Angiotensin inhibition and atrial natriuretic peptide release after acute volume expansion in rats with aortocaval shunt

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Abstract

Objective: In heart failure atrial natriuretic peptide (ANP) release in response to volume expansion is impaired while the renin–angiotensin system is activated. This study was designed to test the hypothesis that ANP release in heart failure is dependent on an activated angiotensin system. Methods: We studied the ANP and renin–angiotensin systems in a rat model of shunt-induced high-output heart failure, in which we rapidly increased circulating fluid volume with a 5 ml hyperoncotic infusion, and evaluated the effects of acute inhibition of the angiotensin converting enzyme as well as of the blockade of angiotensin II type 1 receptors on the ANP release and on renal excretory function. Results: ANP and angiotensin II plasma concentrations prior to volume expansion were elevated ($p < 0.05$) in rats with aortocaval shunt compared to controls. The diuretic response to acute volume expansion (18.5 ± 1.5 vs. 48.2 ± 2.4 μl/min, $p < 0.001$) was markedly blunted. ANP release was attenuated in rats with aortocaval shunt, as was the increase of its second messenger cGMP in plasma and urine. The blunted increase in ANP plasma levels was not due to depleted cardiac stores as cardiac ANP content, as well as ANP synthesis, were increased ($p < 0.05$). Acute inhibition of the angiotensin converting enzyme as well as blockade of the angiotensin II type 1 receptors restored ANP release in response to volume expansion ($p < 0.01$). Moreover, acute inhibition of the renin–angiotensin system completely normalized the diuretic response. Conclusions: Our data suggest that the ANP system is impaired in rats with aortocaval shunt. The activation of the angiotensin system contributes to the impairment of the ANP system. Acute inhibition of the angiotensin II system significantly improved the ability of the ANP system to respond to acute volume expansion. Our findings indicate a hitherto fore unappreciated interaction between both systems and suggest additional mechanisms for the beneficial effects of angiotensin converting enzyme inhibition or angiotensin II type 1 receptor antagonists in heart failure. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Renin–angiotensin system; Natriuretic peptide; Hormones; Heart failure; Experimental; Regulatory systems; Organism; Pathophysiology

1. Introduction

With acute volume expansion, atrial natriuretic peptide (ANP) release normally evokes a natriuretic and diuretic response, as well as a reduced intravascular volume, an increased capillary permeability and a diminished vascular tone (for review see [1–5]). However, in heart failure ANP responses and actions are sharply curtailed. For instance, in dilated cardiomyopathy, the ANP increase with sudden volume expansion was diminished [6] or absent [7]. In heart failure, volume and sodium retention occurred in spite of an activated ANP system. The cardiac production of ANP is increased in heart failure particularly in the ventricles, which results in elevated plasma levels [8–10].
Nevertheless, despite markedly elevated ANP plasma levels, volume and electrolyte homeostasis in heart failure is impaired.

The biological action of ANP is mainly mediated by its second messenger, cyclic guanosine monophosphate (cGMP) [11,12], which is formed by the particulate guanylate cyclase of the natriuretic peptide receptors type A and B [13,14]. Stimulation of the guanylate cyclase with ANP results in elevated cGMP levels in plasma and increased urinary cGMP excretion [15]. The cGMP excretion correlates well with the ANP-induced natriuresis and diuresis. We showed earlier that a dissociation of natriuresis, diuresis, and cGMP excretion could only be achieved with an ANP analog [16].

In heart failure, other regulatory systems, notably the renin–angiotensin system, are activated simultaneously. It has been shown previously by several groups that ANP inhibits the renin–angiotensin–aldosterone system by inhibiting renin release [17] and decreasing aldosterone production [18]. This ANP-mediated effect might be of particular importance in heart failure [19]. However, the angiotensin (Ang) II system remains activated in heart failure in spite of elevated ANP plasma levels which might be an indication for the decreased efficacy of natriuretic peptides in heart failure. We investigated whether or not Ang II modulates the release of ANP and influences its ability to induce diuretic responses in heart failure. To induce heart failure, we relied on the aortocaval shunt, an established model of moderate high-output heart failure [20–22]. To achieve more insight into mechanisms, we analyzed the atrial and ventricular ANP-mRNA expression, the cardiac ANP protein content, the ANP release after an acute volume expansion and the renal responses after inhibition of the renin–angiotensin system with either an angiotensin converting enzyme inhibitor (ACEI) or an angiotensin II receptor type 1 (AT1) antagonist.

