Rats with adenine-induced chronic renal failure develop low-renin, salt-sensitive hypertension and increased aortic stiffness

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CARDIOVASCULAR (CV) DISEASE is the major cause of morbidity and mortality in patients with chronic kidney disease (CKD) (26). There is a graded and inverse relationship between estimated glomerular filtration rate (GFR) and CV risk that is apparent already when GFR falls below 60 ml·min−1·1.73 m2 (8), and the majority of CKD patients die from CV events before developing end-stage renal disease (25). Although the pathophysiological mechanisms causing increased CV risk in CKD are complex, elevated aortic pulse wave velocity (PWV), a measure of aortic stiffness, is per se an independent powerful predictor of CV death in patients with end-stage renal disease (1). In CKD patients, aortic PWV has been shown to be correlated to vascular calcification indices (14, 23, 29), which, in turn, are associated with disorders in bone and mineral metabolism that develop with declining GFR (15). However, increased aortic PWV is associated with mortality also in patient groups with normal kidney function and even in healthy subjects (34).

We have recently analyzed vascular function in a model of severe chronic renal failure produced in Sprague-Dawley rats by feeding animals with chow supplemented with adenine (22), i.e., adenine-induced chronic renal failure (A-CRF). These animals develop tubulointerstitial kidney injury (33) and a more pronounced reduction in GFR compared with the commonly used model of 5/6 nephrectomy (20–22). In addition, animals with A-CRF develop hypertension, disordered mineral metabolism and secondary hyperparathyroidism, oxidative stress, and a marked reduction in the rate of aortic relaxation when assessed ex vivo (22). In view of the resemblance with the uremic syndrome in patients, we consider this model well suited for examining pathophysiological mechanisms that cause CV disease in CKD. The overall objective of this study was to characterize the hemodynamic changes in this model further. The specific aims were first to determine by which mechanisms A-CRF animals develop hypertension, concentrating on the role of the renin-angiotensin system and nitric oxide. Secondly, we hypothesized that the marked decrease in aortic relaxation rate that we previously have observed ex vivo would be associated with increased aortic stiffness in vivo. Reduced aortic compliance may have deleterious effects on the heart by increasing ascending aortic pressure and left ventricular afterload. To examine aortic stiffness we assessed aortic pulse wave velocity in anesthetized animals.

METHODS

General Procedures

Seventy-three male Sprague-Dawley rats (Harlan, Horst, The Netherlands) weighing ~300 g were used and housed in rooms with a controlled temperature of 24–26°C and a 12:12-h dark-light cycle. Chronic renal failure was produced by feeding animals with chow containing adenine, as previously described (22), using a modification of protocols employed by other investigators (16, 20–21). Longitudinal data on body weight, water intake, plasma creatinine concentrations, and blood pressure in this model have been published previously (22). At study start (i.e., day 1 of the study), all animals were randomized to, and provided with, standard pelleted rat chow.
containing adenine [adenine-induced CRF (A-CRF), n = 37] or identical Chow without adenine (pair-fed controls, n = 36). The Chow (R34, Lantmännen, Kinstad, Sweden) contained 0.63% phosphorous, 0.74% calcium, 0.53% potassium, and 0.22% sodium. The concentration of adenine in the Chow was 0.5% for the first 3 wk followed by 0.3% for 2 wk and 0.15%, thereafter, until animals were killed. As pilot studies revealed a reduced food intake in animals consuming adenine-containing Chow, controls were pair-fed and received their daily ration of Chow once daily in the morning. Rats had free access to tap water throughout the study. Chemicals were from Sigma (St. Louis, MO), if not stated otherwise. In protocols A and D (see Protocols), animals were decapitated without anesthesia to eliminate the influence of anesthesia on plasma biomarkers. All experiments were performed in accordance with the American Physiological Society’s guiding principles in the care and use of vertebrate animals in research and training, and they were approved by the regional ethics committee in Gothenburg, Sweden.

