as described above. Animals were sacrificed by decapitation 3 or 6 days after the closure. Blood samples were collected into precooled tubes and immediately centrifuged at 4°C for 10 min at 3000 rpm. Plasma samples were stored at −70°C until analysis. ANP, PRA and AVP levels were compared with the appropriate decompensated animals that did not undergo closure.

2.4. Effects of closure on cardiac hypertrophy and blood pressure

These studies were designed to evaluate the effects of the closure of the A–V fistula on mean arterial blood pressure and cardiac hypertrophy in rats with decompensated CHF. For this purpose, rats with decompensated CHF (n = 6) were anesthetized with pentobarbital sodium (30 mg/kg) and polyethylene tube (PE50) was inserted into the carotid artery for blood pressure measurements. Sham operated animals served as controls for these rats. After the completion of blood pressure monitoring, animals were sacrificed, their chests were opened and the heart was removed, placed in absorbent paper and weighted. Sham-operated controls and rats with decompensated CHF that were not subjected to fistula closure served as controls in the protocol that aimed at examining the development of cardiac hypertrophy.

2.5. Determination of eNOS immunoreactive levels by Western blot analysis

To follow the alterations in immunoreactive levels of eNOS during the development of CHF and after closure of the fistula, rats with decompensated CHF, rats with decompensated CHF plus closure (for 3 and 6 days), and control rats (n = 4–5 for each group) were decapitated, and their kidneys were removed and immediately placed in liquid nitrogen. The renal cortex and medulla were separated and then homogenized with a Polytron homogenizer (PT-2100, Kinematica, Luzern, Switzerland) in 2.5 ml of 10 mM sodium phosphate buffer, pH 7.4, containing 1 mM MgCl2, 30 mM NaCl, 0.02% sodium azide, 20 mg/l bestatin, and 20 mg/l leupeptin. The homogenates were stored at −70°C until assayed. Kaleidoscope prestained standard molecular markers (Bio-Rad, Hercules, CA, USA) were used for determination of the molecular weight of the immunoreactive products. A 10-μl volume of the tissue homogenates (120 μg protein) was treated with 20 μl sample buffer (10% sodiumdodecyl sulfate, 50% glycerol, 1 M Tris, 0.1% bromphenol blue and 1 M dithiothreitol, pH 6.8) and placed in a boiling water bath for 5 min. Samples were then electrophoresed on polycrylamide–Tris–glycine gels 4–20% (Bio-Rad), and transferred electrophoretically to a nitrocellulose membrane at 150 V for 60 min. The blots were blocked in 3% (w/v) dried milk in Tris-buffered saline (TBS) and 0.1% Tween 20 overnight. The nitrocellulose membranes were incubated with 1000-fold-diluted eNOS monoclonal antibodies (Transduction Labs., Lexington, KY, USA) for 120 min, then washed three times with TBS for 5 min each. After washing, the blots were incubated with 5000-fold-diluted peroxidase-conjugated rabbit anti-mouse IgG (Sigma, St. Louis, MO, USA) in TBS containing 0.1% Tween 20 for 60 min. Immunoreactive bands were visualized by the chemiluminescence detection system (Sigma).

2.6. Analytical methods

Sodium concentration in the urine was measured by flame photometry (model 943, Instrumentation Lab., Italy). Plasma ANP and antiuretic hormone levels were measured after extraction by commercial double-antibody kit (Peninsula Labs., Belmont, CA, USA). PRA and AVP were measured by a radioimmunoassay method (Peninsula...
Labs.). Plasma levels of epinephrine and norepinephrine were measured by HPLC on acetic-acid-extracted samples.

2.7. Statistical analysis

Analysis of variance (ANOVA) for repeated measures followed by Dunnett test was utilized to evaluate the difference between the time points and baseline value in each group. Two-way ANOVA was used to compare data of control and CHF groups. Evaluation of the data obtained in the in vitro studies in controls, compensated CHF, and decompensated CHF was done by one-way ANOVA. *P* < 0.05 was considered statistically significant. Data are presented as means±S.E.M.

