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Original Paper

The Role of Renal Vascular Reactivity in the Development of Renal Dysfunction in Compensated and Decompensated Congestive Heart Failure

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Key Words
Congestive heart failure • Renal blood flow • Aorto-caval fistula • Vascular reactivity • Renal dysfunction • Angiotensin II • Norepinephrine • Acetylcholine

Abstract
Background/Aims: Reduction of renal blood flow (RBF) is commonly thought to be a causative factor of renal dysfunction in congestive heart failure (CHF), but the exact mechanism of the renal hypoperfusion is not clear. Apart from the activation of neurohormonal systems controlling intrarenal vascular tone, the cause might be altered reactivity of the renal vasculature to endogenous vasoactive agents. ***Methods:*** To evaluate the role of this mechanism, we assessed by an ultrasonic transient-time flow probe maximum RBF responses to renal artery infusion of angiotensin II (ANG II), norepinephrine (NE) and acetylcholine (Ach) in healthy male rats and animals with compensated and decompensated CHF. CHF was induced by volume overload achieved by the creation of the aorto-caval fistula (ACF) in Hannover Sprague-Dawley rats. ***Results:*** Maximum responses in RBF to ANG II were similar in rats studied five weeks (compensated phase) and 20 weeks (decompensated phase) after ACF creation when compared to sham-operated rats. On the other hand, NE elicited larger maximum decreases in RBF in rats with CHF (five and 20 weeks post-ACF) than in sham-operated controls. We observed greater maximum vasodilatory responses to Ach only in rats with a compensated stage of CHF (five weeks post-ACF). ***Conclusion:*** Greater renal vasoconstrictor responsiveness to ANG II or reduced renal vasodilatation in response to Ach do not play a decisive role in the

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development of renal dysfunction in ACF rats with compensated and decompensated CHF. On the other hand, exaggerated renal vascular responsiveness to NE may be here a contributing causative factor, active in either CHF phase.

Introduction

Congestive heart failure (CHF) is a major public health problem with a worldwide incidence of 1 to 2%, which means that it affects more than 6 million Americans and 9 million Europeans. Notably, the yearly increase in the number of new patients is estimated at 50% [1-5]. Despite an array of therapeutic approaches available, the prognosis of patients with CHF is poor, especially when CHF is associated with impairment of renal hemodynamics and sodium excretion [4, 6-8]. Unfortunately, the current therapeutic regimes usually fail to prevent the development of renal dysfunction in patients with CHF [4, 8,9]. Therefore new treatment strategies targeting renal dysfunction are urgently needed. However, the prerequisite for successful treatment approaches is a better understanding of the mechanism(s) underlying the development of renal dysfunction in CHF.

It is well recognized that a decrease in renal blood flow (RBF) is a common finding in patients with CHF, and it is detected already at a relatively early stage [4, 6,8, 10, 11]. Since adequate, stable perfusion of the kidneys is essential for normal renal function [12, 13], the decrease in RBF is the harbinger of renal dysfunction in CHF. Therefore pathophysiological mechanism(s) leading to the decrease in RBF in CHF have been extensively studied, and evidence was provided for activation, presumably of compensatory value, of neurohormonal vasoconstrictor systems, such as the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS). Such activation might be initially beneficial, however, in the long-term perspective could foster the development of renal dysfunction, especially via deterioration of renal perfusion [9-11, 14-19].

Since activation of the RAS is one of the earliest functional responses in CHF and has a critical role in the pathophysiology and progression of the disease, the relevant research focused on the role of RAS in the development of alterations in RBF [2, 11, 14, 19-22]. Evidence was provided that angiotensin II (ANG II), the most important peptide of the RAS, exerts a substantial influence on renal hemodynamics and excretory function in CHF. It was initially proposed that enhanced intrarenal activity of ANG II alter renal glomerular plasma flow dynamics in CHF [14, 15, 20]. However, the role of increased ANG II concentrations and actions was not unequivocally confirmed and most probably is not the sole causative factor in the development of renal dysfunction in CHF. More likely, the mechanism responsible for the reduction of RBF involves intrarenal interaction between the RAS and other vasoactive system(s) [14, 15, 19, 21, 23-28].

Besides an apparent pathogenic role of changes in the activity of neurohormonal systems involved in the control of renal perfusion, alterations of intrarenal vascular responsiveness to endogenous vasoactive agents must be considered. A major limitation of previous studies of such alterations as a factor in the development of renal dysfunction in CHF was that RBF responses were evaluated after systemic (i.e., intravenous) administration of vasoactive agents, which was associated with changes in mean arterial pressure (MAP) [29-31]. Since an associated MAP alteration is a major confounding factor, to avoid systemic effects in the present study we employed injections of vasoactive agents directly into the renal artery, an approach that was also used in some earlier studies [32-34]. For comparison, RBF and MAP responses to administration of ANG II, norepinephrine (NE) and acetylcholine (Ach) by intravenous route were also examined. In the present study, the rat with aorto-caval fistula (ACF) was employed as a model of CHF.