**Protocols**

**Protocol A: analyses of arterial blood pressure by radiotelemetry.** Approximately 2 wk before the start of adenine or the control diet, 18 rats were anesthetized with isoflurane and equipped with radiotelemetry transmitters (Data Sciences International, St. Paul, MN) for conscious arterial blood pressure (BP) and heart rate monitoring, as described previously in detail (11, 22). Data were collected and analyzed using Dataquest ART version 3.1 (Data Sciences International), and the BP signal was corrected for electronic offset, as described previously (22). During 24-h recordings, data were sampled at 500 Hz for 8 s every 5 min. Four out of eighteen animals were excluded from analyses due to weak transmitter signal, unacceptable offset, or surgical complications leaving eight A-CRF animals and six controls in the study. Data from the first 7 wk of adenine and control diet have previously been reported (22). Results presented here are from experiments examining arterial blood pressure responses to different interventions 7–11 wk after study start. At least a 1 wk wash-out period was allowed between the interventions.

**High NaCl diet.** At week 7 after study start, animals received a 4% NaCl diet for 5 days. Telemetry recordings were carried out for 24 h at baseline (i.e., during 24 h immediately preceding start of high-NaCl diet) and on day 5 of high-NaCl diet. Controls were pair-fed, and hence dietary NaCl intake was matched.

**Treatment with Nω-nitro-l-arginine methyl ester hydrochloride.** At week 9 after study start, animals received the nitric oxide synthase inhibitor Nω-nitro-l-arginine methyl ester hydrochloride (l-NNAME) added to the drinking water (controls 200 mg/l, A-CRF 78 mg/l). A lower concentration of l-NNAME to A-CRF animals was used to correct for increased water intake in this group. These concentrations of l-NNAME had in pilot experiments been shown to produce a daily intake of ~15 mg·kg⁻¹·day⁻¹ in both groups. Telemetry recordings were performed for 24 h at baseline (i.e., during 24 h immediately preceding start of l-NNAME) and on day 3 of l-NNAME treatment.

**Response to the ANG II type-1 receptor antagonist candesartan.** Experiments were carried out 11 wk after study start. Measurements were performed during two consecutive 24-h periods, before (baseline) and after administration of one dose of candesartan cilexetil (AstraZeneca, Mölndal, Sweden; 10 mg/kg, 5 ml/kg, orally by gavage). One week after completion of these studies, animals were killed by decapitation, and free-flowing trunk blood was collected. The heart, kidneys, and left tibia were excised, cleaned, and weighed, and tibia length was measured. Heparin- and EDTA-plasma were obtained following centrifugation (5,000 rpm for 10 min) and were stored at −20° or −80°C until analyzed.

**Protocol B: aortic PWV, aortic pulse wave analysis, and left ventricular end-diastolic pressure.** Experiments were performed on 10 controls and 10 A-CRF animals 12–13 wk after study start. Animals were anesthetized with isoflurane (Pharmacia & Upjohn, Stockholm, Sweden), mixed with air during spontaneous breathing by using a vaporizer (University 1200, Agnths, Lidingö, Sweden). For induction and maintenance of anesthesia, isoflurane concentrations of ~5% and 1.5% (vol/vol), respectively, were used. Rats were placed on a heating table, and rectal temperature was kept at 37°C throughout. Two venous catheters (PE-50) were placed in the femoral veins for fluid and drug administration, and the urinary bladder was catheterized. Isotonic saline was infused throughout in a volume of 6 ml·kg⁻¹·h⁻¹ to replace fluid losses. Through the right femoral artery and left carotid artery, two ultra-miniature fiber optic pressure sensors (Samba preclin 420, sensor diameter 0.42 mm; Harvard Apparatus, Edenbridge, Kent, UK) were placed in the distal abdominal aorta at the level of the aortic bifurcation, and in the ascending aorta immediately above the aortic valve or in the left ventricle. These pressure sensors were used for aortic pulse wave analysis and measurements of aortic PWV and left ventricular end-diastolic pressure (LVEDP) using a sampling frequency of 1,000 Hz. In addition, a third arterial catheter [polyethylene (PE)-50] was placed in the left femoral artery for continuous BP monitoring throughout. After a 15-min equilibration period, baseline recordings of aortic BPs were performed during 5 min. Subsequently, the proximal aortic pressure sensor was gently inserted into the left ventricle for measurements of LVEDP before it was withdrawn and placed in its original position in the ascending aorta. Thereafter, aortic pressures were recorded while BP was first lowered by sodium nitroprusside (SNP; 15 µg·kg⁻¹·min⁻¹ iv) and then raised by phenylephrine (25 µg·kg⁻¹·min⁻¹ iv). Subsequently, rats were killed by an overdose of pentobarbital sodium, and the distance between the tips of the pressure sensors was carefully measured by a fine thread and a ruler. The average distance between the sensor tips was similar in the two groups (97 mm in A-CRF animals and 96 mm in controls) The heart, kidneys, and thoracic aorta were immediately excised, weighed, and immersion-fixed in 4% neutrally buffered formaldehyde (Histolab Products AB, Gothenburg, Sweden).