3. Results

3.1. Urine volume and sodium excretion

The daily urinary sodium excretion patterns in rats with A–V fistula and in sham-operated controls are shown in Fig. 1. As we previously showed [10,11,31], daily sodium excretion before surgery ranged between 1500 and 1600 mequiv./24 h. All groups displayed an immediate postoperative decrement in sodium excretion, most likely due to the anesthesia and surgical stress. However, in sham-operated rats the decrease in sodium excretion lasted for 24 h and then returned to the presurgical baseline levels. In contrast, two distinctly different patterns of sodium excretion were evident in rats with A–V fistula: salt-retaining rats (rats with sodium retention) and compensated subgroups. Decompensated rats (~40% of animals with A–V fistula) developed severe sodium retention and signs of CHF, i.e. dyspnea, lung congestion, pleural effusion, enlarged liver, ascites, and eventually, death of the overwhelming majority of these rats within 7–10 days of the placement of the A–V fistula.

Closure of the A–V fistula was associated with marked diuretic response (urine volume increased from 10.3±3.2 ml/24 h to 28.3±5.7 and 20.0±5.0 ml/24 h, 3 and 6 days after closure, respectively) and natriuretic response (UNaV increased from 177±27 μequiv./24 h to 2563±78 and 1918±246 μequiv./24 h, after 3 and 6 days, respectively), which appeared within the first 24 h after closure, were higher than the basal excretion rates for 2–3 days, and then returned to baseline levels similar to those obtained in sham controls (Fig. 2).

3.2. Hormonal status

Quantitative changes in several endocrine parameters in sham controls and rats with compensated and decompensated CHF are given in Table 1. Most notably, plasma
levels of ANP were significantly higher in rats with A–V fistula than in sham-operated rats. Interestingly, circulating levels of AVP were equally elevated in compensated and decompensated rats with A–V fistula. The high concentrations of ANP were associated with elevated plasma levels of PRA, AVP, epinephrine, and norepinephrine in rats with decompensated CHF versus sham controls. In contrast, PRA and norepinephrine levels were activated to a lesser extent in rats with compensated CHF compared with the decompensated subgroup.

### 3.3. Plasma ANP levels

ANP levels were significantly higher in rats with decompensated CHF compared with control rats (389±135 vs. 94±12 pg/ml, *P*<0.05) (Fig. 3A). The elevated levels of ANP did not decrease 36–48 h after the surgical closure of the fistula (380±90 pg/ml) (Fig. 3). However, on day 6 after closure the ANP levels returned to basal values (92±12 pg/ml), corresponding with the parallel decline in the natriuretic response in these rats.

### 3.4. Plasma renin activity

PRA was very high during the A–V fistula phase (decompensated CHF) [22.1±2.0–47.0±14.0 ng angiotensin I (Ang I)/ml/h, *P*<0.05] (Fig. 3B). The enhanced PRA significantly decreased, although not completely, 3 days after surgical closure of the A–V fistula (29±5 ng Ang I/ml/h, *P*<0.05). A further and more profound decrease in PRA (22±5 ng/ml) was observed on day 6 of the closure.

### 3.5. Plasma AVP levels

Plasma levels of AVP were significantly higher in decompensated rats (37.7±9.6 pg/ml) compared with sham controls (14.2±3.6 pg/ml) (Fig. 3C). Closure of the fistula was associated with significant declines in plasma AVP after 3 and 6 days (20.3±7.1 and 20.0±7.0 pg/ml, respectively).

### 3.6. Effects of fistula closure on blood pressure and cardiac hypertrophy

As shown in Fig. 4A, the placement of a chronic large A–V fistula in rats resulted in expected reduction in mean arterial blood pressure from 127±3 to 75±2 mmHg (*P*<0.01). The hypotensive effect of the placement of an A–V fistula, was associated with cardiac hypertrophy as expressed by the remarkable increase in heart-to-body weight ratio from 0.31±0.009% in sham controls to 0.54±0.029% in rats with decompensated CHF (*P*<0.01) (Fig. 4B). The reduced blood pressure as well as the increased heart

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**Table 1**

<table>
<thead>
<tr>
<th>Animal group</th>
<th>ANP (pg/ml)</th>
<th>PRA (ng Ang I/ml/h)</th>
<th>AVP (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
<th>Norepinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated rats</td>
<td>94±12</td>
<td>22.1±2.0</td>
<td>14.2±3.6</td>
<td>667.5±175.9</td>
<td>184.2±40.5</td>
</tr>
<tr>
<td>Rats with A–V fistula and compensated CHF</td>
<td>382±115*</td>
<td>31.2±20.2</td>
<td>36.1±13.8*</td>
<td>449.0±339.0</td>
<td>481.0±20.0*</td>
</tr>
<tr>
<td>Rats with A–V fistula and decompensated CHF</td>
<td>389±135*</td>
<td>47.0±14.0*</td>
<td>37.7±9.6*</td>
<td>2049.8±496.9*</td>
<td>1112.6±293.2*</td>
</tr>
</tbody>
</table>

* *P*<0.05 vs. sham-operated rats. Values are means±S.E.M.
weight returned toward control values 1 week after the closure of the fistula.