The major aim of this study was to assess renal vascular responses to vasoactive agents to determine if altered renal vascular responsiveness might contribute to the development of renal dysfunction in CHF. Therefore we intrarenally administered ANG II in sham-

operated rats and the animals with ACF-induced CHF during the phase of compensation and decompensation. To determine if the expected alterations of renal vascular responsiveness would be specific for ANG II or merely represent increased reactivity to any endogenous vasoconstrictor, RBF responses to NE were also determined. In addition, to find out if possible alterations in renal vascular responsiveness to endogenous vasoconstrictors run in parallel with an impaired reaction to endogenous vasodilator factors, we also determined RBF responses to Ach, an endothelium-dependent vasodilator. Also to gain a better insight into the possible role of potential compensatory activation of intrarenal neurohormonal systems, kidney concentrations of catecholamines, ANG II and angiotensin-1-7 (ANG 1-7) were determined.

Materials and Methods

Ethical approval, animals, CHF model

The studies were performed in accordance with guidelines and practices established by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine, Prague, which accord with the European Convention on Animal Protection and Guidelines on Research Animal Use. All Hannover Sprague-Dawley (HanSD) rats were bred at the Center of Experimental Medicine of this Institute, which is accredited by the Czech Association for Accreditation of Laboratory Animal Care. The animals were kept on a 12-hour/12-hour light/dark cycle and were fed a normal salt, normal protein diet (0.45 % NaCl, 19-21% protein) manufactured by SEMED (Prague, Czech Republic) and had free access to tap water.

Male rats were used for experiments. At the age of nine weeks they were anesthetized (tiletamine + zolazepam, Virbac SA, Carros Cedex, France, 8 mg/kg; and xylazine, Spofa, Czech Republic, 4 mg/kg intramuscularly) and, to obtain volume overload-dependent CHF, ACF was created using needle technique, as initially described by Garcia and Diebold [35] and validated by many investigators including our group [29, 36-42]. Briefly, after exposure of the abdominal aorta and inferior vena cava between the renal arteries and iliac bifurcation, the aorta was occluded for about 30 seconds. An 18-gauge needle (diameter 1.2 mm) was inserted into the abdominal aorta and advanced across its wall into the inferior vena cava to create ACF. Thereafter the needle was withdrawn, and the puncture site was sealed with cyanoacrylate tissue glue. Successful creation of ACF was confirmed by inspection of pulsatile flow of oxygenated blood from the abdominal aorta into the vena cava. Sham-operated rats underwent an identical procedure but without creating ACF. Previous studies have shown that this model is characterized by cardiac remodeling, congestion and marked activation of the intrarenal RAS with impairment of renal function. Notably, this is a model with many features similar to untreated human CHF [29, 36, 37, 40-42]; its additional advantage is that the onset of compensated and decompensated phase of CHF is here precisely defined and consistent [37, 40, 41]. Five weeks after ACF induction the animals were considered to be in the phase of compensated CHF, and 20 weeks after ACF induction they were regarded as representing the decompensated phase. This division into phases was based on previous studies where the onset of compensated and decompensated CHF in this model was ascertained [29, 36, 41, 42].

Preparations for acute studies of renal and systemic vascular responses to vasoactive agents

The rats were anesthetized with thiopental sodium (80 mg/kg i.p.) and placed on a heated surgical table to maintain body temperature at 37 °C. Tracheostomy was performed to maintain patent airways, and the external end of the tracheal cannula was placed inside a small plastic chamber into which humidified 95% oxygen/5% carbon dioxide mixture was continuously delivered. The right jugular vein was catheterized with polyethylene (PE) 50 tubing for the infusion of solutions and intravenous drug administration. The right femoral artery was cannulated for monitoring of arterial blood pressure. The MAP was monitored using a pressure transducer and recorded using a computerized data acquisition system (PowerLab, ADInstruments, UK). The left kidney was exposed via a flank incision, isolated from the surrounding tissue and placed in a lucite cup. For selective intrarenal administration, a tapered PE-10 catheter was inserted into the aorta via the left femoral artery and passed 1-2 mm down the left renal artery. This catheter was kept patent by a continuous infusion of heparinized isotonic saline at a rate of 2 μl/min throughout the

