Enhanced Renal Vascular Responsiveness to Angiotensin II and Norepinephrine: A Unique Feature of Female Rats with Congestive Heart Failure

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Abstract
Background/Aims: We found recently that the aortocaval fistula (ACF)-induced heart failure (HF) results in higher mortality in female than in male rats. Possibly, the development of renal dysfunction in the females, unlike in males, is associated with altered renal vascular responsiveness to angiotensin II (ANG II). Methods: Five or 20 weeks after ACF creation (compensated and decompensated HF, respectively), we assessed renal blood flow (RBF) responses to intrarenal administration of ANG II, norepinephrine (NE), and acetylcholine (Ach) in female ACF and sham-operated rats. Results: In ACF females, ANG II decreased RBF more than in healthy animals, unlike with earlier published data in male ACF rats that responded similarly. Also, NE decreased RBF more in female ACF rats, whereas Ach increased RBF to the same extent in female ACF and sham-operated rats. RBF responses to intravenous administration of NE and Ach were almost identical in female and male ACF rats. Conclusion: Female ACF rats studied at the onset of HF decompensation reveal, in contrast to male rats, enhanced renal vascular responsiveness to both NE and ANG II. When associated with the demonstrated increased intrarenal ANG II and NE concentrations, such hyperresponsiveness might promote the development of renal dysfunction and accelerate HF decompensation.
Introduction

Heart failure (HF) affects 4% of the adult population in Europe, and without significant progress in the prevention and treatment of HF, a 50% yearly increase in the number of HF patients may be expected [1]. The prognosis of patients with HF is poor, despite important therapeutic advances; this is particularly true for patients with impaired renal function [2–4]. Therefore, it is generally agreed that new treatment strategies are needed. However, the prerequisite of any progress is a detailed understanding of the pathophysiology of the development of renal dysfunction in HF, which is still to come [3].

A decrease in renal blood flow (RBF) is a common finding in patients with HF. It is detected relatively early when the left ventricular function is only minimally impaired and the glomerular filtration rate remains normal. Therefore, decreased RBF is considered as the first cautionary sign of the development of serious renal dysfunction in HF [3, 5, 6]. However, the mechanism(s) responsible for the RBF decrease in HF is unknown. Considering the unsatisfactory results of the pharmacological blockade of the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) as renoprotective measures, it was concluded that even if circulating amounts of angiotensin II (ANG II) and norepinephrine (NE) are increased, decreased RBF is not a simple consequence of these changes [3, 5, 6]. It has been suggested that the culprit could be augmented intrarenal actions of ANG II and NE [7–16].

However, also this hypothesis can be put to doubt in the light by our recent study showing that in male rat model of HF induced by chronic volume overload due to creation of the aorto-caval fistula (ACF), the renal vascular responsiveness to ANG II was not enhanced, similarly in the compensated and in the decompensated phase of HF [14]. Nevertheless, in this context it is important to recognize that even though sex-related differences in the course of HF are known [17–21] and despite appropriate recommendations, experimental researchers commonly employed male animals only. This could generate incomplete if not misleading results, and, unfortunately, such reservation also pertains to the ACF-induced model of HF and our recent study [14, 21–23]. In addition, we found recently that the ACF-induced model of HF exhibits important sex-linked differences with respect to HF-related mortality and the response to pharmacological measures applied to slow down the progression of HF [24, 25].

Considering all that knowledge, we hypothesized that the development of renal dysfunction in the female animals with ACF-induced HF is associated with altered renal vascular responsiveness to ANG II. To test this hypothesis, we compared the renal vascular responsiveness to intrarenal administration of ANG II in female sham-operated (without ACF) and female ACF rats, in the phase of compensated and at the onset of decompensated HF. To find out if the suspected renal vascular hyperresponsiveness to ANG II in female ACF rats is specific or simply represents altered reactivity to diverse endogenous vasoconstrictors and vasodilators, RBF responses to NE and acetylcholine (Ach) were also examined. Moreover, to establish if possible sex-linked differences in the renal vascular responsiveness to vasoconstrictors and vasodilators are not related to a different degree of compensatory activation of intrarenal neurohormonal systems, kidney concentrations of NE, ANG II, and angiotensin-1-7 (ANG 1-7) were determined in female ACF and sham-operated rats and compared to responses previously described by us in male rats [14].

