High NaCl diet impairs dynamic renal blood flow autoregulation in rats with adenine-induced chronic renal failure

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Aso Saeed performed statistical analyses and drafted the manuscript. Gerald F. DiBona performed spectral and transfer function analyses. Elisabeth Grimberg carried out the clearance experiments. Elisabeth Grimberg and Lisa Nguy were responsible for the induction of chronic renal failure and monitoring of the animals throughout the study. Minne Line Nedergaard Mikkelsen and Niels Marcussen performed histological analyses and the semiquantitative scoring. Aso Saeed and Gregor Guron designed and planned the studies and have main responsibility for the final version of the manuscript. All of the authors have read the drafted manuscript and contributed with revisions.

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Abstract

This study examined the effects of two weeks of high NaCl diet on kidney function and dynamic renal blood flow autoregulation (RBFA) in rats with adenine-induced chronic renal failure (ACRF). Male Sprague-Dawley rats received either chow containing adenine or were pair-fed an identical diet without adenine (controls, C). After 10 weeks rats were randomized to either remain on the same diet (0.6% NaCl) or to be switched to high 4% NaCl chow. Two weeks after randomization renal clearance experiments were performed under isoflurane anesthesia and dynamic RBFA, baroreflex sensitivity (BRS), systolic arterial pressure variability (SAPV) and heart rate variability (HRV) were assessed by spectral analytical techniques. Rats with ACRF showed marked reductions in GFR and renal blood flow (RBF) whereas mean arterial pressure and SAPV were significantly elevated. In addition, spontaneous BRS was reduced by approximately 50% in ACRF animals. High NaCl diet significantly increased transfer function fractional gain values between arterial pressure and RBF in the frequency range of the myogenic response (0.06-0.09 Hz) only in ACRF animals (0.3±4.0 vs. -4.4±3.8 dB, P<0.05). Similarly, high NaCl diet significantly increased SAPV in the low frequency range only in ACRF animals. To conclude, a two week period of high NaCl diet in ACRF rats significantly impaired dynamic RBFA in the frequency range of the myogenic response and increased SAPV in the low frequency range. These abnormalities may increase the susceptibility to hypertensive end-organ injury and progressive renal failure by facilitating pressure transmission to the microvasculature.

Key words: chronic renal failure, adenine, renal blood flow autoregulation, arterial pressure variability, baroreflex sensitivity
Introduction

Renal blood flow (RBF) autoregulation, mediated mainly by the myogenic response (MR) and the tubuloglomerular feedback mechanism (TGF), stabilizes RBF and glomerular filtration rate (GFR) despite wide variations in arterial pressure (AP) (9, 21).

Renal blood flow autoregulation (RBFA) may also serve a protective function, particularly in hypertension, by preventing transmission of systemic AP to glomerular capillaries (4, 25). A role for autoregulatory capacity of RBF as a determinant of vulnerability to renal injury has been described in the 5/6 renal ablation model in rats (2, 3, 6, 10) and in genetic models characterized by impaired RBFA (37, 38). Notably, in the rat remnant kidney model a renal mass reduction of more than 75 % was needed to impair RBFA and to produce a marked increase in susceptibility to hypertensive renal injury (4). The same investigators observed that in contrast to severe renal mass reduction, uninephrectomy was associated with preserved RBFA and only a modest increase in the susceptibility to hypertensive renal injury (5). In addition to chronic kidney disease, we and others have shown that a high dietary NaCl intake in models of hypertension (15, 22, 34) may also impair RBFA.

The overall objective of the present study was to examine kidney function and dynamic RBFA in rats with adenine-induced chronic renal failure (ACRF) and to determine the impact of a high dietary NaCl intake. Following dietary intake adenine is metabolized to 2,8-dihydroxyadenine which is freely filtered by the glomeruli and crystallizes in tubular fluid leading to tubular obstruction and chronic tubulointerstitial injury (39). Rats with ACRF develop severe renal insufficiency and metabolic abnormalities resembling those typically observed in patients with uremia (14, 23, 24, 27-31), making it an attractive model for investigating pathophysiological mechanisms in chronic renal failure (CRF). The model of ACRF has mainly been used to examine the effects of severe renal failure on mineral and bone metabolism and extrasosseous calcifications (14, 23, 24, 27-29). Only a limited number
of studies have investigated kidney function and renal hemodynamics in detail in this model (13, 19). The model of ACRF may show a higher resemblance to the clinical situation in CRF patients compared to the remnant kidney model as rats typically develop more pronounced reductions in GFR (30, 31). In addition, kidney failure in ACRF rats is caused by homogenous parenchymal injury and not by removal of tissue mass by surgery or renal infarction.

The primary aim of the present study was to assess kidney function and renal hemodynamics, focusing on RBFA, in animals with ACRF and to determine the influence of high NaCl intake. Secondly, using spectral analytical techniques we investigated spontaneous baroreflex sensitivity and variability of heart rate and systolic AP.
Methods

General procedures and protocol

Thirty-nine 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 (30, 31) using a modification of protocols employed by other investigators (14, 23, 24, 27-29, 39). At study start (i.e. day one of the study) animals were provided with standard pelleted rat chow containing adenine (adenine-induced CRF [ACRF], n=20) or identical chow without adenine (pair-fed controls [C], n=19). The chow (R34, Lantmännen, Kimstad, Sweden) contained 0.63 % phosphorous, 0.74 % calcium, 0.53 % potassium and 0.6 % sodium chloride (NaCl). The concentration of adenine in the chow was 0.5 % for the first three weeks followed by 0.3 % for two weeks and 0.15 % thereafter until clearance experiments were performed at about 10-12 weeks after study start and the study terminated. 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 as previously described (30, 31). Rats had free access to tap water throughout the study.

Two weeks prior to clearance experiments animals were randomized either to continue with the same chow as described above with normal NaCl content (NNa, 0.6 % NaCl), or to switch to an identical chow with high NaCl content (HNa, 4.0 % NaCl). Hence, clearance experiments were carried out on four groups of animals: (1) C-NNa (n=9); (2) C-HNa (n=10); (3) ACRF-NNa (n=10) and (4) ACRF-HNa (n=10).