experiment. During the surgery, animals received an intravenous infusion of 0.9% saline solution containing 6% bovine serum albumin (Sigma Chemical Co., Prague, Czech Republic) at a rate of 20 µl/min. With the surgery completed, an isotonic saline solution was infused to compensate for fluid losses. An ultrasonic transient-time flow probe (1RB, Transonic Systems, Altron Medical Electronic GmbH, Germany), connected to a Transonic flowmeter, was placed on the left renal artery and RBF was continuously recorded. On completion of surgery, a 45-min equilibration period was allowed. The protocol consisted of evaluating MAP and RBF responses to systemic intravenous and intrarenal bolus doses of vasoactive agents. Vasoactive agents were loaded in a small volume (20 µl) to a Cheminert valve and then rapidly infused into the animal by a bolus of saline solution (150 µl for intrarenal and 200 µl for intravenous boluses) at a rate of 90 µl/min. Two different doses of each vasoactive agent were administrated in a random order. A baseline value of RBF and MAP was assessed prior to each infusion. Minimum or maximum (depending on vasoactive substance) value of RBF and MAP following a bolus of a vasoactive agent was recorded. The changes in RBF and MAP were then expressed in percent as a difference between baseline value and maximum or minimum value divided by baseline value. This experimental approach was employed and validated by previous studies, including ours [32-34].

Determination of RAS peptides and catecholamines

Since kidney ANG II concentrations under anesthesia are higher than those measured in conscious rats after decapitation, and there are also marked differences in renin secretion in response to anesthesia and surgery [43-47], in this study ANG II, ANG 1-7 and catecholamine concentrations were measured in samples from decapitated animals.

Measurement of tissue ANG II concentrations. Immediately after decapitation and blood collection, the kidneys were removed, dried, weighed and 0.5 g of the tissue was homogenized in 3 ml precooled methanol. The tube with homogenate was kept on ice and then centrifuged at 4 °C and 3000 g for 10 min. The supernatant was evaporated using Savant SpeedVac vacuum centrifuge. Dried samples were stored at -20 °C or lower until purification by solid-phase extraction. Dried kidney samples were reconstituted with 4 ml of 50 mM sodium phosphate buffer (pH 7.4) containing 267 mg bovine serum albumin (BSA)/l and kept on ice. Phenyl-bonded solid phase extraction columns (SPE) (Bond-Elut®PH, Agilent) were preconditioned with methanol (3 ml), followed by distilled water (2 x 3 ml). After that, reconstituted samples were applied to pre-washed columns. The columns were sequentially washed with distilled water (3 ml), hexane (3 ml) and chloroform (3 ml). Water removes salts and other polar substances from the columns, hexane, and chloroform elute contaminating lipids and hydrophobic material from the columns but do not affect angiotensin peptides recovery. In the end, angiotensin peptides were eluted from SPE columns using 2 x 1 ml flush of methanol. The eluates were evaporated to dryness using a vacuum centrifuge. Dried samples were stored at -20 °C or lower until assayed. ANG II levels were measured by competitive radioimmunoassay (RIA), using the commercially available RIA kit (ED29051, IBL Int., Hamburg, Germany).

Measurement of tissue ANG 1-7 concentrations. Kidney ANG 1-7 levels were measured by competitive radioimmunoassay using the custom-made RIA kit (BeckmanCoulter, Prague, Czech Republic). The samples were prepared as follows. Kidney samples were purified by SPE. Dried kidney samples were reconstituted with 4 ml of 50 mM sodium phosphate buffer (pH 7.4) containing 267 mg BSA/l and kept on ice. C18-bonded SPE columns (Bond-Elut®C18, Agilent) were preconditioned with mixture of ethanol + distilled water + 4 % acetic acid (83:13:4 by volume; 5 ml), methanol (5 ml), distilled water (5 ml) and with 4 % acetic acid (5 ml). Thereafter, reconstituted samples were applied to pre-washed columns. The columns were sequentially washed with distilled water (5 ml) and acetone (5 ml). At the end, ANG peptides were eluted from SPE columns with 2 x 1ml + 1 x 1.5 ml of mixture of ethanol + distilled water + 4 % acetic acid (83:13:4 by volume). The eluates were evaporated to dryness using a vacuum centrifuge.

Measurement of tissue catecholamine concentrations. Kidney tissue samples were frozen and stored at -80 °C until assayed. At the time of analysis, kidney tissue was homogenized in phosphate buffer 1:3 (supplemented with protease inhibitors and ascorbic acid) using oscillation mill and centrifuged at 4 °C and 3000 g for 10 min. For analysis, 20 µg of tissue homogenate was required. At the beginning of the assay, the extraction of all samples, standards, and controls was performed. The catecholamine concentrations were measured by a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle,

using the commercially available ELISA kit (RE59395, IBL International, Hamburg, Germany). All methods are routinely employed in our laboratories [43, 45-47].