Methods

Animals, Ethical Approval

Female Hannover Sprague-Dawley (HanSD) rats were bred and housed at the Center of Experimental Medicine of Institute of Clinical and Experimental Medicine, which is accredited
by the Czech Association for Accreditation of Laboratory Animal Care. Rats were housed under standard conditions and had access to standard rat chow and water ad libitum. All animal experiments were approved by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine, Prague, in accordance with guidelines and practices established by the European Convention on Animal Protection and Guidelines on Research Animal Use.

**HF Model, Exclusion Criteria**

Nine weeks old female rats were anesthetized with an intraperitoneal injection of ketamine/midazolam mixture (Calypsol, Gedeon Richter, Hungary, 160 mg/kg and Dormicum, Roche, France, 160 mg/kg). HF due to volume overload was then induced by creating an ACF using a needle technique. This procedure is routinely performed at our laboratory and details of the technique were reported by us previously [26]. Sham-operated rats underwent an identical procedure but without creating ACF. If a technical error occurred during the ACF procedure or if a pulsatile flow in the inferior vena cava could not be confirmed, suggesting incomplete ACF creation, animals were consequently excluded from the study.

**Experimental Design**

Subsequently after either ACF induction or sham operation, all rats were divided into groups to study vascular responsiveness to vasoactive agents as follows ($n = 10$ in each group):

1. HanSD female rats 5 weeks after sham operation
2. HanSD female rats 5 weeks after ACF operation (i.e., the phase of compensated HF)
3. HanSD female rats 20 weeks after sham operation
4. HanSD female rats 20 weeks after ACF operation (i.e., the phase of decompensated HF)

This division into phases is based on previous studies where the onset of compensated and decompensated HF in this model was ascertained [7, 14, 26, 27]. Separate groups of rats ($n = 8$ in each group) were used to evaluate the degree of activation of the 2 axes of the RAS: the vasoconstrictor axis represented by ANG II and the vasodilator axis represented by ANG 1-7, along with determination of the degree of sympathorenal activation assessed by kidney concentrations of NE. We used identical study design as in our previous study to compare between male and female rats with ACF [14].

**Renal and Systemic Vascular Responsiveness to Vasoactive Agents**

Rats were anesthetized with thiopental sodium (80 mg/kg i.p.) and prepared for acute studies in which renal and systemic vascular responses to vasoactive agents were determined. We used the same experimental protocol as in our previous study [14]. Rats were allowed to recover after completion of surgery, and RBF responses to 2 intrarenal doses of ANG II (2 and 8 ng), NE (20 and 60 ng), and Ach (10 and 40 ng) were determined. In the same experimental animals, mean arterial pressure (MAP) and RBF responses to 2 intravenous doses of ANG II (20 and 40 ng), NE (100 and 200 ng), and Ach (50 and 200 ng) were determined.

**Kidney ANG II, ANG 1-7, and Noradrenaline Concentrations**

To determine kidney levels of ANG II, ANG 1-7, and noradrenaline, samples of renal tissue were obtained from decapitated animals. The samples were handled as described previously [14], and kidney ANG II levels were measured by competitive radioimmunoassay (RIA), using the commercially available RIA kit (ED29051, IBL Int., Hamburg, Germany), kidney ANG 1-7 levels were measured by competitive RIA using the custom-made RIA kit (BeckmanCoulter, Prague, Czech Republic), and noradrenaline levels were measured by a solid-phase enzyme-
linked immunosorbent assay, using the commercially available enzyme-linked immunosorbent assay kit (RES9395, IBL International, Hamburg, Germany). All methods are routinely employed in our laboratory [14, 28–31].