Chemicals were from Sigma (St. Louis, MO, USA) if not stated otherwise. 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 were approved by the regional ethics committee in Gothenburg, Sweden.

Clearance experiments

Animals were anaesthetized with isoflurane (Pharmacia & Upjohn, Stockholm, Sweden) mixed with air during spontaneous breathing by using an isoflurane vaporizer (Univentor-1200, Agnthos, Lidingö, Sweden). For induction and maintenance of anesthesia isoflurane concentrations of approximately 5 % and 1.5 % (vol/vol), respectively, were used. Rats were placed on a heating table and surgically prepared for renal clearance experiments as previously described (32, 34). In brief, a polyethylene catheter (PE 50) inserted via the femoral artery was connected to a pressure transducer (Smiths Medical, Kirchseeon, Germany) for monitoring of arterial pressure [AP, pulsatile and mean (MAP)] and heart rate (HR) using a data acquisition program (Biopac MP 150, Biopac Systems, Santa Barbara, CA, USA). The sample rate for acquisition of AP was 62.5 Hz and no filtering was performed. Infusions of saline and $^{51}$Cr-EDTA ($^{51}$Cr-ethylenediamine tetraacetic acid, Amersham Laboratories, Buckinghamshire, UK) were administered through a femoral vein catheter (PE 50). The left kidney was exposed by a flank incision, immobilized in a plastic cup and the ureter was catheterized (PE 25) for urine collection. A perivascular ultrasonic transit-time flow probe (0.7 VB) was placed around the left renal artery and connected to a flowmeter (Transonic Systems Inc., Ithaca, NY, USA model T206) for measurement of RBF. The sample rate for acquisition of RBF was 62.5 Hz and a lowpass analog prefilter with a cut-off frequency of 30 Hz was used. Glomerular filtration rate (GFR) was determined by measuring renal $^{51}$Cr-EDTA clearance as described (32, 34). Blood was sampled at the start and completion of each 20-minute clearance period and mean values of plasma radioactivity were used to calculate GFR. Arterial blood samples (0.25 ml) were replaced by equivalent volumes of 4 % bovine serum albumin in isotonic saline. Rats were infused with a total volume of 10
ml/kg/h of isotonic saline throughout. Rectal and kidney temperatures were kept at 37° C. Kidney temperature was monitored by a thermocouple probe connected to a digital thermometer model CIE-305 (Obiat Pty. Ltd., Silverwater, Australia). The temperature-sensing probe tip was in direct contact with the kidney surface throughout the experiment and was placed in the cup that was used for immobilization.

After a 45 minute equilibration period two consecutive 20 minute clearance periods were performed after which rats were killed by an overdose of pentobarbital sodium. The hematocrit (Htc) was determined on arterial blood (50 µl) at the start of the first clearance period (ABL 510; Radiometer, Copenhagen, Denmark). The heart and kidneys were immediately excised, weighed and immersion-fixed in 4% neutrally buffered formaldehyde (Histolab Products AB, Gothenburg, Sweden). Renal vascular resistance (RVR) was calculated as MAP (mmHg)/RBF (ml/min/100g body weight [BW]), and filtration fraction (FF) was estimated as GFR/renal plasma flow (RPF) where RPF = RBF x (1-Htc).

Transfer function analysis of RBFA and baroreflex sensitivity

To evaluate dynamic RBFA, data on AP (mmHg) and RBF (ml/min) were sampled at 62.5 Hz from the two clearance periods. After subtracting the mean values from the AP and RBF data files, they were digitally low-pass filtered (5.0 Hz cut-off frequency, finite-impulse response, order 50) and then decimated to a rate of 12.5 Hz. These 12.5 Hz data files were split into blocks of 4,096 data points (frequency discrimination of 0.003 Hz) and were subjected to power spectral and transfer function analyses using methods previously described in detail (34). Processing of data was performed offline using software routines written for Matlab 7.14 (The MathWorks Inc., Natick, MA, USA).

Arterial pressure and RBF data over the range of frequencies for the MR (0.08-0.18) and the TGF mechanism (0.03-0.06) were analyzed (9, 21, 34). The slope of gain decrease in the frequency range of the MR was determined by least squares fitting of the linear regression.
of gain decrease and the phase peak was estimated as the average phase value within the same
frequency interval. In addition, to assess the contribution of the MR to RBFA, mean gain
values in the frequency range of 0.06-0.09 Hz were used to minimize corruption by TGF
(<0.06 Hz) and by myogenic transients (>0.09 Hz) (34, 38).

For assessment of BRS, SAP peaks were identified and the time interval
between successive SAP peaks was taken as the pulse interval (PI). PI values were converted
to instantaneous heart rate values (HR). Artifacts were removed from the SAP peaks and HR
data sets. These irregularly spaced data sets were cubic spline interpolated at 20 Hz, the linear
trends were removed and the date sets were divided into blocks of 8192 data points with 50%
overlap. A Hanning window was applied and power spectral and transfer function analysis
between SBP as input and HR as output were performed using methods similar to those
previously reported (1, 7, 34). Frequency resolution was 0.0012 Hz and the highest frequency
was 5.0 Hz. Within the low frequency range, defined as 0.25 to 0.50 Hz, the frequency with
the highest value of coherence was identified and the values for coherence, gain (bpm/mm
Hg), phase (degrees) and time delay (seconds, calculated from phase) for that frequency were
recorded. Negative phase and time delay values demonstrate that the HR response occurred
after the peak systolic pressure.

Systolic AP variability and heart rate variability by spectral analyses

For spectral analysis of variability of SAP (SAPV) and HR (HRV), two data sets
of 36,000 points (9.6 minutes each) were constructed from each of the two 20 minute
clearance periods, yielding four data sets whose analytical results were averaged. SAP peaks
were identified and the time interval between successive SAP peaks was taken as the pulse
interval (PI), which is equivalent to the R-R interval on the electrocardiogram. Artifacts were
removed from the PI data set, the irregularly spaced PI data set was cubic spline interpolated
at 20 Hz, the linear trend was removed and the data set was divided into blocks of 8192 data
points with 50% overlap. A Hanning window was applied and the power spectral density was calculated using the Welch algorithm. A similar approach was used for the SAP data set.