Experimental design, exclusion criteria

The rats (males) were prepared as described above and RBF responses to two 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 MAP and RBF responses to two intravenous doses of ANG II (20 and 40 ng), NE (100 and 200 ng) and Ach (50 and 200 ng) were determined in the following experimental groups (n = 10 in each group):

1. HanSD rats 5 weeks after sham operation
2. HanSD rats 5 weeks after ACF induction
3. HanSD rats 20 weeks after sham operation
4. HanSD rats 20 weeks after ACF induction

Separate groups of animals (n = 8 in each group) were used to evaluate the degree of activation of the two axes of the RAS: the vasoconstrictor axis represented by ANG II, and the vasodilator axis represented by ANG 1-7, along with determination the degree of sympathorenal activation assessed b kidney concentrations of NE, epinephrine and dopamine.

A total of 72 out of 80 rats were selected to enter the experiment. Animals were excluded from the study if successful ACF induction could not be confirmed by inspecting pulsatile flow in the vena cava, or if rats did not show signs related to CHF (i.e., bilateral cardiac hypertrophy, lung edema), or if a technical error occurred during surgical preparation.

Statistical analysis

All values are expressed as mean ± SEM. Statistical analysis of the data was performed using Graph-Pad Prism software (Graph Pad Software, San Diego, California, USA) employing one-way ANOVA and two-way ANOVA followed by Student-Newman-Keuls test where appropriate. The values exceeding 95% probability limits ($p<0.05$) were considered statistically significant.

Results

Table 1 summarizes body and organ weight parameters. As shown, the rats five and 20 weeks after induction of ACF exhibited marked bilateral cardiac hypertrophy [expressed as whole heart weight (HW), left ventricle (with septum) weight (LVW), and right ventricle weight (RVW)] as compared with sham-operated rats. Interestingly, the degree of right ventricle hypertrophy was in all post-ACF rats higher than that of the left ventricle (as evident from the increases in RVW to LVW ratio). Moreover, ACF rats five and 20 weeks after induction of ACF displayed significantly higher lung weight as compared with sham-operated rats, which indicated the development of lung congestion.

As shown in Fig. 1A, five and 20 weeks after induction of ACF the kidney ANG II levels were significantly higher than in sham-operated rats. Likewise, kidney ANG 1-7 levels in ACF

Table 1. Basal characteristics of body and organ weights and its individual structural components (determined five and 20 weeks after induction of aorto-caval fistula or after sham-operation). Values are means ± SEM. ACF, aorto-caval fistula. *P<0.05 vs. sham-operated rats in the same week. † P<0.05 rats 20 weeks vs. rats 5 weeks after ACF

Parameter	Group			
	Sham-operated rats 5 weeks	Sham-operated rats 20 weeks	ACF rats 5 weeks	ACF rats 20 weeks
Body Weight (g)	473 ± 13	546 ± 13	463 ± 4	569 ± 15
Heart weight (mg)	1351 ± 18	1431 ± 42	2074 ± 52*	2640 ± 10*†
Left ventricle weight (mg)	990 ± 11	1029 ± 27	1394 ± 40*	1722 ± 55*†
Right ventricle weight (mg)	258 ± 5	291 ± 14	442 ± 12*	593 ± 27*†
Right ventricle weight/Left ventricle weight	0.261 ± 0.005	0.282 ± 0.01	0.318 ± 0.009*	0.344 ± 0.009*†
Lung weight (mg)	1878 ± 46	1835 ± 66	2136 ± 65*	2264 ± 71*
Liver weight (mg)	16692 ± 1026	18012 ± 1075	15871 ± 944	17826 ± 1172
Kidney weight (mg)	1629 ± 41	1660 ± 51	1573 ± 42	1689 ± 49

rats, both five and 20 weeks after induction of ACF, were significantly higher than in sham-operated rats (Fig. 1B). Fig. 1C shows that kidney NE levels were significantly higher in ACF rats twenty weeks after induction of ACF than in sham-operated rats. Renal concentrations of epinephrine and dopamine exhibited a similar pattern (data not shown).

Fig. 2 summarizes the basal MAP, RBF, and renal vascular resistance in rats five and 20 weeks after induction of ACF and in their sham-operated counterparts. There were no significant differences in MAP between sham-operated rats studied five and 20 weeks after sham-operation (Fig. 2A). A creation of ACF resulted in similar decreases in MAP when measured five and 20 weeks after induction of ACF. In either case, MAP remained within the range of renal autoregulatory capacity.

There were no significant differences in RBF between sham-operated rats studied five and 20 weeks after sham-operation. ACF creation caused significant RBF decreases, similar after five and 20 weeks (Fig. 2B). However, the renal vascular resistance (RVR) was significantly elevated only in rats studied five weeks after ACF creation (Fig. 2C).