Aortic and left ventricular pressure data were collected and analyzed by the acquisition program Biopac MP 150 (Biopac Systems, Santa Barbara, CA). From the data recorded, heart rate, systolic BP, mean arterial pressure (MAP), diastolic BP, and pulse pressure (PP) were extracted in real-time using the built-in routines. Aortic PWV (m/s) was calculated using the foot-to-foot method, the foot being objectively defined by the peak of the second derivative of the pressure curve during each pressure waveform (13, 19). Augmented aortic pressure was calculated as the difference between systolic BP and the pressure at the inflection point in the pressure waveform representing the arrival of the reflected pulse wave (13). Aortic augmentation index (AI, %) was calculated as augmented pressure divided by PP × 100. The inflection point in the pressure waveform was identified by the zero crossing-point of the second derivative as described previously (13). Left ventricular end-diastolic pressure was determined by identifying the peak of the second derivative of the left ventricular pressure curve during each pressure waveform. Aortic PWV and AI, and LVEDP, were determined by postprocessing of data using the Biopac MP 150 features. These variables were determined for all pressure waveform(s) during 4–6 consecutive respiratory cycles (corresponding to ~25–40 pressure waveforms) for each animal and during each intervention (i.e., baseline, SNP, and phenylephrine), and average values are presented.

**Protocol C: kidney function and perfusion fixation of the aorta.** Experiments were performed on eight controls and eight A-CRF animals 12–13 wk after study start. Rats were placed individually in metabolic cages and after 24 h of equilibration measurements were performed during two consecutive 24-h periods. At the end of experiments, blood was sampled from the tail vein for plasma analyses of creatinine and electrolytes, and animals were transferred to normal cages.
RESULTS

Organ Weights and Plasma Analyses

There were no statistically significant differences between groups in body weight or tibia length (Table 1). Left ventricular weight was markedly elevated in A-CRF animals, whereas there was no significant difference between groups in right ventricular weight (Table 1). Plasma concentrations of creatinine, potassium, and phosphate were clearly elevated in A-CRF animals, while plasma calcium levels were significantly reduced (Table 1). Plasma renin activity (PRA) was markedly suppressed in A-CRF animals vs. controls (Table 1). Both ADMA and SDMA plasma levels were significantly elevated in A-CRF rats vs. controls, although there was no statistically significant difference between groups in L-arginine concentrations (Table 1).

Plasma nitrite levels tended to be reduced in A-CRF animals vs. controls, although not reaching statistical significance (Table 1; P = 0.06).