Three frequency intervals were defined; a very low frequency band (VLF, 0.04-0.2 Hz); a low frequency band (LF, 0.2-0.6 Hz); and a high frequency band (HF, 1.0-3.0 Hz). With respect to SAPV, the VLF band mainly contains power that reflects myogenic vascular function (20, 35). The LF band predominantly encompasses the operating frequency of the arterial baroreflex control of peripheral sympathetic nerve activity and its modulatory effects on the arterial resistance vasculature (20, 35).

**Kidney histology**

Kidneys were processed using routine techniques and 3 μm thick transverse sections through the hilar area were prepared and stained with hematoxylin and eosin, periodic acid-Schiff (PAS) and elastin-vanGieson’s. Histopathological changes were scored semiquantitatively (0-3) by investigators (M-L. N. M. and N. M.) blinded to treatment group. The score 0 was used when no pathologic changes were present, 1 when few of the structures showed changes and the changes were mild, 2 when moderate changes were present, and 3 when severe changes were present in the structures under investigation. From each animal 100 glomeruli were examined and the percent of affected glomeruli was determined. Regarding scoring of interstitial fibrosis and inflammation the following criteria were used: 0, no changes; 1, changes affecting up to 10% of parenchymal area; 2, changes affecting 11 to 25% of parenchymal area, and 3, changes affecting >25% of parenchymal area. The following variables were assessed: intimal thickening of arteries, media thickening of arteries, hyaline deposition/necrosis of arteries, arteriolar hyperplasia, arteriolar hyalinosis, glomerular ischemia, focal glomerulosclerosis, global glomerulosclerosis, interstitial fibrosis, interstitial inflammation, tubular dilatation, tubular atrophy, and medullary atrophy.

**Biochemical analyses**
Plasma and urinary concentrations of sodium, potassium, calcium and phosphate were determined by a Modular P800 Cobas C 701/502 analyzer (Roche/Hitachi, Roche Diagnostics GmbH, Mannheim, Germany).

**Statistical analysis**

Values are means±SD if not stated otherwise. Analyses were performed using two factorial analysis of variance (ANOVA). Normality was tested with the Shapiro-Wilk test and equality of variances was assessed by Levene’s test. For histopathological variables Kruskal-Wallis one-way ANOVA on ranks was used followed by Mann–Whitney U test between pairs of groups (ACRF-NNa versus C-NNa and ACRF-HNa versus ACRF-NNa) when appropriate. A P value <0.05 was considered statistically significant. The statistical software SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) was used.
Results

Organ weights and blood analyses (Table 1)

During the equilibration period of clearance experiments two animals in group ACRF-HNa developed hypotension and died. As expected there was no statistically significant effect of adenine on body weight (BW) as controls were pair fed. Body weights were slightly reduced in groups subjected to high NaCl diet at the time of clearance experimentation. This finding was explained by reduced BWs in both high NaCl groups at study start compared to groups on a normal NaCl diet and did not reflect responses to the actual two week challenge with high NaCl diet. Adenine produced statistically significant increases in left (LVW) and right (RVW) ventricular weight. There was a statistically significant between factors interaction in LVW that was explained by high NaCl intake causing an increase in LVW specifically in ACRF animals. Both wet and dry kidney weights, and kidney water content, were significantly elevated in ACRF animals. High NaCl intake caused a more pronounced increase in wet kidney weight in ACRF animals compared to in controls as indicated by a statistically significant between factors interaction.

The hematocrit (Hct) was significantly reduced in ACRF rats versus controls. High NaCl diet caused a marked reduction in Hct in ACRF animals but not in controls leading to a statistically significant between factors interaction. Plasma levels of potassium and phosphate were significantly elevated in ACRF animals versus controls whereas sodium and calcium concentrations did not differ significantly between groups. There was a statistically significant effect of NaCl intake on plasma phosphate levels in that high NaCl intake tended to increase phosphate concentrations.

Kidney function and renal hemodynamics (Table 2)

Average values for the two consecutive clearance periods are presented.

Adenine produced statistically significant increases in MAP and RVR, and significant
reductions in RBF and GFR. In ACRF animals, the reduction in GFR was relatively more pronounced compared to that in RBF leading to marked reductions in FF. There were statistically significant between factors interactions in GFR, RBF and RVR as high NaCl intake tended to increase GFR and RBF in controls while opposite effects were generally observed in ACRF animals. Adenine produced statistically significant increases in urine output and in fractional urinary excretion of sodium (\( FE_Na \)), potassium (\( FE_K \)) and phosphate (\( FE_Pi \)). High NaCl intake increased urinary sodium excretion (\( UNaV \)), \( FE_Na \) and \( FE_Pi \) specifically in ACRF animals leading to statistically significant between factors interactions.

We observed that MAP decreased during the equilibration period in all groups and hence also analyzed MAP during the initial 5 minutes of the equilibration period. Mean arterial pressures during this period were 110±14, 108±9, 138±12 and 159±13 mmHg in groups C-NNa, C-HNa, ACRF-NNa and ACRF-HNa, respectively (adenine effect \( P<0.001 \), between factors interaction \( P<0.01 \)).

**Dynamic renal blood flow autoregulation (Table 3, Figure 1)**

In the frequency range of the MR (0.08–0.18 Hz), the normal positive-to-negative transition in fractional gain (with decreasing frequency) occurred in all groups and the slope of fractional gain reduction did not differ significantly between groups. However, there were statistically significant between factors interactions in fractional gain (frequency interval 0.06-0.09 Hz) and phase (frequency interval 0.08-0.18 Hz) indicating that high NaCl intake affected the MR differently in ACRF and control animals. The transition in fractional gain from positive to negative values in the frequency range of the MR was attenuated specifically in group ACRF-HNa (right upper panel), and the corresponding local maximum in phase was blunted (right lower panel), indicating an adverse effect of high NaCl intake on the MR specifically in ACRF animals. The signature of the TGF mechanism, characterized by
a peak in fractional gain centered at about 0.03-0.06 Hz, was not clearly detectable in any of
the groups making it difficult to assess the TGF mechanism in the present study.