3.7. Immunoreactive levels of renal eNOS before and after closure

In line with our previous report [21], a protein fraction of 140 kDa was clearly recognized in the renal medulla and, to a lesser extent, in the renal cortex, after the incubation of cortical and medullary homogenates from kidneys of sham controls and decompensated CHF, before and after 3 or 6 days of closure, with anti-eNOS antibodies (Fig. 5). The immunoreactive levels of eNOS increased 3-fold in the medullary tissue, but not in the cortex, of rats with decompensated CHF. The elevated levels of medullary immunoreactive eNOS gradually and significantly declined 3 and 6 days after the surgical closure of the A–V fistula, suggesting that the placement of large fistula enhanced the expression of eNOS in the medullary region, whereas surgical closure eliminated this increase.

4. Discussion

The present study provides important insight into the mechanism of sodium and water retention in CHF. As we have previously shown, rats with aortocaval fistula display...
two distinct patterns of urinary sodium excretion: some animals show progressive renal sodium retention, whereas others compensate and return to normal sodium balance [10,11]. Although plasma ANP levels are similarly elevated in both subgroups, only rats with decompensated CHF showed remarkable activation of neurohormonal systems such as PRA and catecholamines [10,11]. Due to the antinatriuretic activity of these systems, their activation may overcome the diuretic and natriuretic effects of ANP in rats with sodium retention. Indeed, we found that closure of the A–V fistula resulted in a remarkable natriuresis that was associated with a significant decline in the plasma levels of sodium-retainng systems within 3 days, whereas plasma concentrations of ANP remained elevated for several days after the surgical closure of the fistula. Only after 6 days and the end of the natriuretic phase did the plasma levels return to normal. Taken together, these data suggest that the balance between the above-mentioned opposing hormonal systems is a major determinant of sodium balance in this experimental model of heart failure. Consistent with our earlier report [21], the immunoreactive levels of medullary eNOS, an important endogenous vasodilator that plays a crucial role in the regulation of medullary blood flow and sodium excretion, were elevated in rats with decompensated CHF. The closure of the fistula was accompanied by a significant and gradual decline in the medullary eNOS after 3 and 6 days. This finding suggests that eNOS may compose a major homeostatic mechanism in the maintenance of medullary blood supply, particularly under pathophysiological conditions characterized by renal hypoperfusion such as CHF.

In agreement with our previous reports [10,11], rats with A–V fistula displayed high levels of ANP in their plasma, similar to those reported in other forms of clinical CHF [32,33]. ANP is released into the circulation in response to volume load and atrial distention [34,35]. For instance, large A–V fistulas in humans increase venous return, preload, and cardiac output and therefore provoke enhanced secretion of ANP, mainly from the right atrium [28]. However, a slow decrease in plasma ANP levels occurred only on day 6 following surgical closure of the A–V fistula, most likely due to reduced venous return and to regression of the cardiac hypertrophy. A similar slow decline in plasma ANP concentrations was also observed after the closure of a large fistula in two patients with CHF [28]. These findings are in contrast with the reported immediate decrease in ANP levels after repair of mitral stenosis [36]. We observed that circulatory levels of ANP persistently remained elevated even 3 days after the closure, and the natriuretic phase that occurred after closure of the A–V fistula may be attributed to those levels. Numerous studies have established the role of ANP in the regulation of fluid and sodium balance [34,35]. Besides its powerful natriuretic activity, ANP also inhibits the activity of the renin–angiotensin–aldosterone system and other vasoconstrictor systems such as endothelin and AVP [34,35,37]. Therefore, the return to normal sodium balance, which was observed after closure, required not only elevated circulating levels of immunoreactive ANP, but a progressive fall in PRA [11].