2. Methods

2.1. Animals

Male Wistar rats (230–250 g) from Moellegaard Animal Farms (Schoenwalde, Germany) were fed normal rat chow and allowed free access to tap water. The animals were kept on a 12-h light–dark cycle. All experiments were performed between 7 and 12 a.m. The studies were approved and performed according to Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). A first set of experiments was performed with 8–12 animals per group (12 controls, 8–9 in each group of rats with aortocaval shunt) for volume expansion, hemodynamic and renal measurements and angiotensin inhibition. A second set of experiments was performed with 9 animals per group for the measurements of ANP tissue content and mRNA-expression.

2.2. Shunt Operation

The aortocaval shunt was induced under ether anesthesia by a modified method developed by Garcia [23]. Briefly, a laparotomy was performed and the aorta was punctured with a 1.2 mm disposable needle (external diameter, Braun Melsungen, Melsungen, Germany) distal to the renal arteries. The needle was advanced into the adjacent inferior vena cava. After temporarily clamping the vessels, the needle was withdrawn and the aortic puncture site was sealed with a drop of cyanoacrylate glue (Instant Krazy Glue, Borden Company, Willowdale, Ontario, Canada). Perioperative mortality was less than 3%. Sham-operated control animals were treated identically, except that no puncture of the vessels was performed.

2.3. Acute volume expansion

An acute volume expansion was performed in anesthetized rats (chloralhydrate 400 mg/kg) 30 days after shunt production. For measurements of diuresis and cGMP-excretion, a PE-50 polyethylene catheter was inserted into the bladder and urine was collected in 20 min periods. Sodium chloride (0.9%) was infused at a flow rate of 1.5 ml/h throughout the whole experiment. As illustrated in Fig. 1, surgery was followed by a 20 min equilibration period before baseline values were obtained during the following 20 min. Acute volume expansion was then performed with 5 ml of hyperoncotic solution (HAES 10%, Braun Melsungen, Melsungen, Germany) infused within 5 min. Urine for this period (t1) was collected during the 5 min of infusion of hyperoncotic solution and during the following 15 min. The experiment was continued for two additional collection periods of 20 min each (t2 and t3). Urine volume was collected for the entire 20 min periods for measurements of diuresis and cGMP excretion. Hemodynamic values were analyzed at the end of each period and in addition at the end of the acute volume expansion.

2.4. Determination of C- and N-terminal ANP, cGMP, and Angiotensin II (Ang II)

Blood samples for ANP (800 μl) were withdrawn from the carotid artery at the end of each observation period in Na-EDTA preloaded (final concentration 7 mM) and prechilled tubes. Degradation of ANP was prevented with phenyl-methyl-sulfonyl fluoride (final concentration 10 μM) and pepstatin (3 μM). The blood was centrifuged at 4°C at 2000 g for 10 min immediately after withdrawal and the plasma was kept at ~80°C until extraction. The blood was replaced with the same amount of blood from donor animals (shunt- or sham-operated rats, respectively).
C-terminal ANP plasma samples were extracted with C18 Sep-Pak columns, which had been equilibrated with acetonitrile and ammonium acetate (0.2%, pH 4.0). After loading of plasma, the columns were washed with ammonium acetate and ANP was eluted with acetonitrile (60%) and ammonium acetate (40%), following a previously described protocol [24]. Samples were then measured by radioimmunoassay [24] which was performed with antibodies kindly provided by Dr. J. Gutkowska, Montreal, Canada. Cardiac concentrations of ANP were diluted and directly determined by RIA in the supernatants which were kept at -80°C after RNA extraction with the lithium chloride method [25]. Plasma samples for the N-terminal ANP (1–98) were directly determined by RIA [26]. Prior to determination by radioimmunoassay, plasma cGMP was extracted with alumina (AG7) and Dowex (AG50W-X8) columns, while urinary cGMP was determined directly using a specific radioimmunoassay [27]. Antibodies were donated by Dr. P. Hamet, Montreal, Canada. For Ang II measurements, blood was collected in prechilled Na-EDTA tubes (final concentration 7 mM) at baseline. O-phenanthrolin (Merck, Darmstadt, Germany, final concentration 1.25 mM) was added and Ang II was measured by radioimmunoassay as described previously in detail [28,29]. Mean intra-assay variabilities were 11% and mean inter-assay variabilities were 15%. The cross-reactivity to Angiotensin I and other peptides was less than 0.01% [28]. Hematocrit, which might have changed after acute volume expansion, was not measured and the plasma hormone concentrations could thus have been underestimated.