Baroreflex sensitivity (Figure 2, Table 4)

Maximal coherence was high (>0.85) in all groups. Baroreflex gain, reflecting
baroreflex sensitivity, was significantly reduced by approximately 50 % in both ACRF groups
versus controls.

Systolic arterial pressure variability and heart rate variability (Table 5)

Adenine increased SAPV in both the VLF and LF bands. In addition, there was
a statistically significant between factors interaction in the LF band that was a consequence of
a much more pronounced effect of high NaCl intake on SAPV in ACRF animals compared to
controls. There were no statistically significant effects of adenine or NaCl intake on HRV.

Kidney histology (Figure 3, Table 6)

Arteries and cortical afferent and efferent arterioles appeared normal or showed
very discrete abnormalities in all animals and there were no statistically significant
differences between groups in vascular morphology. There were statistically significant
differences between groups in global glomerulosclerosis and glomerular ischemia, but not in
focal glomerulosclerosis. Global glomerulosclerosis and glomerular ischemia, indicated by
partial or total collapse of the glomerular tuft, were only detected in group ACRF-HNa but
abnormalities were mild and affected less than 5 % of all glomeruli in all rats. Animals with
ACRF showed pronounced, and statistically significant, tubulointerstitial injury characterized
by inflammation, fibrosis, tubular dilatation and atrophy, and medullary atrophy
Discussion

The main finding of the present study was that two weeks of high NaCl diet caused an impairment of the myogenic component of dynamic RBFA specifically in ACRF rats. In addition, SAPV in LF and VLF intervals was increased in ACRF rats versus controls and high NaCl diet aggravated SAPV in the LF range specifically in rats with renal failure.

In the present study we showed that GFR values in our model of ACRF was ≈15% of those in healthy control animals and that the FF was markedly reduced as a consequence of RBF and RPF being less diminished than GFR (RBF in ACRF animals was ≈50% of control values). Presumably, this could be explained by the fact that renal injury in ACRF is caused by tubular obstruction produced by 2, 8-dihydroxyadenine crystals leading to tubulointerstitial damage. Hence, increased hydrostatic pressure in Bowman’s space and a reduced filtration pressure gradient across the glomerular capillary wall probably explains why GFR was relatively more affected. In accord with the severe tubulointerstitial injury in ACRF animals there was a ≈5-fold increase in urine output. Notably, in spite of marked reductions in GFR, normal absolute urinary excretion rates of sodium, potassium and P\textsubscript{i} were maintained in ACRF as a consequence of prominent increases in fractional urinary excretion rates of these ions. We have previously shown that ACRF rats on a normal dietary sodium intake have elevated plasma levels of aldosterone and parathyroid hormone (31) and likely these hormonal changes, together with tubular cell injury per se, contributed to the high rates of FE\textsubscript{K} and FE\textsubscript{Pi}. The single kidney GFR values in the present study are in good agreement with our previously published data (30) on total renal creatinine clearance obtained on conscious rats using metabolic cages (≈0.8 and ≈0.1 ml/min/100 g BW in controls and ACRF rats, respectively). These data indicate that anesthesia had no major effect on GFR in the present study. Notably, during clearance experiments absolute rates of urinary sodium excretion did not differ as much as expected between groups on high and normal NaCl diets.
One likely explanation for this discrepancy, at least in ACRF rats, was the relatively more pronounced fall in AP in group ACRF-HNa vs. ACRF-NNa under anesthesia during the equilibration period. The greater AP reduction in ACRF-HNa is anticipated to reduce urinary sodium excretion e.g. by the pressure-natriuresis mechanism.

In agreement with our previous studies, ACRF rats were hypertensive, had left ventricular hypertrophy and increased RVR (30, 31). There was no statistically significant effect of adenine on transfer function fractional gain values (AP to RBF) in the frequency range of the MR or the TGF indicating that dynamic RBFA was not altered by ACRF per se. However, the effect of high NaCl diet on dynamic RBFA was clearly different in ACRF animals compared to controls and was characterized by elevated transfer function fractional gain values, and a diminished phase peak, in the frequency range of the MR (Figure 1). These results demonstrate a synergistic, detrimental, effect of the combination of high NaCl and ACRF on the myogenic component of dynamic RBFA. This abnormality is anticipated to make kidneys of ACRF-HNa animals more vulnerable to fluctuations in AP and could contribute to progressive kidney injury by facilitating transmission of systemic AP to the renal microvasculature (4). Indeed, although glomerular histological changes were modest in ACRF animals in the present study, glomerulosclerosis and glomerular ischemia that are pathological features characteristic of hypertensive renal injury, were only detected in the ACRF-HNa group (Table 6). One may speculate that a duration of high NaCl intake longer than two weeks might have caused more pronounced glomerular abnormalities. Previous studies by Bidani and co-workers (2, 3, 6, 10) have shown that RBFA is impaired in the 5/6 renal ablation model in rats and associated with a marked increase in susceptibility to hypertensive renal injury. However, uninephrectomized rats, with preserved RBFA, showed only a modest increase in susceptibility to injury (5). These studies demonstrate a relationship between impaired RBFA and susceptibility to hypertensive renal injury in kidney disease models.
Increased susceptibility to hypertensive renal injury has also been documented in genetic models exhibiting impaired RBFA in which there was no pre-existing kidney disease (37, 38). In contrast to findings in the remnant kidney model, ACRF rats on a normal dietary NaCl intake did not develop abnormal dynamic RBFA in spite of having marked reductions in GFR. This is most likely explained by the apparent differences between the models where the ACRF model is characterized by uniform tubulointerstitial injury. On the contrary, the remnant kidney model, which is produced by a severe reduction of functional renal mass by surgery or renal infarction, is typified by adaptive hyperfiltration and glomerular hypertension in remaining viable nephrons (11, 12). These adaptive changes, which most likely are less in the ACRF model, are a consequence of preglomerular vasodilation that may interfere with normal RBFA.