Kidney Function and Fluid-Handling

As previously reported (22), A-CRF animals showed a marked increase in urine output (∼4-fold vs. controls) and a corresponding elevation in water intake (Table 2). Renal creatinine clearance was reduced in A-CRF animals to about 10% of control values (Table 2). There were no statistically significant differences between groups in the absolute rate of urinary sodium and potassium excretion (Table 2). However, fractional urinary sodium and potassium excretion were markedly elevated in A-CRF animals (Table 2).

Blood Pressure and Heart Rate in Freely Moving Animals Assessed by Radiotelemetry

The 24-h profile of MAP and heart rate measured at 7 wk after study start is shown in Fig. 1. Average MAP for the 24-h period was significantly elevated in the A-CRF group (119 ± 6 mmHg vs. 106 ± 6 mmHg in controls).

| Table 1. Organ weights and blood analyses at time of euthanasia |
|-----------------|-----------------|
|                 | Controls (n)    | A-CRF (n)     |
| BW, g           | 387 ± 46 (16)   | 368 ± 36 (17) |
| Tibia length, mm| 40.1 ± 1.1 (15) | 40.6 ± 1.4 (18) |
| LVW/tibia, mg/mm| 21.9 ± 2.1 (15) | 26.8 ± 3.8 (18)** |
| RVW/tibia, mg/mm| 4.9 ± 1.1 (15)  | 5.1 ± 1.0 (18)  |
| P-arginine, μmol/l| 31 ± 4 (23)   | 285 ± 80 (26)** |
| P-sodium, mmol/l| 143 ± 3 (18)    | 142 ± 3 (17)    |
| P-potassium, mmol/l| 4.1 ± 0.3 (18) | 6.0 ± 0.7 (17)** |
| P-calcium, mmol/l| 2.5 ± 0.1 (18)  | 2.2 ± 0.3 (17)** |
| P-phosphorus, mmol/l| 1.7 ± 0.2 (18) | 2.5 ± 1.1 (17)** |
| PRA, μg·l⁻¹·h⁻¹| 12.3 ± 7.3 (9)  | 0.6 ± 0.5 (11)** |
| ADMA, μmol/l    | 0.45 ± 0.02 (3) | 0.65 ± 0.05 (9)** |
| SDMA, μmol/l    | 0.32 ± 0.03 (3) | 1.25 ± 0.12 (9)** |
| L-Arg, μmol/l   | 152 ± 11 (3)    | 137 ± 16 (9)    |
| P-nitrite, mmol/l| 161 ± 80 (10)   | 104 ± 32 (9)    |

Values are expressed as means ± SD. Data from pair-fed control rats (controls) and animals with adenine-induced chronic renal failure (A-CRF) at the time of acute experimentation 10–13 wk after study start (animals from protocols A, C, and D, see METHODS). The numbers of animals per group are presented in parentheses. BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; P, plasma; PRA, plasma renin activity; ADMA, asymmetric dimethyl arginine; SDMA, symmetric dimethyl arginine; L-Arg, L-arginine. *P < 0.05, **P < 0.01, and ***P < 0.001.
9 vs. 101 ± 10 mmHg, P < 0.01) while heart rate did not differ significantly between groups (311 ± 19 vs. 311 ± 21 bpm in A-CRF animals and controls, respectively). There were no statistically significant differences between day (light) and night (dark) values within groups for any of the measured variables.

Responses to 4% NaCl diet. A-CRF animals showed a marked increase in MAP in response to 5 days of 4% NaCl diet (from 119 ± 9 to 143 ± 9 mmHg, P < 0.001), whereas MAP did not change significantly in controls (Fig. 2A). Notably, although there was a pronounced increase in MAP in A-CRF animals, this was not accompanied by a compensatory reduction in heart rate; instead, heart rate tended to increase (Fig. 2A). During the 5-day period of 4% NaCl diet, there was no significant difference between groups in NaCl intake, as controls were pair-fed. However, A-CRF animals showed a more pronounced increase in body weight during the 5-day period (23 ± 9 vs. 9 ± 6 g in A-CRF animals and controls, respectively, P < 0.01), indicative of sodium and water retention.