2.5. Hemodynamic measurements

Hemodynamic studies were performed under chloral hydrate anesthesia (400 mg/kg) 30 days after shunt production. A PE-50 tubing catheter was inserted via the right jugular vein into the superior vena cava for measurements of central venous pressure. Arterial blood pressure was measured by cannulating the right carotid artery. Both pressures were registered at baseline, during and after volume expansion using a Statham P23XL transducer and a Gould AMP 4600 amplifier. Heart rate was derived from the arterial blood pressure signal.

2.6. Angiotensin converting enzyme inhibition (ACEI) and AT1-blockade

Thirty days after shunt induction, the angiotensin converting enzyme was acutely inhibited with ramiprilat given intravenously (50 μg/kg, dissolved in 150 μl of NaHCO₃ 0.83%) immediately before the beginning of the baseline period. As we aimed to analyze the effect of angiotensin inhibition without any decrease in blood pressure, the dose of 50 μg/kg has been chosen following several publications indicating that 1 mg/kg induced a sustained hypotensive effect [30] and 50 μg/kg did not induce any hemodynamic changes [31]. AT1 receptors were blocked with valsartan (0.3 mg/kg, dissolved in 150 μl of NaHCO₃ 0.83%) intravenously. This dose had previously been demonstrated to be without hypotensive effects [32]. Vehicle treated animals received the same amount of NaHCO₃ 0.83%.

2.7. RNA extraction and hybridization

In a second set of experiments, the hearts of sham-operated controls and rats with aortocaval shunt were excised 30 days after shunt production for RNA measurements. RNA was extracted following the lithium-chloride method [25]. The supernatants were kept at -80°C for measurements of ANP tissue concentrations. For northern blotting, 2 μg (atria) and 15 μg (ventricles) of spectrophotometrically determined total RNA was used. Denaturation was performed with glyoxal buffer (1 M glyoxal, 50% dimethylsulphoxide, 10 mM sodium dihydrogenphosphate, pH 7.0). The RNA was electrophoresed through a denaturing 1.2% agarose gel (4 h, 100 V) and transferred to a 13 N Nylon membrane. An ANP specific cRNA probe was prepared from the prepro-ANP cDNA fragment which
had been provided by Bloch and Seidman (Boston, MA, USA) and was cloned in a pSP64 plasmid. ANP-mRNA expression was compared to β-actin-mRNA expression, which was not different in the samples of rats with aorto caval shunt and sham-operated controls. Hybridization was performed with radioactive labeled 32P-UTP probes (100 μCi) in 50% formamide, 50 mM sodium phosphate (pH 6.8), 1 M NaCl, 200 μg/ml salmon sperm DNA, 5x Denhardt’s solution, 0.1% SDS, and 10 mM EDTA for 18 h at 65°C. Filters were washed twice for 5 min with 1x SSC, 0.1% SDS at 65°C and once with 0.1x SSC and 0.1% SDS for 15 min at 65°C. The filters were exposed for 24 h at −70°C for autoradiography and quantified with a laser densitometer.

2.8. Statistical analysis

Differences between groups were evaluated with the unpaired Student’s t-test and the Wilcoxon rank sum test where appropriate. The responses to acute volume expansion were compared using two-way analysis of variance with a posteriori comparison (Bonferroni). The significance level was set at p<0.05. All data are expressed as means±SE (standard error of the mean).

3. Results

3.1. Development of hypertrophy

After 30 days of aortocaval shunt, the absolute and relative heart weights had significantly increased (Table 1) compared to sham-operated control rats. All heart chambers contributed to this significant increase. We did not observe any signs of overt heart failure, like edema or ascites.