The mechanisms by which high NaCl impaired the myogenic component of dynamic RBFA in ACRF animals in the present study remain to be elucidated. Groups ACRF-HNa and ACRF-NNa showed similar reductions in GFR indicating that worsened renal failure was not a cause. Although MAP was comparable during clearance periods in groups ACRF-HNa and ACRF-NNa we observed that MAP was significantly elevated in the ACRF-HNa group early during the equilibration period soon after arterial catheterization suggesting that animals in this group might have had higher AP in the wake state and that they were more sensitive to isoflurane anesthesia. In agreement with these observations we have previously demonstrated by radiotelemetry technique that unanesthetized ACRF rats develop more severe hypertension in response to a 4 % NaCl diet (30). Thus, it is possible that a more severe hypertension in group ACRF-HNa disrupted the functional integrity of the renal resistance vasculature and impaired the MR. However, our histological analysis did not reveal any significant changes between groups in renal arterial morphology making this hypothesis less probable. In addition, one may speculate that the more pronounced reduction in AP in
ACRF-HNa rats during the equilibration period might have interfered with the myogenic component of RBFA by producing preglomerular vasodilation. However, it is important to recognize that our transfer function analysis data reflect dynamic changes in RBF in response to spontaneous fluctuations in AP, i.e. dynamic RBFA. Thus, dynamic RBFA data do not indicate a certain steady-state level of RBF at a certain steady-state level of AP but more likely predicts whether the dynamic response of RBF following a dynamic change in AP will be normal regardless of the prevailing arterial tone. Similar to the present study we have previously described a synergistic negative effect of chronic angiotensin II infusion and high dietary NaCl intake on the MR of RBFA in rats (34). The ACRF model is however characterized by suppressed plasma renin activity (30, 31) suggesting that other mechanisms were involved in the present study. It is possible that plasma volume expansion caused by elevated dietary NaCl intake in ACRF rats triggered adaptive responses in glomerular hemodynamics in viable nephrons leading to hyperfiltration in an attempt to maintain volume homeostasis and that these adaptations impaired RBFA. Thus, similar pathophysiological mechanism as in 5/6 nephrectomy rats on a normal NaCl intake might be involved in ACRF rats challenged with a NaCl load.

ACRF rats showed a marked reduction in cardiac baroreflex sensitivity and increased SAPV in the LF (0.2-0.6 Hz) and VLF (0.04-0.2 Hz) intervals. In addition, high NaCl diet enhanced SAPV significantly in the LF interval, and tended to do so also in the VLF range, only in ACRF rats. The reduced cardiac baroreflex sensitivity in ACRF animals could have been caused by chronically increased AP considering the well-established relation between hypertension and the development of baroreflex dysfunction (8). We have previously shown that ACRF rats develop elevated aortic pulse-wave velocity indicative of enhanced aortic stiffness (30), and increased media thickness of the thoracic aorta (30, 31), and these changes presumably could have contributed to the blunted baroreflex sensitivity.
Interestingly, reduced baroreflex sensitivity has been demonstrated in patients with CRF (17, 36) and has also been shown to be an independent risk factor for sudden cardiac death in this patient group (18) underlining the clinical relevance of our model. Similarly, enhanced AP variability has been identified as an independent cardiovascular risk factor by enhancing end-organ damage in hypertensive individuals (33) and also in patients with chronic kidney disease (26). The increased SAPV in the LF interval suggests an increased modulation of arterial resistance by the sympathetic nervous system in ACRF animals considering previous studies demonstrating that AP fluctuations in this frequency interval are mainly attributed to the influence of sympathetic activity (20). Thus, our data indirectly indicate an increased sympathetic activity to the vasculature in ACRF rats, in particular in the group subjected to high NaCl intake, and suggest a role for increased sympathetic activity as a cause of increased AP variability and possibly also hypertension. The increased SAPV in the LF interval might also be a direct consequence of a blunted arterial baroreflex as previous studies have shown that sinoaortic deafferentation increases AP variability in the LF interval (16). The mechanisms responsible for AP fluctuations in the VLF are less well understood and a number of factors, including vascular myogenic activity, have been suggested to be involved (35).

There are some limitations of the present study. First, previous studies (5) have shown that the susceptibility to hypertensive renal injury in models of reduced renal mass correlates with reduced RBFA capacity demonstrated by traditional steady state methods using pressure ladders, and not by abnormalities detected by dynamic transfer function techniques. This discrepancy may be explained by the fact that dynamic studies of RBFA do not assess total autoregulatory capacity but instead mainly provide information on the mechanisms of RBFA and its dynamic properties. Taken together, our results should be interpreted with caution as the defects in dynamic RBFA may not translate into an increased
vulnerability to hypertensive glomerular injury. Secondly, as renal injury in the ACRF model is characterized by chronic tubulointerstitial damage secondary to tubular obstruction the clinical implications of our results are mainly restricted to comparable kidney diseases such as chronic obstructive nephropathy. One should also take into consideration that the pathophysiological importance of impairments in RBFA for disease progression has mainly been demonstrated in kidneys diseases that primarily affect the glomeruli.

In conclusion, a two week period of high NaCl diet in ACRF rats significantly impaired dynamic RBFA in the frequency range of the myogenic response and aggravated SAPV in the LF range. Thus, high NaCl diet in rats with CRF caused, or aggravated, abnormalities in cardiovascular regulation that are likely to produce an added risk of end-organ damage beyond the effects of increased average MAP per se.

**Perspectives and Significance**

In chronic kidney disease hypertension is common and may, if not effectively treated, exacerbate renal injury causing a vicious circle. The pathophysiological mechanisms of hypertensive renal injury are multiple and incompletely understood (3, 4, 17, 25, 33). The findings in the present study suggest that high NaCl intake in an experimental model of chronic tubulointerstitial disease increase the susceptibility to hypertensive renal injury by enhancing SAPV and impairing RBFA. Thus, in patients with hypertension and chronic kidney disease caused by tubulointerstitial injury the avoidance of high NaCl intake might protect from progressive renal injury. Further investigations are needed to elucidate exact mechanisms underlying these abnormalities. Presumably, novel therapies targeting these mechanisms could have renoprotective effects of clinical relevance.
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Disclosures

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


Conte G, and Zoccali C. Long-term visit-to-visit office blood pressure variability increases


Figure legends

Figure 1. Transfer function gain and phase between arterial pressure and renal blood flow.