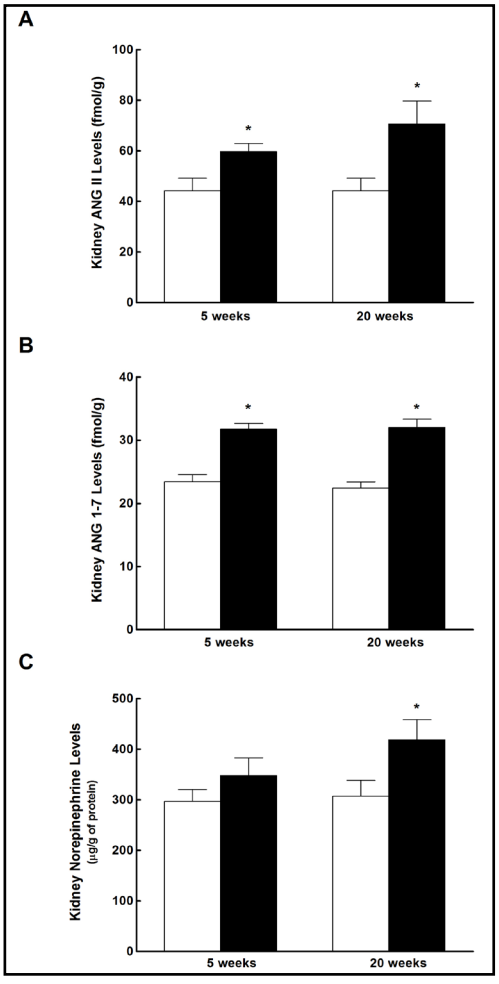

Fig. 1. Kidney angiotensin II (ANG II) levels (A), kidney angiotensin 1-7 (ANG 1-7) levels (B) and kidney norepinephrine (NE) levels (C) in sham-operated rats (open bars) and rats with ACF (solid bars) studied five and 20 weeks after induction of ACF or sham-operation. * P<0.05 versus sham-operated rats at the same time point.

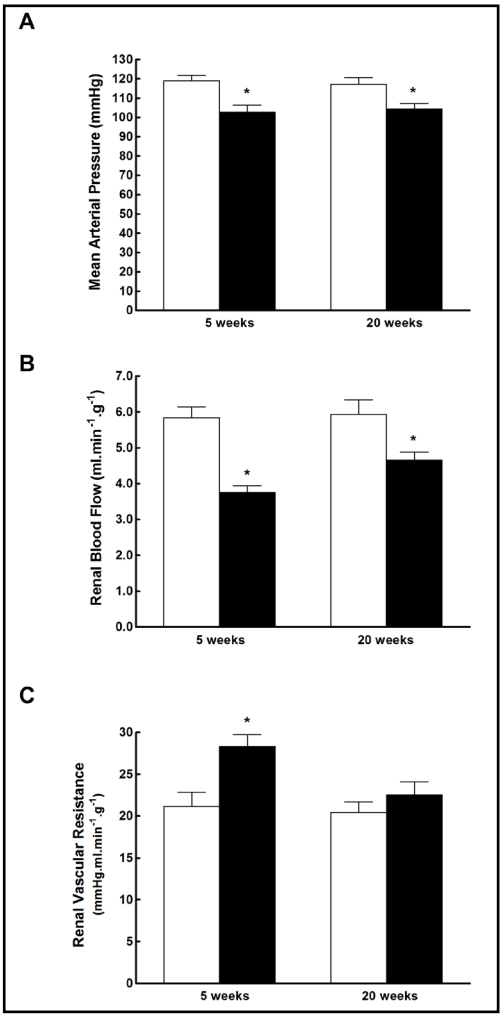

Fig. 2. Mean arterial pressure (A), renal blood flow (B), and renal vascular resistance (C) in sham-operated rats (open bars) and rats with ACF (solid bars) studied five and 20 weeks after induction of ACF or sham-operation. * P<0.05 versus sham-operated rats at the same time point.

Selective intrarenal administration of ANG II, NE and Ach did not alter MAP in any experimental group. As shown in Fig. 3A, administration of 2 and 8 ng of ANG II produced similar dose-dependent decreases in RBF in rats studied five and 20 weeks after sham-operation and after the creation of ACF.

Fig. 3B shows that intrarenal administration of NE at the doses of 20 and 60 ng caused dose-dependent RBF decreases that were almost always greater in ACF rats than in sham-operated counterparts. An exception was a similar decrease in RBF with the low NE dose when studied 20 weeks after creation of ACF or sham operation.