Responses to L-NAME. Inhibition of nitric oxide synthase by L-NAME significantly increased MAP in both groups (from 100 ± 11 to 124 ± 13 mmHg in controls, P < 0.001; and from 112 ± 10 to 149 ± 15 mmHg in A-CRF animals, P < 0.001). The increase in MAP was significantly more pronounced in A-CRF animals vs. controls both in absolute values (Fig. 2B) and when expressed in percent change (33 ± 8 vs. 25 ± 4% in A-CRF animals and controls, respectively, P < 0.05). Heart rate decreased in both groups to a similar extent (Fig. 2B).

Responses to candesartan. Candesartan reduced MAP significantly in both controls (from 99 ± 12 to 88 ± 12 mmHg, P < 0.001) and A-CRF animals (128 ± 20 to 123 ± 18 mmHg, P < 0.05). However, the reduction in MAP was significantly more pronounced in controls vs. A-CRF rats (Fig. 2C). Heart rate increased in controls (300 ± 22 to 327 ± 26 bpm, P < 0.001) and in A-CRF animals (319 ± 15 to 332 ± 14 bpm, P < 0.001). The increase in heart rate was significantly more prominent in controls vs. A-CRF animals (Fig. 2C).

Aortic Pulse Wave Velocity

Aortic PWV was significantly elevated in A-CRF animals vs. controls at baseline (5.10 ± 0.51 vs. 4.58 ± 0.17 m/s, P < 0.05) and during maximal vasodilatation with SNP (4.04 ± 0.31 vs. 3.75 ± 0.20 m/s, P < 0.05), while there was no statistically significant difference between groups during phenylephrine infusion (Fig. 3). Notably, whereas MAP was significantly increased in group A-CRF during PWV measurements at baseline (124 ± 12 vs. 108 ± 10 mmHg, P < 0.05), there were no statistically significant differences between groups in MAP during infusion of SNP (46 ± 5 vs. 48 ± 6 mmHg, in A-CRF and controls, respectively) and phenylephrine (173 ± 19 vs. 161 ± 7 mmHg, in A-CRF and controls, respectively). The above-mentioned MAP data are average values of those recorded simultaneously in the ascending aorta and at the aortic bifurcation during PWV measurements. There was no statistically significant difference between groups in heart rate during baseline or during SNP or phenylephrine infusion (data not shown).

Aortic Blood Pressures and Left Ventricular End-Diastolic Pressure

Both in the ascending aorta and at the aortic bifurcation in the distal abdominal aorta, systolic blood pressure (SBP), PP, MAP, and AI were significantly elevated in A-CRF animals vs. controls (Table 3). In addition, diastolic blood pressure (DBP) was significantly increased in A-CRF animals vs. controls at
the aortic bifurcation (Table 3). As expected, DBP and MAP were significantly lower, and AI significantly elevated, at the distal recording site vs. the proximal site when analyzed within groups (Table 3). However, in A-CRF animals, the increases in SBP (+14 ± 7 vs. −2 ± 7 mmHg, in A-CRF animals and controls, respectively, \( P < 0.001 \)) and in PP (+20 ± 7 vs. +7 ± 7 mmHg, in A-CRF animals and controls, respectively, \( P < 0.001 \)) along the length of the aorta were significantly more pronounced than in controls.

SBP and PP were significantly elevated in A-CRF animals during SNP infusion both in the ascending aorta (SBP: 84 ± 10 vs. 75 ± 7 mmHg, \( P < 0.05 \); PP: 53 ± 7 vs. 40 ± 4 mmHg, \( P < 0.001 \)) and at the aortic bifurcation (SBP: 65 ± 7 vs. 58 ± 8 mmHg, \( P < 0.05 \); PP: 36 ± 5 vs. 23 ± 5 mmHg, \( P < 0.001 \)), although there were no statistically significant differences between groups in heart rate or MAP at either site (data not shown).