Fractional gain (upper panels) and phase (lower panels) between arterial pressure and renal blood flow in isoflurane-anesthetized Sprague-Dawley rats with adenine-induced chronic renal failure (ACRF) or pair-fed controls. Data are from clearance experiments performed approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). The transition in fractional gain from positive to negative values in the frequency range of the myogenic response (MR, 0.08-0.18 Hz) was attenuated specifically in group ACRF-HNa (right upper panel), and the corresponding local maximum in phase was blunted (right lower panel), indicating an adverse effect of high NaCl intake on the MR specifically in ACRF animals. Values are means±SEM. Numerical data (mean ±SD) for gain, phase and the slope of fractional gain reduction in the frequency range of the MR are presented in table 3.

Figure 2. Transfer function gain between arterial pressure and heart rate (baroreflex sensitivity).

Transfer function gain between arterial pressure and heart rate, a measure of baroreflex sensitivity, in isoflurane-anesthetized Sprague-Dawley rats with adenine-induced chronic renal failure (ACRF) or pair-fed controls (C). Data are from clearance experiments performed approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Gain values were reduced by approximately 50 %
in ACRF groups versus controls and two factorial ANOVA revealed a statistically significant

effect of adenine (P<0.05). Numerical data for the transfer function analysis are presented in

Table 4. Values are means±SD.

**Figure 3. Kidney histology**

Kidney cortex of representative animals from groups C-Na (A), ACRF-Na (B) and ACRF-HNa (C). Sections were stained with periodic acid-Schiff (PAS) and

magnifications are x 200. Kidneys from ACRF animals showed a grossly distorted

architecture mainly characterized by tubular atrophy and dilatation and interstitial fibrosis and

inflammation. Arrowheads show tubular casts consisting of 2,8 dihydroxyadenine. Glomeruli

and arteries (arrows) in ACRF animals had a normal appearance or showed very mild

abnormalities. There were no significant differences between groups ACRF-Na and ACRF-

HNa. Semiquantitative histological data are presented in Table 6.
Table legends

Table 1. Organ weights, hematocrit and plasma electrolytes.

Data are from clearance experiments performed approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Presented kidney weights (KW) are from the left kidney. Data on hematocrit and plasma electrolytes are average values of two consecutive 20 min clearance periods. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; LV, left ventricle; RV, right ventricle; and Hct, hematocrit.

Table 2. Left kidney function and renal hemodynamics

Clearance experiments were performed on the left kidney of isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Single (left) kidney data are presented as average values of two consecutive 20 min clearance periods. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; MAP, mean arterial pressure; GFR, glomerular filtration rate; BW, body weight; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; UV, urine flow rate; U_{NaV}, urinary Na excretion; U_{K,V}, urinary potassium excretion; U_{Pi,V}, urinary phosphate excretion; FE_{Na}, fractional urinary excretion of Na; FE_{K}, fractional urinary excretion of potassium; FE_{Pi}, fractional urinary excretion of phosphate.
Table 3. Characteristics of the transfer function between arterial pressure and renal blood flow.

Clearance experiments were performed on isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Data are from the left kidney and are average values of two consecutive 20 min clearance periods. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. Fractional gain values in the frequency range 0.06-0.18 Hz reflect the regulatory action of the myogenic response. ACRF indicates adenine-induced chronic renal failure; and C pair-fed controls.

Table 4. Characteristics of the transfer function between arterial pressure and heart rate

Data are from clearance experiments performed on isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Spontaneous baroreflex function was assessed by transfer function analyses within the low frequency range, defined as 0.25 to 0.50 Hz (see Methods). All measures were taken at the frequencies of maximal coherence. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; and C pair-fed controls.

Table 5. Systolic arterial pressure variability and heart rate variability

Data are from clearance experiments performed on isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow
with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Presented data are systolic arterial pressure variability (SAPV) and heart rate variability (HRV) within different frequency intervals (see Methods). Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; VLF, very low frequency; LF, low frequency; and HF, high frequency.

Table 6. Kidney histology

Animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet or to be switched to high (4 %) NaCl chow. Following clearance experiments kidneys were immersion fixed and histological abnormalities were scored semiquantitatively using a scale from 0 to 3 (see Methods). Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; NNa, normal NaCl chow; HNa, high NaCl chow, K-W test, Kruskal-Wallis test. * denotes P<0.05 ACRF-NNa vs. C-NNa by Mann-Whitney test. There were no statistically significant differences between groups ACRF-HNa and ACRF-NNa in any of the examined variables.
Figure 2.
Figure 3.
Table 1. Organ weights, hematocrit and plasma electrolytes.

<table>
<thead>
<tr>
<th></th>
<th>C-NNa (n=9)</th>
<th>C-HNa (n=10)</th>
<th>ACRF-NNa (n=10)</th>
<th>ACRF-HNa (n=8)</th>
<th>ANOVA effects:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADENINE</td>
<td>NaCl intake</td>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>369±31</td>
<td>343±23</td>
<td>387±27</td>
<td>346±32</td>
<td>ns</td>
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<tr>
<td>LVW, g/kg BW</td>
<td>2.33±0.24</td>
<td>2.25±0.12</td>
<td>3.24±0.35</td>
<td>3.54±0.40</td>
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<td>RVW, g/kg BW</td>
<td>0.54±0.09</td>
<td>0.56±0.09</td>
<td>0.66±0.08</td>
<td>0.71±0.13</td>
<td>P&lt;0.001</td>
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<tr>
<td>KWwet, g/kg BW</td>
<td>2.68±0.30</td>
<td>2.74±0.20</td>
<td>5.03±1.06</td>
<td>6.20±0.67</td>
<td>P&lt;0.001</td>
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<tr>
<td>KWdry, g/kg BW</td>
<td>1.18±0.10</td>
<td>1.15±0.06</td>
<td>1.51±0.29</td>
<td>1.60±0.18</td>
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<td>Kidney water, %</td>
<td>56±3</td>
<td>58±2</td>
<td>69±5</td>
<td>74±2</td>
<td>P&lt;0.001</td>
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<tr>
<td>Htc, %</td>
<td>43±2</td>
<td>43±2</td>
<td>31±6</td>
<td>16±3</td>
<td>P&lt;0.001</td>
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<tr>
<td>P-Na, mmol/l</td>
<td>144±1</td>
<td>144±3</td>
<td>143±2</td>
<td>145±2</td>
<td>ns</td>
</tr>
<tr>
<td>P-K, mmol/l</td>
<td>4.5±0.2</td>
<td>4.7±0.2</td>
<td>6.5±1.0</td>
<td>5.8±0.8</td>
<td>P&lt;0.001</td>
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<tr>
<td>P-Ca, mmol/l</td>
<td>2.35±0.06</td>
<td>2.40±0.07</td>
<td>2.31±0.08</td>
<td>2.28±0.36</td>
<td>ns</td>
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<tr>
<td>P-Pi, mmol/l</td>
<td>1.7±0.1</td>
<td>1.8±0.1</td>
<td>2.2±0.2</td>
<td>2.6±0.6</td>
<td>P&lt;0.001</td>
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</tbody>
</table>