The intrarenal administration of 10 and 40 ng of Ach in ACF rats tested five weeks after creation of ACF elicited significantly higher increases in RBF than in sham-operated rats (Fig. 3C). Dissimilarly, comparable increases in RBF were observed in sham-operated and ACF rats when tested after 20 weeks (Fig. 3C).

Fig. 4 and 5 summarize MAP and RBF responses to intravenous bolus administration of ANG II, NE and Ach.

As shown in Fig. 4A-left, both doses of ANG II-induced increases in MAP that were significantly smaller in ACF than in sham-operated rats, irrespective of the time of measurement (five or 20 weeks after the operation). By analogy, both ANG II doses induced decreases in RBF that were substantially smaller in ACF than in sham-operated rats (Fig. 4B-left).

NE at the doses of 100 and 200 ng elicited significantly smaller increases in MAP in ACF than in sham-operated rats (both after five and 20 weeks) (Fig. 4A-right). As shown in Fig. 4B-right, both NE doses decreased RBF distinctly less in ACF rats than their sham-operated counterparts, similarly after five and 20 weeks.

As shown in Fig. 5A, Ach at doses of 50 and 200 ng elicited smaller MAP decreases in ACF rats than in their sham-operated controls, both after five and 20 weeks. Both Ach doses increased RBF significantly less in ACF rats than in the sham-operated controls (Fig. 5B).

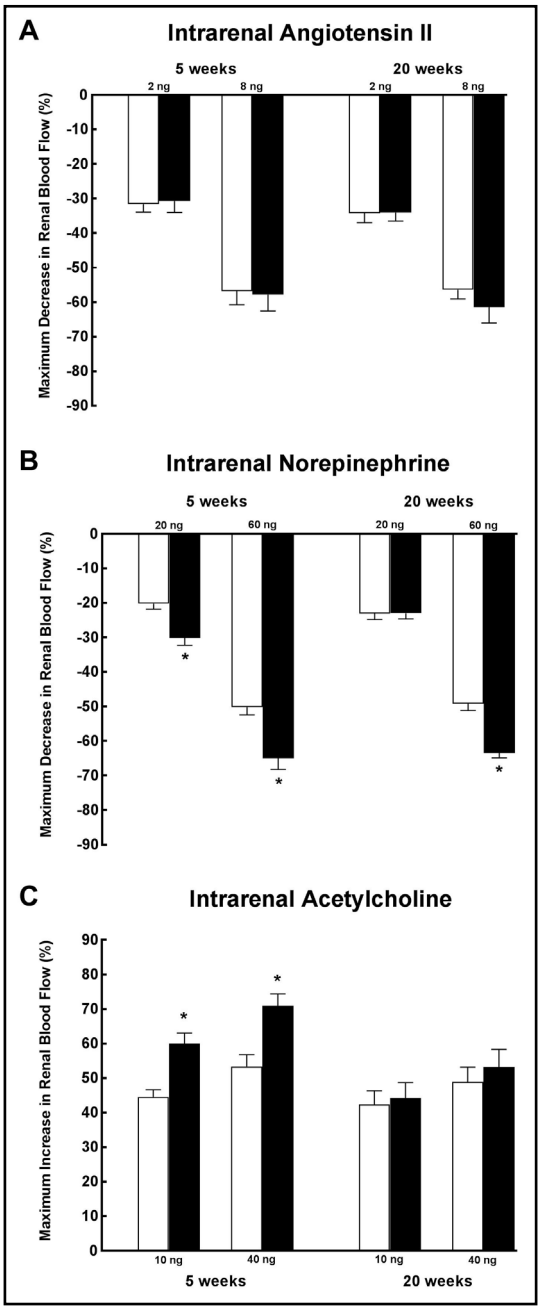

Fig. 3. Maximum change in renal blood flow elicited by selective intrarenal bolus administration of angiotensin II (2 and 8 ng) (A), norepinephrine (20 and 60 ng) (B) and acetylcholine (10 and 40 ng) (C) in sham-operated rats (open bars) and rats with ACF (solid bars) studied five and 20 weeks after induction of ACF or sham-operation. * $P<0.05$ versus sham-operated rats at the same time point.