Several reports have indicated that activation of PRA is involved in deranged renal function and augmented tubular sodium retention in heart failure [1,10,19,37]. We have used rats with CHF [10–12,24], and others have utilized dogs with the same experimental model [15], to demonstrate that the exaggerated sodium retention in this model is largely dependent on the increased activity of renin. Angiotensin II contributes significantly to the decrease in renal perfusion and the diminished glomerular filtration rate through its vasoconstrictor effect on the efferent and afferent arterioles as well as by promoting mesangial contraction [1,19]. In addition, angiotensin II and aldosterone have a direct stimulatory effect on tubular sodium reabsorption [1,18]. Of interest was the current observation that natriuretic response to closure on days 2–3 was significantly elevated over normal basal sodium excretion and returned to basal excretion rates afterward. This pattern of sodium excretion may be attributed to the elevated levels of ANP that overcame the antinatriuretic effects of angiotensin II and aldosterone, leading to enhanced sodium excretion in the first few days after closure to get rid of the positive sodium balance.

Previously, we demonstrated that the natriuretic response to exogenous ANP administration was attenuated in rats with A–V fistula compared with controls, although rats with compensated CHF responded more favorably than the decompensated group [10,11,24,30]. Interestingly, plasma levels of ANP were similarly elevated in both subgroups, indicating that the differences in sodium excretion in the two subgroups was not related to alterations in ANP secretion, but more likely to a change in renal sensitivity to ANP [10,11]. We later demonstrated that PRA played a major role in the retention of water and sodium in rats with decompensated CHF [11,12,24]. Support for this notion was derived from our findings that removal of the influence of PRA — by pharmacological inhibition of angiotensin-converting enzyme [11] or with specific angiotensin AT-1 receptor antagonists such as losartan or eprosartan [3,12] — significantly improved the natriuretic response to exogenous ANP. Furthermore, these blockers also restored daily sodium excretion to normal in rats with decompensated CHF, suggesting that the response to the high endogenous levels of the hormone was improved as well [3,10,11,24]. Similarly, in a model of ovine heart failure, acute administration of losartan was able to maintain urinary sodium excretion despite a fall in renal perfusion pressure [38]. Likewise, in dogs with CHF due to rapid atrial pacing, chronic administration of TVC-116, an angiotensin AT-1 antagonist, prevented sodium retention [39]. Taken together with the previous data, the present findings suggest that, analogous to pharmacological intervention, suppression of PRA following closure is
crucial for the expression of the natriuretic and diuretic actions of ANP.

An additional factor that may be involved in the development of positive salt and water balance and edema formation is AVP [17,18]. The present study demonstrated that rats with A-V fistula had significantly higher levels of AVP compared with controls. Increased plasma levels of AVP have been demonstrated in humans with CHF and in some experimental models of this disease [1,18]. Elevated concentrations of AVP cause a decrease in free water clearance [18]. In a study by Mulnari et al. [40], administration of AVP antagonist to rats with ischemic CHF induced by left coronary ligation resulted in a rise in urine output of 4–10-fold over baseline, confirming the role of AVP in water retention. Administration of selective AVP-V2 receptor antagonists of peptidic and non-peptidic nature to rats with inferior vena cava constriction, to dogs with CHF induced by rapid pacing, and to rats with CHF induced by coronary ligation resulted in correction of the impaired urinary dilution in response to acute water load [18]. Our finding that A-V fistula closure was associated with reduction of AVP levels and exaggerated diuretic response support the notion that activation of the AVP system contributes to water retention and subsequently to edema formation.