Data are from clearance experiments performed approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on
the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Presented kidney weights (KW) are from the left kidney. Data on hematocrit and plasma electrolytes are average values of two consecutive 20 min clearance periods. Values are means±SD. Main effects and between factors interaction from two factorial ANOVA are presented. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; LV, left ventricle; RV, right ventricle; and Hct, hematocrit.
Table 2. Left kidney function and renal hemodynamics

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<th>ACRF-HNa (n=8)</th>
<th>ANOVA effects:</th>
<th>Adenine</th>
<th>NaCl intake</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>98±6</td>
<td>92±6</td>
<td>111±6</td>
<td>110±14</td>
<td>P&lt;0.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>343±15</td>
<td>303±19</td>
<td>324±13</td>
<td>299±45</td>
<td>ns</td>
<td>P&lt;0.001</td>
<td>ns</td>
<td>P&lt;0.01</td>
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<tr>
<td>GFR, ml/min/100g BW</td>
<td>0.24±0.06</td>
<td>0.31±0.03</td>
<td>0.04±0.03</td>
<td>0.04±0.01</td>
<td>P&lt;0.001</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
<td>ns</td>
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<td>RBF, ml/min/100g BW</td>
<td>1.34±0.33</td>
<td>1.71±0.25</td>
<td>0.87±0.22</td>
<td>0.74±0.14</td>
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<td>ns</td>
<td>P&lt;0.01</td>
<td>ns</td>
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<td>RVR, mmHg/ml/min/100g BW</td>
<td>77±21</td>
<td>55±10</td>
<td>133±29</td>
<td>152±28</td>
<td>P&lt;0.001</td>
<td>ns</td>
<td>P&lt;0.05</td>
<td>ns</td>
</tr>
<tr>
<td>FF, %</td>
<td>33±5</td>
<td>33±4</td>
<td>7±5</td>
<td>6±3</td>
<td>P&lt;0.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>UV, ul/min/100g BW</td>
<td>1.04±0.41</td>
<td>1.08±0.27</td>
<td>4.69±1.94</td>
<td>5.73±1.80</td>
<td>P&lt;0.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>UNaV, umol/min/100g BW</td>
<td>0.12±0.08</td>
<td>0.17±0.08</td>
<td>0.15±0.11</td>
<td>0.42±0.18</td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
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<tr>
<td>UKV, umol/min/100g BW</td>
<td>0.27±0.08</td>
<td>0.27±0.08</td>
<td>0.32±0.15</td>
<td>0.23±0.06</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>UPiV, umol/min/100g BW</td>
<td>0.05±0.02</td>
<td>0.05±0.03</td>
<td>0.03±0.02</td>
<td>0.06±0.02</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FENa, %</td>
<td>0.34±0.24</td>
<td>0.38±0.14</td>
<td>3.14±2.76</td>
<td>8.69±2.81</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>FEK, %</td>
<td>26±9</td>
<td>18±4</td>
<td>147±51</td>
<td>123±27</td>
<td>P&lt;0.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>FEPi, %</td>
<td>10±3</td>
<td>8±6</td>
<td>46±19</td>
<td>68±17</td>
<td>P&lt;0.001</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
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</tr>
</tbody>
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Clearance experiments were performed on the left kidney of isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6%) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4%) NaCl chow (HNa). Single (left) kidney data are presented as average values of two consecutive 20 min clearance periods. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; MAP, mean arterial pressure; GFR, glomerular filtration rate; BW, body weight; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; UV, urine flow rate; $U_{Na}V$, urinary Na excretion; $U_{K}V$, urinary potassium excretion; $U_{Pi}V$, urinary phosphate excretion; $FE_{Na}$, fractional urinary excretion of Na; $FE_{K}$, fractional urinary excretion of potassium; $FE_{Pi}$, fractional urinary excretion of phosphate.
Table 3. Characteristics of the transfer function between arterial pressure and renal blood flow

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<th>ACRF-HNa (n=8)</th>
<th>ANOVA effects:</th>
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</thead>
<tbody>
<tr>
<td>Gain slope 0.08-0.18 Hz, dB/decade</td>
<td>17.7±8.6</td>
<td>11.4±13.7</td>
<td>19.4±16.9</td>
<td>10.3±8.5</td>
<td>ns</td>
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<tr>
<td>Gain 0.06-0.09 Hz, dB</td>
<td>-3.1±2.7</td>
<td>-3.9±3.1</td>
<td>-4.4±3.8</td>
<td>0.3±4.0</td>
<td>ns</td>
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<tr>
<td>Phase 0.08-0.18 Hz, degrees</td>
<td>45.7±17.1</td>
<td>43.5±12.8</td>
<td>60.0±17.6</td>
<td>36.0±15.4</td>
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</table>