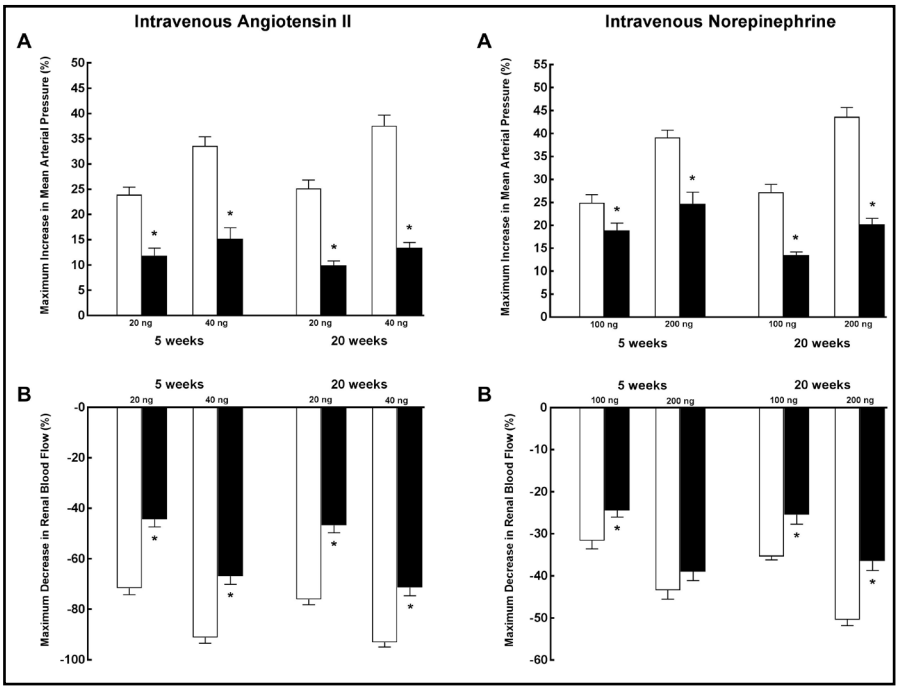

Fig. 4. Maximum change in mean arterial pressure (A) and renal blood flow (B) elicited by intravenous bolus administration of angiotensin II (20 and 40 ng) and norepinephrine (100 and 200 ng) in sham-operated rats (open bars) and rats with ACF (solid bars) studied five and 20 weeks after induction of ACF or sham-operation. * P<0.05 versus sham-operated rats at the same time point.

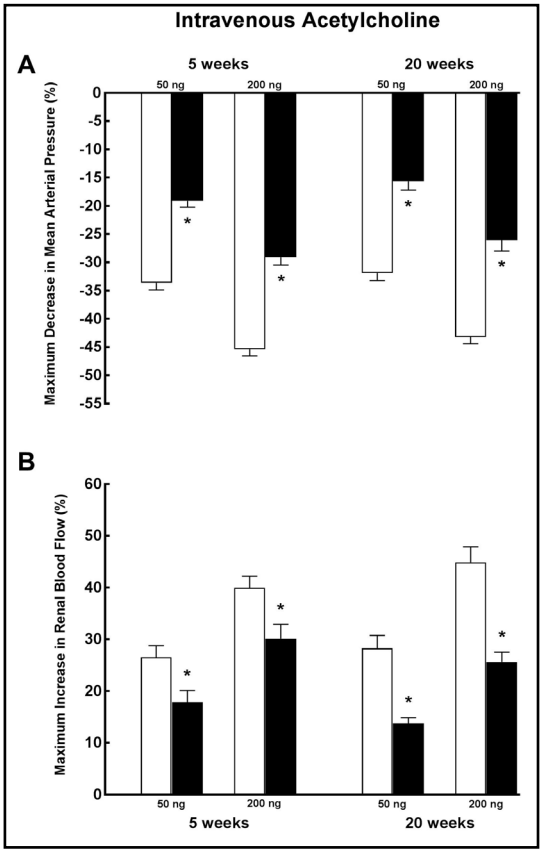

Fig. 5. Maximum change in mean arterial pressure (A) and renal blood flow (B) elicited by intravenous bolus administration of acetylcholine (50 and 200 ng) in sham-operated rats (open bars) and rats with ACF (solid bars) studied five and 20 weeks after induction of ACF or sham-operation. * P<0.05 versus sham-operated rats at the same time point.

Discussion

In the present study we show that as soon as five weeks after creation of ACF, the HanSD rats displayed signs of pronounced bilateral cardiac hypertrophy associated with marked lung congestion, indicating left ventricle failure. In addition, ACF rats exhibited an elevation of ANG II, ANG 1-7 and catecholamine concentrations in the kidney, when assessed during the compensated (i.e., five weeks after creation of ACF) and decompensated CHF (20 weeks after ACF operation). These results are in agreement with recent findings in this model of CHF, and strongly suggest a marked activation of both vasoconstrictor/sodium retaining and the vasodilatory/natriuretic axis of the RAS in the kidney [29, 38, 39], along with activation of the sympathorenal axis.

Altogether our findings support the notion that CHF is not a purely hemodynamic disorder that markedly affects renal hemodynamics. Thus neurohormonal activation of the RAS and SNS are important determinants of further CHF progression [16, 17, 20-22, 24-28].