3.2. Hemodynamic measurements

Hemodynamic measurements before acute volume expansion (Table 2) showed no significant differences in heart rate or mean arterial blood pressure between rats with aorto caval shunt and control rats. The right atrial pressure was significantly higher in rats with aorto caval shunt (5.9±2.2 mmHg) compared to control rats (3.0±0.6 mmHg, p<0.05). Treatment with an ACEI or AT1-antagonist did not influence baseline hemodynamic variables. Acute volume expansion induced a significant increase in right atrial pressures in control rats as well as in rats with aorto caval shunt treated with vehicle, ACEI and AT1-antagonist. No significant changes in mean arterial blood pressure or heart rate occurred after acute volume expansion in any group.

3.3. Angiotensin II plasma levels

In rats with aorto caval shunt, Ang II plasma levels were significantly increased compared to sham-operated rats (360±82 vs. 146±17 pmol/l, p<0.05), indicating activation of the renin–angiotensin system. We had previously shown that plasma renin activity was increased in parallel to the aorto caval shunt model [33]. In the ACEI treated group, the Ang II plasma concentrations decreased to 116±26 pmol/l (p<0.01), whereas the AT1-antagonist

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Table 1

<table>
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<tr>
<td>LV/BW, mg/100 g</td>
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<td>256±29***</td>
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Values are means±SE, n=9 shunt rats, n=9 control rats. BW, body weight; BW/HW, relative heart weight; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; RV/BW, relative right ventricular weight; LV/BW, relative left ventricular weight. Experiments were performed 30 days after shunt induction.

**=p<0.01.
***=p<0.001 vs. control.

Table 2

<table>
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<tr>
<th></th>
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<th>Shunt/AT1</th>
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<td>Baseline/AT1</td>
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<td>444±23</td>
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</table>

Values are means±SE, n=12 control rats, n=8-9 rats with aorto caval shunt in each therapy group. ACEI, ACE-Inhibitor; AT1 Angiotensin II receptor type 1 antagonist; RAP, right atrial pressure; MBP mean blood pressure; HR, heart rate. Experiments were performed 30 days after shunt induction.

*=p<0.05 vs. control.
*=p<0.05 vs. baseline.
Increased Ang II plasma levels to 563±19 pmol/l (p<0.05), which is consistent with previous reports [34]. Acute volume expansion did not significantly change Ang II levels in any group.

3.4. ANP release, diuretic and cGMP response after acute volume expansion

Baseline plasma levels of C-terminal ANP were elevated about 8-fold in rats with aortocaval shunt compared to sham-operated controls (380±33 vs. 47±3 pmol/l, p<0.001, Fig. 2a). While C-terminal ANP levels in control animals increased almost four-fold in response to acute volume expansion (from 47±3 to 203±12 pmol/l, p<0.001), no significant increase could be observed in rats with aortocaval shunt. The lack of C-terminal ANP to respond to acute volume expansion in rats with aortocaval shunt was paralleled by unchanged N-terminal ANP plasma levels indicating a blunted cardiac release of the peptide (Fig. 2b). Measurements of plasma concentrations of C- and N-terminal ANP at the end of the following 20 min periods (t2, t3, data not shown) demonstrated that the maximal increase in ANP plasma concentrations occurred at the end of t1, excluding a delayed increase in ANP plasma concentrations.

The baseline plasma concentrations of cGMP, a marker of the ANP-activated guanylate cyclase, were higher in rats with aortocaval shunt than in control rats (Fig. 2c, p<0.001), but did not show any further increase after volume expansion (51±7 to 50±6 nmol/l vs. 12±2 to 39±5 nmol/l in control rats, p<0.001). Similarly, the increase in urinary cGMP excretion after acute volume expansion was significantly less (increase of 16.6±3.8 vs. 39.1±4.1 pmol/min, p<0.05, Fig. 3a) in rats with aortocaval shunt (from 26.8±3.5 to 43.4±3.1 pmol/min) compared to control rats (from 22.6±2.2 to 61.7±3.9 pmol/min). Baseline cGMP excretion (in contrast to urinary cGMP concentration) was not elevated in rats with aortocaval shunt, which might be due to the lower baseline diuresis observed in this model. The baseline diuresis in rats with aortocaval shunt was lower than in sham-operated control rats (Fig. 3b). The diuretic response to acute volume expansion was markedly diminished during all observation periods in rats with aortocaval shunt (p<0.01).