Clearance experiments were performed on isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Data are from the left kidney and are average values of two consecutive 20 min clearance periods. Presented data in the frequency range 0.06-0.18 Hz reflect the regulatory action of the myogenic response. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; and C pair-fed controls.
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<th>ACRF-HNa (n=8)</th>
<th>ANOVA effects:</th>
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<tr>
<td>Maximal coherence</td>
<td>0.92±0.05</td>
<td>0.94±0.05</td>
<td>0.85±0.12</td>
<td>0.96±0.04</td>
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<tr>
<td>Frequency at maximal coherence, Hz</td>
<td>0.32±0.05</td>
<td>0.32±0.05</td>
<td>0.33±0.07</td>
<td>0.33±0.05</td>
<td>ns</td>
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<tr>
<td>Gain, bpm/mmHg</td>
<td>0.84±0.80</td>
<td>0.92±0.62</td>
<td>0.40±0.35</td>
<td>0.43±0.25</td>
<td>P&lt;0.05</td>
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<tr>
<td>Phase, degrees</td>
<td>-98.5±53.2</td>
<td>-102.8±63.2</td>
<td>-113.2±47.3</td>
<td>-72.2±44.0</td>
<td>ns</td>
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<tr>
<td>Time delay, s</td>
<td>-0.85±0.47</td>
<td>-0.90±0.56</td>
<td>-1.0±0.47</td>
<td>-0.61±0.32</td>
<td>ns</td>
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</tbody>
</table>

Data are from clearance experiments performed on isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Spontaneous baroreflex function was assessed by transfer function analyses within the low frequency range, defined as 0.25 to 0.50 Hz (see Methods). All measures were taken at the frequencies of maximal coherence. Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; and C pair-fed controls.
Table 5. Systolic arterial pressure variability and heart rate variability

<table>
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<th>C-NNa (n=9)</th>
<th>C-HNa (n=10)</th>
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<th>ACRF-HNa (n=8)</th>
<th>ANOVA effects:</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAPV, VLF (0.04-0.2 Hz), mmHg²</td>
<td>1.90±1.16</td>
<td>2.45±1.58</td>
<td>3.37±1.74</td>
<td>12.13±16.38</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>SAPV, LF (0.2-0.6 Hz), mmHg²</td>
<td>5.17±3.44</td>
<td>5.28±1.37</td>
<td>8.57±6.82</td>
<td>34.10±29.86</td>
<td>P&lt;0.01 P&lt;0.05</td>
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<td>HRV, VLF (0.04-0.2 Hz), ms²</td>
<td>0.41±0.26</td>
<td>0.37±0.19</td>
<td>1.46±2.79</td>
<td>0.23±0.20</td>
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<td>HRV, LF (0.2-0.6 Hz), ms²</td>
<td>0.81±0.44</td>
<td>0.87±0.81</td>
<td>1.26±0.65</td>
<td>0.92±1.02</td>
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<tr>
<td>HRV, HF (1.0-3.0 Hz), ms²</td>
<td>4.48±2.48</td>
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<td>4.22±2.51</td>
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<tr>
<td>HRV, LF/HF</td>
<td>0.26±0.23</td>
<td>0.37±0.56</td>
<td>0.40±0.35</td>
<td>0.34±0.29</td>
<td>ns ns ns</td>
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</table>

Data are from clearance experiments performed on isoflurane-anesthetized animals approximately 12 weeks after study start (see Methods). All animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet (NNa) or to be switched to high (4 %) NaCl chow (HNa). Presented data are systolic arterial pressure variability (SAPV) and heart rate variability (HRV) within different frequency intervals (see Methods). Main effects and between factors interaction from two factorial ANOVA are presented. Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; VLF, very low frequency; LF, low frequency; and HF, high frequency.
### Table 6. Kidney histology

<table>
<thead>
<tr>
<th>Condition</th>
<th>C-NNa (n=4)</th>
<th>C-HNa (n=10)</th>
<th>ACRF-NNa (n=6)</th>
<th>ACRF-HNa (n=10)</th>
<th>K-W test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar hyperplasia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td>Arteriolar hyalinosis</td>
<td>0</td>
<td>0</td>
<td>0.17±0.41</td>
<td>0.40±0.70</td>
<td>ns</td>
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<tr>
<td>Intimal thickening of arteries</td>
<td>0</td>
<td>0.10±0.32</td>
<td>0</td>
<td>0.10±0.32</td>
<td>ns</td>
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<tr>
<td>Medial thickening of arteries</td>
<td>0</td>
<td>0.70±0.48</td>
<td>0.33±0.52</td>
<td>0.50±0.53</td>
<td>ns</td>
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<tr>
<td>Hyaline deposition/necrosis of arteries</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.30±0.67</td>
<td>ns</td>
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<tr>
<td>Global glomerulosclerosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.40±0.52</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Focal glomerulosclerosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.20±0.42</td>
<td>ns</td>
</tr>
<tr>
<td>Glomerular ischemia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.50±0.53</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0</td>
<td>0</td>
<td>2.50±0.55*</td>
<td>2.30±0.48</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>0</td>
<td>0</td>
<td>2.50±0.55*</td>
<td>2.90±0.32</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Tubular dilatation</td>
<td>0</td>
<td>0</td>
<td>2.00±0*</td>
<td>2.50±0.53</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0</td>
<td>0</td>
<td>2.50±0.55*</td>
<td>2.40±0.52</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Medullary atrophy</td>
<td>0</td>
<td>0</td>
<td>1.67±0.52*</td>
<td>2.40±0.52</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>
Animals received chow with a normal (0.6 %) NaCl content from study start until two weeks prior to clearance experiments when animals were randomized to either remain on the same diet or to be switched to high (4 %) NaCl chow. Following clearance experiments kidneys were immersion fixed and histological abnormalities were scored semiquantitatively using a scale from 0 to 3 (see Methods). Values are means±SD. ACRF indicates adenine-induced chronic renal failure; C, pair-fed controls; NNa, normal NaCl chow; HNa, high NaCl chow, K-W test, Kruskal-Wallis test. * denotes P<0.05 ACRF-NNa vs. C-NNa by Mann-Whitney test. There were no statistically significant differences between groups ACRF-HNa and ACRF-NNa in any of the examined variables.