Rats with CHF displayed an increase in renal vascular resistance and a substantial decrease in RBF five weeks after the ACF creation, indicating established renal dysfunction already at this early stage of CHF. It can be noticed that we and others reported that in this model of CHF the decompensation phase (marked by the occurrence of mortality) started around 20 weeks after creation of ACF [37, 40-42]. In our original study undertaken to describe the course of CHF-related morbidity and mortality in HanSD rats, the median for survival rate was 43 weeks after ACF creation [41]. Obviously, in ACF model renal dysfunction is present already in the early stage of CHF and it plays an important role in the disease progression. Additionally, our present findings accord with our earlier suggestion that persistent impairment of renal hemodynamics and sodium excretion rather than progressing cardiac remodeling determines long-term survival rate in ACF-induced model of CHF [39].

What is the mechanism underlying the decrease in RBF in ACF rats? The evidence obtained from previous studies suggested that enhanced renal vascular responsiveness to ANG II combined with impaired endothelium-dependent vascular response could be responsible [29-31]. However, these studies were performed in a very early time period after ACF creation (5 – 7 days) and used systemic administration of substances, which alters MAP. Nevertheless, we found that there was no difference in renal vascular responses to ANG II between ACF and sham-operated group studied five and 20 weeks after the operation. This indicates that ACF rats do not exhibit exaggerated renal vascular responsiveness to ANG II. Also, rats five weeks after ACF operation displayed augmented responses to intrarenally infused Ach when compared to control animals. This might be a compensatory mechanism to increased renal vascular resistance that mostly disappears in the decompensated phase (20 weeks post ACF) of CHF, in which ACF rats responded almost equally to an intrarenal bolus of Ach as did sham-operated animals. Thus, ACF rats do not show impaired renal vasodilator responses to Ach, at least when studied five and 20 weeks post-ACF. These observations indicate that altered renal vascular responsiveness to ANG II and Ach is not responsible for the reduction of RBF in ACF-induced model of CHF.

On the other side, our present findings show that ACF rats studied five and 20 weeks after creation of ACF exhibited exaggerated renal vascular responsiveness to NE when compared to sham-operated rats. Such hyperreactivity might contribute to the development of renal dysfunction during the compensated as well as decompensated CHF. This finding is in agreement with the notion that inappropriate activation of SNS, especially in the kidney, accelerates the development of renal dysfunction and contributes to the progression of CHF [24-28].

Another important set of findings relates to MAP and RBF response to systemic (intravenous) administration of vasoactive agents. We show that the rats studied five and 20 weeks after creation of ACF exhibited attenuated peripheral vascular (MAP) responses to vasoconstrictors (ANG II and NE) and endothelium-dependent vasodilator Ach. The same pattern of changes was also observed for RBF, which indicates that the responses to ANG II, NE and Ach were also attenuated in the kidney, in direct contrast with the pattern of responses to intrarenal administration of these agents. These findings demonstrate how crucial in such studies the route of administration is. Presumably, MAP alterations seen with the intravenous route initiate some indirect effects. Somewhat similarly, the studies that evaluated effects of nitric oxide synthase inhibition and pharmacological blockade of the RAS on renal function and renal sodium excretion have clearly demonstrated that the actual result depends on the route of administration (systemic vs. intrarenal) of the blocker: also here a different response seen with systemic administration was attributed to the associated effects on MAP [48]. This explains the discrepancy between previous observations and our results showing that in the ACF-induced CHF the renal vascular responsiveness to NE and Ach is attenuated and not augmented, as is actually the case.

Therefore, our present studies, admittedly using exogenous stimulation, strongly suggest that ACF rats in the compensated and decompensated CHF display attenuated peripheral vascular responsiveness to vasoconstrictors (ANG II and NE) and Ach, an endothelium-dependent vasodilator. In contrast, renal vascular responses to ANG II, NE and Ach are sustained in CHF rats and even augmented in the case of NE.

Conclusion

The data demonstrate that ACF rats with CHF, in the phase of compensation as well as decompensation, do not exhibit exaggerated renal vascular responsiveness to ANG II. We observed an augmented responses to intrarenal Ach bolus in ACF rats five weeks post ACF, probably compensatory to increased RVR. However, even 20 weeks after ACF operation the rats did not show impaired renal vasodilator responses to Ach. Furthermore, the data show that the rats with ACF-induced CHF exhibit enhanced renal vascular responsiveness to NE. In summary, we suggest that exaggerated renal vascular responsiveness to ANG II and impaired renal endothelium-dependent vasodilatation do not play a decisive role in the development of renal dysfunction in this model of CHF. On the other hand, augmented renal vascular sensitivity to NE might be here a contributing factor active in either phase of CHF.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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