3.5. ANP synthesis and content in atria and ventricles

We analyzed ANP synthesis and tissue content to exclude depleted cardiac stores as a cause for the blunted release of ANP after acute volume expansion and determined the ANP-mRNA expression in atria and ventricles. β-actin-mRNA expression was analyzed to exclude a nonspecific increase of mRNA synthesis. Fig. 4 shows a typical Northern blot of ANP-mRNA and β-actin-mRNA expression in left ventricles of rats with aortocaval shunt and control rats. The ANP-mRNA to β-actin-mRNA ratio was significantly increased in rats with aortocaval shunt in both ventricles (Table 3). Measurements of cardiac ANP content indicated higher amounts in both atria and in the left ventricle. When expressed as ANP concentrations per tissue weight, no significant changes between rats with aortocaval shunt and control rats were found. This apparent difference between ANP content and ANP concentration is due to an increased cardiac weight in rats with aortocaval
3.6. Effect of ACEI and AT1 blockade on ANP release and cGMP plasma levels

Having shown that the renin–angiotensin system was activated in rats with aortocaval shunt, we then analyzed whether its inhibition may be able to improve the blunted ANP release after acute volume expansion. Compared to vehicle treated rats, C-terminal ANP plasma levels were lower in rats treated with ACEI (Fig. 2a, 206 ± 36 vs. 379 ± 33 pmol/l with vehicle, $p < 0.01$). After acute volume expansion, C-terminal ANP plasma levels increased significantly in rats treated with ACEI. The increase in ANP plasma concentrations after acute volume expansion, which was blunted in vehicle treated rats with aortocaval shunt, was completely restored with ACEI, compared to sham-operated rats. Similarly, the AT1-antagonist normalized the ANP release after volume expansion (Fig. 2a). The increase in C-terminal ANP with ACEI was paralleled by a similar increase in N-terminal ANP levels (Fig. 2b). These data indicate that a facilitated release, rather than a diminished degradation, was responsible for the rise in ANP plasma levels after acute volume expansion. While plasma cGMP after acute volume expansion did not change in vehicle treated rats with aortocaval shunt, ACEI as well as AT1 blockade induced a significant increase in plasma cGMP (Fig. 2c) after acute volume expansion. Thus, inhibition of the renin–angiotensin system by either ACEI or AT1-antagonists restored the blunted ANP release and cGMP response after acute volume expansion in rats with aortocaval shunt.

3.7. Relationship between increased ANP and changes in right atrial pressures

The increases in right atrial pressures after acute volume expansion and the corresponding release of N-terminal ANP are shown in Fig. 5. The ANP release per increase in right atrial pressure was blunted as indicated by an almost horizontal slope in the vehicle treated rats with aortocaval shunt. Acute ACEI or AT1 blockade restored the ANP release per increase in right atrial pressure after volume expansion resulting in a slope steeper than in vehicle

![Fig. 3. The effects of ACEI on cGMP excretion (a) and diuresis (b) are shown before (baseline, b) and after acute volume expansion (t1–t3) in control rats and in rats with aortocaval shunt treated with either vehicle, ACEI or AT1-antagonist. Data are expressed as excretion per min and t1–t3 is the mean of the three periods t1, t2 and t3. The blunted renal responses to acute volume expansion in vehicle treated rats with aortocaval shunt were restored by ACEI and AT1-antagonist. Data are presented as mean±SE, n=8–12 in each group, * =p<0.05, **=p<0.01 and ***=p<0.001 (vs. vehicle treated rats with aortocaval shunt).](https://academic.oup.com/cardiovascres/article-abstract/42/3/733/283399)

![Fig. 4. The ANP-mRNA and β-actin-mRNA expression in the left ventricle are shown 30 days after shunt induction for four rats with aortocaval shunt (left) and 4 control rats (right). St.=internal atrial standard. While the β-actin-mRNA band was not different in both groups, the ANP-mRNA expression was increased in rats with aortocaval shunt.](https://academic.oup.com/cardiovascres/article-abstract/42/3/733/283399)
Table 3
ANP content, concentration and ANP-mRNA in the heart