During baseline conditions, LVEDP was almost doubled in A-CRF animals vs. controls (15.1 ± 5.0 vs. 8.4 ± 0.9 mmHg, \( P < 0.001 \), Fig. 4).

### Table 3. Aortic blood pressures and augmentation index during baseline conditions

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>A-CRF (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>347 ± 29</td>
<td>324 ± 26</td>
</tr>
<tr>
<td>Ascending aorta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>130 ± 9</td>
<td>150 ± 14*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>93 ± 7</td>
<td>101 ± 11</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>37 ± 4</td>
<td>49 ± 5*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>112 ± 7</td>
<td>126 ± 12*</td>
</tr>
<tr>
<td>AI, %</td>
<td>11 ± 6</td>
<td>26 ± 7*</td>
</tr>
<tr>
<td>Aortic bifurcation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>128 ± 12</td>
<td>164 ± 17*†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>84 ± 11†</td>
<td>96 ± 12*†</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>43 ± 5†</td>
<td>69 ± 9*†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>104 ± 12†</td>
<td>123 ± 13*†</td>
</tr>
<tr>
<td>AI, %</td>
<td>36 ± 16†</td>
<td>53 ± 4*†</td>
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</tbody>
</table>

Values are expressed as means ± SD. Blood pressures were measured simultaneously in the ascending aorta and in the distal abdominal aorta at the level of the aortic bifurcation during isoflurane anesthesia (see METHODS). Experiments were performed 12–13 wk after study start. Presented data are from baseline measurements before administration of sodium nitroprusside and phenylephrine. Augmentation index (AI) was calculated as described in METHODS. A-CRF: adenine-induced chronic renal failure; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; and MAP, mean arterial blood pressure. *\( P < 0.05 \) A-CRF vs. controls. †\( P < 0.05 \) ascending aorta vs. aortic bifurcation within group.
Aortic Morphology

Morphometrical analyses of perfusion-fixed thoracic aortas revealed no statistically significant differences between groups in media thickness (123 ± 18 vs. 105 ± 16 μm, in A-CRF and controls, respectively) or lumen radius (818 ± 145 vs. 827 ± 122 μm, in A-CRF and controls, respectively), although media thickness tended to be increased in A-CRF animals. However, media thickness/lumen radius ratio was significantly elevated in A-CRF animals vs. controls (0.152 ± 0.022 vs. 0.127 ± 0.007, respectively, P < 0.05). Figure 5 illustrates a perfusion-fixed thoracic aorta from an A-CRF animal with clear media thickening. Semiquantitative assessment of thoracic aortas showed medial calcifications by von Kossa staining in only 1 out of 10 A-CRF animals subjected to PWV analyses, whereas no vascular calcifications were detected in remaining animals (P = not significant between groups). However, the medial layer of aortas from 7 out of 10 examined A-CRF animals displayed an abnormal architecture with fragmentation of elastic lamellae and disorganized VSMCs, whereas these abnormalities were virtually absent in aortas from controls (P < 0.01 between groups, Fig. 5). The medial changes in A-CRF animals had a focal appearance and were not evenly distributed throughout the aortic circumference. There were no apparent alterations in the intima or adventitia of A-CRF animals.

DISCUSSION

The main findings in the present study were that rats with A-CRF develop hypertension that is not renin-dependent and exaggerated by the high-NaCl diet. In addition, A-CRF animals had increased aortic PWV both during baseline conditions and during SNP infusion, clearly indicative of elevated aortic stiffness in vivo. Notably, the increase in aortic PWV occurred although media calcifications were present in only ~10% of A-CRF animals, suggesting that other pathophysiological mechanisms caused the increase in aortic stiffness.