**Statistical Analysis**

All values are shown as means ± SEM. GraphPad Prism 7 software (GraphPad software, San Diego, CA, USA) was used to perform statistical analysis of the data. Statistical testing included one-way ANOVA or two-way ANOVA followed by Tukey’s post hoc test where appropriate. All results that exceeded 95% probability limits (a p value lower than 0.05) were considered to be statistically significant.

**Results**

Table 1 collects the organ weight parameters. As presented, female rats studied 5 and 20 weeks after induction of ACF displayed marked cardiac hypertrophy as apparent from the whole heart weight and increases in the left ventricle (LV; including the interventricular septum) and right ventricle (RV) weight compared with sham-operated rats. Moreover, ACF rats displayed marked lung congestion as seen from an increase of wet lung weight. In addition, the degree of RV hypertrophy was in ACF rats more pronounced than that of LV, as indicated by the increase in the ratio of RV to LV weight. These organ responses to the induction of ACF were principally identical to those observed by us recently in male ACF animals [14]. However, of special interest is the finding that female ACF rats studied 20 weeks after induction of ACF showed significantly greater bilateral cardiac hypertrophy when compared with male ACF at the same time point [male data taken from ref. 14]; this was seen for whole heart weight (+107 ± 4% vs. +85 ± 3%, p < 0.05), LV weight (+90 ± 3% vs. +66 ± 4%, p < 0.05), and RV weight (+147 ± 5% vs. +102 ± 4%, p < 0.05). RV hypertrophy was also more pronounced in female than in male ACF rats (again analyzed as the ratio of RV to the LV weight; +37 ± 2% vs. +22 ± 2%, p < 0.05), when compared with the values for sham-

### Table 1. Basal characteristics of body and organ weight and its individual structural components in female rats, determined 5 and 20 weeks after creation of the ACF or after sham operation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>sham-operated rats</th>
<th>ACF rats</th>
<th>sham-operated rats</th>
<th>ACF rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>5 weeks</td>
<td>270±4</td>
<td>281±5</td>
<td>20 weeks</td>
<td>316±6</td>
</tr>
<tr>
<td>HW, mg/body weight, g</td>
<td>5 weeks</td>
<td>3.23±0.12</td>
<td>5.09±0.21*</td>
<td>20 weeks</td>
<td>6.39±0.22**</td>
</tr>
<tr>
<td>LV weight, mg/body weight, g</td>
<td>5 weeks</td>
<td>2.32±0.08</td>
<td>3.43±0.12*</td>
<td>20 weeks</td>
<td>4.43±0.11**</td>
</tr>
<tr>
<td>RV weight, mg/body weight, g</td>
<td>5 weeks</td>
<td>0.66±0.07</td>
<td>1.12±0.10*</td>
<td>20 weeks</td>
<td>1.41±0.11**</td>
</tr>
<tr>
<td>RV weight/LV weight</td>
<td>5 weeks</td>
<td>0.28±0.007</td>
<td>0.32±0.006*</td>
<td>20 weeks</td>
<td>0.33±0.012**</td>
</tr>
<tr>
<td>Lung weight, mg/body weight, g</td>
<td>5 weeks</td>
<td>4.97±0.29</td>
<td>5.71±0.18*</td>
<td>20 weeks</td>
<td>5.56±0.21*</td>
</tr>
<tr>
<td>Liver weight, mg/body weight, g</td>
<td>5 weeks</td>
<td>31.59±2.05</td>
<td>31.45±2.15</td>
<td>20 weeks</td>
<td>29.96±1.99</td>
</tr>
<tr>
<td>Kidney weight, mg/body weight, g</td>
<td>5 weeks</td>
<td>3.63±0.38</td>
<td>3.46±0.36</td>
<td>20 weeks</td>
<td>3.22±0.29</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* p < 0.05 versus sham-operated rats in the same week.