<table>
<thead>
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<th>ANP content</th>
<th>ANP concentration</th>
<th>ANP-mRNA</th>
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<td></td>
<td>Control</td>
<td>Shunt</td>
<td>Control</td>
</tr>
<tr>
<td>RA</td>
<td>8.4±0.7</td>
<td>13.9±1.4*</td>
<td>282.0±20.0</td>
</tr>
<tr>
<td></td>
<td>4.6±0.6</td>
<td>7.8±1.0*</td>
<td>154.0±23.0</td>
</tr>
<tr>
<td>RV</td>
<td>2.1±0.2</td>
<td>2.0±0.2</td>
<td>13.1±1.8</td>
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<tr>
<td>LV</td>
<td>0.4±0.1</td>
<td>0.7±0.1*</td>
<td>0.62±0.2</td>
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Values are means±SE, n=9 shunt rats, n=9 control rats. Values are ANP contents per μg per heart chamber and ANP concentrations in μg/g tissue; ANP-mRNA is normalized as ratio of ANP-mRNA/Actin-mRNA. RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle. Hearts were excised and measured 30 days after shunt induction.

3.8. Effect of ACEI and AT1 blockade on renal responses

Fig. 3a shows the excretion of the second messenger cGMP at baseline and after acute volume expansion compared to vehicle treated rats. Again, the blunted increase in cGMP excretion in rats with aortocaval shunt was restored by either inhibition of ACE or AT1 blockade. As shown in Fig. 3b, the diuresis at baseline more than doubled (p<0.001), compared to vehicle treated rats with aortocaval shunt. In parallel to the enhanced cGMP excretion after acute volume expansion, the diuretic response was significantly improved by ACEI (p<0.001) and was not statistically different from the diuretic response measured in sham-operated animals. Similarly, the diuretic response after acute volume expansion could be improved with the AT1-antagonist.

4. Discussion

Our major new observation is the effect of ACEI and AT1 blockade on ANP release after acute volume expansion. The renin–angiotensin system is activated in heart failure, as reflected by increased Ang II levels in our study. Aldosterone production as well as renin release are known to be inhibited by ANP [17,18]. Acute ACEI or AT1 blockade led to lower basal ANP plasma levels, which is consistent with previous studies [35–37]. The dosages of ACEI or AT1-antagonist used in our study did not induce any hemodynamic changes. The blunted C-terminal ANP increase after acute volume expansion was restored with ACEI. The parallel increase in N-terminal ANP plasma levels, as a more specific marker for endogenous ANP secretion [38], supports the notion of a facilitated ANP release rather than an altered degradation.

We showed that the diuretic response to acute volume expansion was blunted in rats with aortocaval shunt, an established model of moderate high-output heart failure [20–22]. The blunted diuretic response to acute volume expansion was at least partly due to a diminished ANP release. The atria are the most important source for ANP release in response to acute volume expansion. We found that atrial ANP-mRNA expression was not decreased. In both ventricles, the ANP-mRNA was significantly increased. We and others have described that atrial and ventricular ANP synthesis was markedly increased in heart failure rats and in the failing human heart [8–10,40]. The ANP, which is released after acute volume expansion, originates mainly from the atria [39,41]. ANP contents were elevated in both atria and in the left ventricle of rats with aortocaval shunt compared to sham-operated controls. In the aortocaval shunt model,
atrial ANP was reported to show a tendency towards higher levels and ventricular ANP increased [42], which is consistent with our observations. Our findings therefore suggest that depleted cardiac and atrial stores were not responsible for the attenuated ANP release after acute volume expansion. ANP release in response to volume expansion depends on the atrial pressure and wall stress [1,43]. We observed a significant increase in right atrial pressures in rats with aortocaval shunt after acute volume expansion, which was not different from the increase in control rats. Therefore, neither depleted cardiac ANP stores, nor a blunted elevation of atrial pressures could explain the attenuated ANP release.

ANP evokes the stimulation of the particulate guanylate cyclase and results in elevated cGMP levels in plasma and increased urinary cGMP excretion [11,12,15]. In the present study, the ANP release in response to acute volume expansion was followed by an increase in plasma and urinary cGMP in control rats. In rats with aortocaval shunt, however, the diuretic response to acute volume expansion was reduced to about one third of the control values. The ANP release was blunted and consequently, the increase in urinary cGMP excretion was diminished and plasma cGMP did not increase. The diminished release of ANP is therefore likely to be a cause for the blunted renal response to acute volume expansion.