The model of A-CRF is characterized by severe tubulo-interstitial kidney injury and marked reductions in GFR, and it has frequently been used to investigate disorders in mineral and bone metabolism, as well as mechanisms of vascular calcifications in severe renal failure (10, 12, 16, 18, 20–22). However, although increased BP has been reported in a few studies on animals with A-CRF (17, 22), we are unaware of previous studies investigating the pathophysiological mechanisms of hypertension in this model. In the present study, PRA was suppressed in hypertensive A-CRF animals during baseline conditions when animals had free access to chow with normal NaCl content. In addition, in response to the ANG II type-1 receptor antagonist candesartan A-CRF animals showed only a minor reduction in MAP that was less pronounced than the decrease observed in normotensive controls. These results clearly indicate that the hypertension in A-CRF animals is not mediated by the renin-angiotensin system. During 5 days of the 4% NaCl diet, A-CRF animals showed a marked increase in MAP that was accompanied by a significant weight gain compared with controls. These findings indicate that the increase in MAP during a high-NaCl diet was at least partially caused by sodium retention and extracellular fluid volume expansion. It is reasonable to hypothesize that sodium retention...
and extracellular fluid volume expansion contributed to hypertension also when dietary NaCl intake was normal, considering the marked reduction in GFR (C_{creatinine} was 10% of control values) and the suppressed PRA in these animals. Hypothetically, another explanation for the low PRA levels in A-CRF animals could be the damage of renin-producing juxtaglomerular cells caused directly by adenine or its metabolites. However, by light microscopy, we have been unable to detect any significant abnormalities in renal arterial or arteriolar morphology in A-CRF animals (unpublished observation). The preserved renal arterial morphology was anticipated as adenine, via its metabolite 2,8-dihydroxyadenine causes renal failure by tubular obstruction and subsequent tubulointerstitial injury. Interestingly, the increase in MAP during the high-salt diet in A-CRF animals was accompanied by an increase in heart rate, while the expected baroreflex-mediated decrease in heart rate was observed in response to l-NAME. These observations suggest an impaired baroreflex control of heart rate, particularly during high-salt intake in A-CRF animals. Similar findings of salt-induced impairments in baroreflex functions have been demonstrated in salt-sensitive hypertensive models (3, 9).

Confirming our previous results in this model (22), plasma levels of ADMA, an analog of l-arginine that inhibits nitric oxide synthase (30), and SDMA, which may interfere with nitric oxide synthesis by competing with l-arginine for transmembrane transport (4), were both significantly elevated in A-CRF animals. In addition, plasma nitrite levels tended to be reduced in A-CRF animals, lending further support for the notion that this model is associated with reduced nitric oxide bioavailability. Hence, we speculated that nitric oxide deficiency could contribute to the hypertension observed in A-CRF animals and that these rats would show an attenuated increase in BP in response to l-NAME treatment. However, on the contrary, A-CRF animals showed a more pronounced increase in MAP compared with controls in response to l-NAME, suggesting that nitric oxide bioavailability in A-CRF animals was at least similar to that in controls. A hypothetical explanation could be that l-NAME-induced vasoconstriction in hypertensive A-CRF animals produced a more pronounced increase in vascular resistance compared with that in controls as a consequence of structural adaptation of resistance arteries with increased wall-to-lumen ratios (7). However, additional studies are clearly needed to explain this finding.

Unexpectedly, there was no difference between day and night regarding BP and heart rate data collected by radiotelemetry, indicating a disturbed circadian rhythm. Notably, this lack of circadian rhythm was observed in both A-CRF animals and controls and is most likely explained by the fact that food intake and feeding patterns were altered as a consequence of the experimental protocol. Food intake is moderately reduced in A-CRF animals consuming a chow containing 0.15% adenine (our unpublished observations) and averaged 16–17 g per day in the present study. In addition, pair-fed rats were administered their daily ration of chow once daily, which obviously had a major impact on the feeding pattern of these animals. Previous studies have shown that food restriction markedly attenuates circadian variations in BP and heart rate (31).