# p < 0.05 ACF rats 20 weeks versus ACF rats 5 weeks.

ACF, aortocaval fistula; LV, left ventricle; HW, heart weight; RV, right ventricle.
operated female and male rats at the same time point (i.e., 20 weeks after sham operation). In contrast, our present findings show that female ACF rats studied 20 weeks after induction of ACF exhibited lesser lung congestion when compared with male ACF at the same time point (male data taken from ref. 14; $+14 \pm 1\%$ vs. $+23 \pm 1\%$, $p < 0.05$), again, the percent
increases are compared with the values for sham-operated female and male rats at the same time point (i.e., 20 weeks after sham operation).

Figure 1a shows that 5 and 20 weeks after induction of ACF, the kidney ANG II levels were significantly higher than that in sham-operated rats. Likewise, kidney ANG 1-7 levels in ACF rats were also significantly higher (Fig. 1b). As shown in Figure 1c, renal concentrations of NE in ACF rats, both 5 and 20 weeks after induction of ACF, were significantly higher than that in sham-operated rats. The renal concentrations of ANG II, ANG 1-7, and NE showed a similar pattern as that observed in male ACF and sham-operated rats [14].

Fig. 2. MAP (A), RBF (B), and RVR (C) in sham-operated female rats (open bars) and female rats with ACF (solid bars) studied 5 and 20 weeks after induction of ACF or sham operation. * p < 0.05 versus sham-operated rats at the same time point. MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance.
Fig. 3. Maximum change in RBF elicited by selective intrarenal bolus administration of ANG II (2 and 8 ng; A), norepinephrine (20 and 60 ng; B), and Ach (10 and 40 ng; C) in sham-operated female rats (open bars) and female rats with ACF (solid bars) studied 5 and 20 weeks after induction of ACF or sham operation. * p < 0.05 versus sham-operated rats at the same time point. ANG II, angiotensin II; RBF, renal blood flow; NE, norepinephrine; Ach, acetylcholine.
Figure 2 shows that there were no significant differences in MAP, RBF, and renal vascular resistance (RVR) between sham-operated female rats studied 5 and 20 weeks after sham operation. ACF induction caused significant decreases in MAP and RBF, similar after 5 and 20 weeks (Fig. 2b, c). RVR was in female ACF rats slightly, but significantly, elevated only when studied 5 weeks after ACF creation (Fig. 2c).

Figure 3a shows that intrarenal administration of 2 and 8 ng of ANG II produced dose-dependent decreases in RBF in female rats studied 5 and 20 weeks after sham operation and after the creation of ACF. However, both doses of ANG II caused greater RBF decreases in ACF rats as compared with sham-operated rats when tested 20 weeks after the creation of ACF (–60 ± 1% vs. –42 ± 2% and –90 ± 1% vs. –67 ± 2%, p < 0.05 in both cases). This finding is of special interest because in our recent study [14], we found similar decreases in RBF elicited by identical doses of ANG II in ACF and sham-operated male rats studied both 5 and 20 weeks after surgery.

Figure 3b shows that intrarenal administration of NE at the doses of 20 and 60 ng elicited dose-dependent RBF decreases in sham-operated as well as in ACF female rats. As with ANG II, both doses of NE caused greater RBF decreases in ACF as compared with sham-operated
rats when tested 20 weeks after the creation of ACF (−49 ± 2% vs. −29 ± 3% and −88 ± 2% vs. −64 ± 3%, \( p < 0.05 \) in both cases). Our present findings in female rats follow a similar pattern of changes as in male ACF and sham-operated rats [14].

Figure 3c shows that intrarenal administration of 10 and 40 ng of Ach elicited comparable increases in RBF in sham-operated and ACF female rats studied 5 and 20 weeks after sham operation and after the creation of ACF. We did not observe enhanced responses to Ach at 5 weeks after surgery as in ACF male rats; nevertheless, the results from female rats obtained 20 weeks after the operation are in line with the findings recently reported by us in male sham-operated and ACF rats [14].

Remarkably, selective intrarenal administration of ANG II, NE, and Ach did not change MAP in any experimental group (data not shown).