An additional hallmark of rats with decompensated CHF is the higher levels of catecholamines in their circulation. Increased activity of the sympathetic nervous system is one of the earliest and most consistent findings in CHF [6–8]. Studies using the renal norepinephrine spillover technique demonstrated that the basal sympathetic outflow to the kidney is significantly increased in patients with CHF [41]. Moreover, elevated plasma norepinephrine levels are frequently observed and have been thought to be a prognostic marker in patients with CHF [1]. In light of the sodium-retaining force of the sympathetic nervous system, activation of this system may contribute to sodium retention and to the attenuated renal responsiveness to ANP. In line with this concept, in rats with experimental heart failure due to coronary artery ligation, renal denervation resulted in improvement in renal function [9]. Similarly, in dogs with low cardiac output induced by vena-cava constriction, administration of a ganglionic blocker resulted in a marked increase in sodium excretion [42]. Likewise, the blunted diuretic/natriuretic actions of ANP in rats with CHF were restored either by prior renal denervation [6] or by administration of clonidine [18,42]. Administration of dibenamine (an α-adrenoceptor blocker) to patients with CHF [43] or of metoprolol (a mixed α- and β-adrenoceptor blocker) to animals with experimental CHF [44] caused an increase in sodium excretion. However, other studies failed to show an ameliorative effect of renal denervation on renal hemodynamics and sodium excretion in CHF. In a study by Mizelle et al. [45] there were no differences in renal hemodynamics or electrolyte excretion between innervated and denervated kidneys following chronic unilateral denervation, in conscious dogs with CHF induced by rapid ventricular pacing. Similarly, in dogs with reduced cardiac output due to pulmonary constriction there were no significant differences in renal hemodynamics or sodium excretion between the denervated and intact kidney [46]. These discrepant results are probably due to species differences, the presence or absence of anesthesia, and the method of inducing heart failure. Taken together, it is widely accepted that activation of the sympathetic nervous system in CHF contributes significantly to the associated abnormalities of renal function, which, in turn, lead to sodium retention and edema formation.

Finally, of interest was the observation that decompensated CHF was accompanied by increased immunoreactivity levels of eNOS in the renal medulla. Our data demonstrated that surgical closure of the A-V fistula reduced the medullary eNOS immunoreactivity to basal levels. We previously showed that, in contrast to the cortex, the renal medulla contains high levels of eNOS under normal conditions, where it plays an important role in maintaining the limited blood supply to the medulla [21]. Among its various features, CHF is characterized by a marked decrease in renal blood flow [1,4]. However, analysis of intrarenal blood flow measurements in rats with CHF revealed that while cortical blood flow was markedly diminished, medullary blood flow appeared to be preserved [21–23]. Due to its vasodilatory properties, it is has been speculated that the overexpression of eNOS in the renal medulla may play an important role in the maintenance of medullary blood flow under CHF condition [21]. This finding also explains the exquisite sensitivity of the medullary circulation to blockade of NOS by L-NAME, in doses which do not cause any measurable decrease in cortical blood flow [21,26]. The renal tissue contains additional isoform of NOS, namely neuronal NOS, which is present in the macula densa and plays an important role in the juxtaglomerular apparatus-mediated tubuloglomerular feedback response [47]. Since, the immunoreactive levels of neuronal NOS in the renal tissue were not determined before and after the A-V fistula closure in the current study, we could not exclude a potential role of this isoform of NOS in the regulation of the renal hemodynamics and excretory function of the kidney in our model.

Our finding that the closure of the A-V fistula was associated with a remarkable increase in blood pressure is in agreement with those of Humphreys et al. [27], who demonstrated that closure of fistula in dogs is accompanied by circulatory adjustments such as decreased cardiac output and heart rate and increase in blood pressure. Most likely, restoration of blood pressure to normal levels is responsible for increasing glomerular filtration rate and sodium excretion. Improving the renal perfusion that presumably took place on closure of the fistula, in association with the decline in the circulating vasoconstrictors,
protected the medullary circulation from deterioration and allowed the eNOS immunoreactivity in this tissue to return to normal levels. Moreover, the regression of the cardiac hypertrophy following the closure of the A–V fistula, which was also reported by Martin-Gerdes et al. [48] in similar model, may improve the cardiac output and subsequently the renal function. The reduction in heart weight following the closure could be attributed to the decline in the levels of antinatriuretic vasoconstrictors which are known as growth promoting agents.

In summary, the present study has demonstrated that closure of large A–V fistula is associated with remarkable diuretic and natriuretic responses that are associated with elevated levels of plasma ANP and declines in PRA, AVP, and the medullary NO system. These findings are consistent with the concept that the degree of sodium retention in rats with experimental CHF is largely determined by the balance between these two antagonistic hormonal systems. In addition, these results support the beneficial role of ANP in the ability of rats with heart failure to compensate and return to normal sodium balance as soon as the antinatriuretic effects of RAS, AVP, and catecholamines are eliminated. By analogy, it is assumable that the elevated plasma levels of ANP in CHF patients may play a role in the recovery from the edema and positive sodium balance by facilitating diuresis and natriuresis after the primary cause of CHF is removed.

References