Rats with A-CRF developed increased aortic PWV, indicative of elevated aortic stiffness, independently of vascular wall calcifications. In addition, pulse wave analysis revealed that aortic AI was elevated both in the ascending aorta and at the aortic bifurcation of A-CRF animals, demonstrating that reflected pressure waves from the periphery contributed more to aortic PWV at least partly contributed to increased AI by causing a more rapid return of reflected pressure waves from the periphery, which, in turn, led to a considerable overlap of forward-directed waves and reflected pressure waves in the aorta already during systole. Importantly, as MAP per se has a major impact on aortic PWV (32), we were able to demonstrate that aortic PWV, as well as aortic PP and SBP, were significantly elevated in A-CRF animals during SNP infusion when MAP levels were similar to those in controls. Thus, our observations indicate that elevated aortic stiffness in A-CRF animals was independent of vascular calcifications and increased MAP. However, from the present experiments we cannot rule out that hypertension in A-CRF animals contributed to the development of increased aortic stiffness.

Evidently, the elevations in aortic AI and SBP in A-CRF animals caused an increase in left ventricular afterload that was associated with left ventricular hypertrophy and increased LVEDP. The increase in LVEDP indicates diastolic dysfunction that could be a consequence of impaired myocardial relaxation or decreased compliance. Interestingly, our results are in line with clinical studies demonstrating that enhanced arterial stiffness is an independent risk factor for the development of left ventricular diastolic dysfunction (24).

We have previously shown, using an identical protocol as in the present study, that thoracic aortas from A-CRF animals display a marked reduction in the rate of relaxation ex vivo in response to a variety of different vasodilator stimuli (22). This abnormality was independent of the endothelium and was not present in mesenteric resistance arteries, suggesting an abnormality in vascular smooth muscle cells of the aorta (22). Hence, we speculate that the increase in aortic stiffness observed in A-CRF animals in the present study could be caused by the same functional abnormality in vascular smooth muscle function that was responsible for reduced aortic relaxation rate in our previous report (22). We are at present elucidating these mechanisms further. However, also morphological changes in the aortic wall could at least partly contribute to the increase in aortic stiffness observed in the present study. In addition to an increase in media thickness to lumen radius, A-CRF animals showed fragmentation of elastic lamellae and disorganized smooth muscle cells in the media. Elastin is abundant in the extracellular matrix of the aorta and contributes importantly to the passive elastic properties of large “Windkessel” arteries (6). Hence, the fragmentation of elastic lamellae that was observed in aortas of A-CRF animals may be a factor causing increased vascular stiffness. In our model of A-CRF the vast majority of rats do not develop aortic media calcifications in contrast to rats in other studies (27), in which adenine often has been used specifically to cause severe alterations in mineral and bone metabolism accompanied by vascular calcifications. The discrepant findings are likely explained by the higher concentration of adenine in the chow (0.75%), which routinely has been used by others, leading to more severe renal failure and more pronounced hyperphosphatemia and hyperparathyroidism (27). In general, other investigators have also used chow with higher concentrations of calcium and phosphorous.

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In conclusion, hypertension in A-CRF animals is not renin-dependent and is exaggerated by increased dietary NaCl intake. In addition, A-CRF animals develop increased aortic PWV, clearly indicative of elevated aortic stiffness, in the absence of aortic calcifications.

**Perspectives and Significance**

The major cause of death in CKD is cardiovascular disease, and hypertension and indices of elevated aortic stiffness are powerful risk factors. The experimental model used in the present study bears many resemblances to the clinical syndrome of uremia and may serve as a promising tool for investigating cardiovascular disease mechanisms in CKD. Our results provide novel insights into the mechanisms causing hypertension in this model. Future studies using this model may identify novel more effective antihypertensive therapies in patients with reduced kidney function. In addition, we demonstrated that A-CRF animals developed increased aortic stiffness, which underlines the clinical relevance of this model. Interestingly, aortic stiffness was elevated in the absence of vascular calcifications, indicating that other mechanisms are involved. On the basis of our previous results, we hypothesize that abnormalities in aortic vascular smooth muscle function, which need to be examined further, cause this increase in aortic stiffness.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