Figures 4–6 summarize MAP and RBF responses to intravenous bolus administration of ANG II, NE, and Ach. As shown in Figure 4, in female ACF rats both doses of ANG II elicited significantly smaller increases in MAP and decreases in RBF than in sham-operated rats studied 5 or 20 weeks after the operation.
Likewise, as shown in Figure 5, NE at the doses of 100 and 200 ng caused significantly smaller increases in MAP and smaller decreases in RBF in ACF rats than in their sham-operated counterparts, irrespective of the time of measurement.

As shown in Figure 6, Ach at the doses of 50 and 200 ng caused smaller MAP decreases and significantly smaller increases in RBF in ACF rats than in their sham-operated counterparts, as studied 5 or 20 weeks after induction of ACF or sham operation. * p < 0.05 versus sham-operated rats at the same time point. MAP, mean arterial pressure; RBF, renal blood flow; Ach, acetylcholine.

**Discussion**

The most important and novel finding of the present study is that during the decompensated phase of HF (i.e., 20 weeks after the creation of ACF), selective intrarenal administration of ANG II and NE to female ACF, at doses that do not alter MAP, elicited greater decreases in
RBF than in their sham-operated counterparts. On the other hand, RBF responses to intra-renal administration of Ach were similar in female ACF and sham-operated rats. Overall, the present data indicate that at the onset of the decompensated phase of HF, female ACF rats exhibit enhanced renal vasoconstrictor responses to ANG II and NE. These findings, particularly the exaggerated renal vascular responsiveness to ANG II, may support our hypothesis that such enhanced renal vascular responsiveness to endogenous vasoconstrictors contributes to the development of renal dysfunction in female ACF rats, and this in turn might accelerate the progression of HF decompensation toward the lethal end. However, as discussed later, we did not observe exaggerated responsiveness to vasoconstrictors with intravenous administration. The novel finding is that of exaggerated renal vascular responsiveness to intrarenally administered ANG II in female ACF at the onset of the decompensated phase of HF. However, we found recently that altered renal vascular responsiveness to ANG II is not responsible for the reduction of RBF in the male rats with ACF-induced HF [14]. Of particular interest is also our finding that female rats studied 20 weeks after induction of ACF (i.e., at the onset of the decompensated HF) displayed bilateral cardiac hypertrophy greater than that observed in male ACF rats but exhibited less lung congestion than did the male ACF rats [14]. Altogether, the present findings support our earlier suggestion that the lasting impairment of renal hemodynamics rather than progressing cardiac remodeling is the main determinant of the long-term survival rate in ACF-induced model of HF [9, 10].

Nevertheless, the mechanisms of the sex-related differences in the renal vascular responsiveness to ANG II and in the degree of heart hypertrophy, as observed in the volume-overload induced HF, remain unclear. Consideration of several potentially relevant issues might provide some insight into the background of these differences.

First, it is possible that the renal vascular responsiveness to vasoactive agents could depend on the degree of activation of intrarenal neurohormonal systems, different in ACF male and female rats. This notion could be derived from the knowledge that the ANG 1-7, the biologically active peptide of the vasodilatory/natriuretic axis of the RAS, exhibits important modulatory effects on the vasoconstrictor actions of ANGII[32–34]. Therefore, it is conceivable that the differences in intrarenal concentrations of ANG II, NE, and ANG 1-7 could be responsible, at least in part, for the different renal vascular responsiveness to ANG II in male and female ACF rats. However, our present findings show that intrarenal concentrations of those endogenous vasoactive agents are almost identical with those recently found in male ACF rats exposed to the same experimental protocol [14]. Therefore, it is unlikely that differential intrarenal compensatory activation of the said neurohormonal systems would be responsible for the sex-related differences in the intrarenal vascular reactivity. Nevertheless, we show that female ACF rats also display striking activation of the vasoconstrictor/sodium retaining axis of the RAS, along with activation of the sympathorenal axis and the vasodilatory/natriuretic axis of the RAS in the kidney, in agreement with previous findings in male ACF rats [7, 9, 10, 14, 35]. The present results offer probably the first evidence in support of the notion that, also in female ACF rats, HF is not simply a hemodynamic disorder but also a neurohormonal syndrome, with similar activation of the RAS and SNS as observed in male ACF rats.