The increase in ANP plasma levels after acute volume expansion was restored by acute inhibition of the renin–angiotensin system via ACEI or AT1 blockade indicating a novel interaction between these two humoral systems. The observation that ACEI restores the blunted ANP release in rats with aortocaval shunt appears to contradict earlier studies, which reported that the ANP release was stimulated by Ang II in neonatal cardiomyocytes [44]. In superfused isolated atria, the reports are contradictory [45,46]. In isolated perfused hearts, Ang II induced ANP release along with elevated ventricular pressures [47]. Since these studies were performed in isolated cells and organs, it may not be possible to transpose them to intact animals or humans. Several studies analyzed Ang II effects in vivo and described increased ANP plasma levels in rats [46,48] and men [49,50]. However, systolic blood pressure increased in parallel in these studies [46,48,49] and a similar ANP release was achieved with other pressor agents as well [50]. When the blood pressure effect of Ang II was blocked [51], or when a nonpressor dose of Ang II was infused [46], no effect on basal ANP plasma concentrations could be observed. These observations suggest that the effect of Ang II on the ANP release might have been obscured by its vasoconstricting and hypertensive action.

Previous authors had blocked the renin–angiotensin system with ACEI or AT1-antagonists and had failed to observe any effect on ANP release [52,53]. However, these studies were performed in conscious and healthy rats without any previous activation of the renin–angiotensin system. Our study was performed in anesthetized animals with chronic activation of the renin–angiotensin system. Thus, we cannot exclude the possibility that a further stimulated renin–angiotensin system might have contributed to the results obtained. Analysis of renin transgenic rats demonstrated a blunted ANP release in response to acute volume load [54], indicating that genetic modification of the renin–angiotensin system influences the ANP release.

These findings suggest that the effect of angiotensin, as well as of ACEI and AT1-antagonists, might be different in a state of a chronically activated angiotensin system. Consistent with this notion, ACEI restored the ANP response to acute volume expansion in patients with cardiomyopathy [7]. Although not investigated in this study, it is possible that various other mechanisms might be involved in the release of natriuretic peptides [55] and the relative contribution of endocrine (angiotensin, endothelin) and mechanical factors [55] may be different in the healthy state compared to heart failure. In particular, it is intriguing to speculate on the effect of ET-1 and endothelin receptor blockade on ANP release in the normal and heart failure animals.

We observed a restored ANP release under acute inhibition of the renin–angiotensin system. We did not investigate the mechanism by which Ang II modulates the ANP release. A possible direct interaction between Ang II and ANP release at the cellular level in the heart is intriguing. However, we did not demonstrate that the interaction between both humoral systems occurs directly on the cardiomyocytes.

To assess the biological importance of the restored ANP release, we measured diuresis and cGMP excretion during ACEI and AT1 blockade. We had previously shown that the basal diuresis and the diuretic response to acute volume load was diminished in rats with an aortocaval shunt, compared to control rats [33]. The basal diuresis was increased with ACEI, even though ANP values were lower compared to vehicle treated rats. The lack of statistical effect on baseline diuresis with the AT1-antagonist could be due to a dose, which might not have been equipotent to the ACEI. The ACEI effect may be explained by the contribution of bradykinin, which is known to increase after ACEI. In contrast to the baseline diuresis and natriuresis, the response to acute volume expansion was most likely ANP dependent. We observed that the restored ANP release resulted in improved renal excretory responses. The second messenger cGMP increased in plasma. Increased plasma cGMP concentrations have been reported to be induced by ANP via a stimulation of particulate guanylate cyclase [56], but not by nitric oxide-stimulated soluble guanylate cyclase. Furthermore, the renal excretion of cGMP was enhanced. These findings suggest that the restored renal excretory response to volume expansion is mediated at least in part by the reestablished ANP release. We previously demonstrated that inhibition of the neutral endopeptidase, the ANP
degrading enzyme, improved the renal response to acute volume load. It is therefore intriguing to speculate on a possible additional or potentiating effect of the combined inhibition of neutral endopeptidase and the angiotensin system. In summary, we showed that the blunted ANP release and the renal excretory responses were normalized by acute inhibition of the renin–angiotensin system. The most likely mechanism by which ACEI and the AT1-antagonist restored the renal responses to acute volume expansion was by facilitating release of ANP. Our data suggest an additional hitherto fore unappreciated beneficial effect of renin–angiotensin inhibition in heart failure. In addition to the well known effects on myocardial function and survival in heart failure, ACEI restores basal ANP and volume-mediated release to normal. These effects may be termed a ‘resetting’ of the ANP system.

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References