Second, considering the important sex-related differences in the regulation of RAS and, in general, of blood pressure [34, 36–39], different renal vascular responsiveness to ANG II in male and female ACF rats could be related to the status of sex hormones. Previous studies revealed that the treatment with estrogens attenuated vascular responsiveness to ANG II [40, 41] and that, in general, estrogens exhibit protective action against the development of hypertension, especially its ANG II-induced form [36, 38, 42]. Therefore, one can assume that intact female ACF rats should exhibit attenuated renal vascular responsiveness to ANG II as compared with the male rats [14]. Moreover, we found previously that castration did not change the survival rate in male ACF HanSD rats but significantly
worsened it in female ACF HanSD rats [24]. This suggests that ovarian hormones display some protective actions in this model of HF. Thus, it seems unlikely that different sex hormonal environment is responsible for the sex-linked differences in renal vascular responsiveness to ANG II in ACF rats. One could even hypothesize that in the absence of protective actions of estrogens, the enhanced renal vascular responsiveness to ANG II in female ACF rats could be further exaggerated. It is evident that instead of all that speculation, comprehensive studies are needed in animals after gonadectomy, after gonadectomy with substitution of appropriate sex hormones, and gonadectomy with the administration of steroid hormones of the opposite sex. There is also a need for studies of post-menopausal females, without and with hormonal supplementation. Such animal groups should be exposed to the identical experimental protocol as employed in the present study. It is evident that the present results provide only a necessary basis for future comprehensive research.

The second set of findings relates to MAP and RBF responses to systemic (i.e., intravenous) administration of vasoactive agents. We found that female ACF rats exhibited attenuated total peripheral vascular responses to vasoactive agents (as seen from the changes in MAP) and attenuated renal vascular responses (as seen from the changes in RBF) as compared with sham-operated (i.e., healthy) rats. This is in direct contrast to the exaggerated responses to intrarenal administration of these vasoactive agents. The same discrepancy pattern was found in our recent study in male ACF rats [14], which further supports the view that for studies evaluating renal vascular responsiveness, the route of administration of vasoactive drugs is of critical importance. It is likely that MAP changes after intravenous administration mobilize compensatory mechanisms, such as activation of SNS followed by its effects on peripheral and RVR [43, 44], which confounds interpretation of the results. The discrepancy between intravenous versus intrarenal administration is especially evident in the case of ANG II. Intravenously administered ANG II resulted in attenuated RBF responses in ACF female rats when compared to sham-operated rats. However, when ANG II was administered intrarenally, it elicited comparable or even greater RBF decreases in ACF female rats than in their sham-operated counterparts. Multiple reasons can explain this discrepancy. Since MAP changes after a bolus of ANG II differ significantly between ACF and sham-operated rats, they elicit nonuniform effects of MAP alterations on RBF responses. Alternatively, unequal distribution of the bolus after intravenous administration might be responsible for this discrepancy. To summarize, considering the significant differences between the RBF responses to intrarenal and intravenous injections of vasoconstrictor agents [14, 45], the route of administration should always be considered in the interpretation of the results.

Conclusion

In conclusion, the present results show that female ACF HanSD rats studied at the onset of the decompensated phase of HF reveal, in contrast to male ACF HanSD rats, enhanced renal vascular responsiveness to intrarenal NE and ANG II. When associated with the demonstrated increased intrarenal NE and ANG II levels, the exaggerated renal vascular responsiveness to these vasoconstrictor agents may play an important role in the development of renal dysfunction and can accelerate the onset of HF decompensation. In general, these findings further support the notion that sex-linked differences have to be considered in the studies aimed at evaluation of the pathophysiology of HF and of the effectiveness of the therapeutic measures applied to slow it down.
Acknowledgments

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Disclosure Statement

The authors have no conflicts of interest to declare.

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