Arrhythmogenic Remodeling in Murine Models of Deoxycorticosterone Acetate-Salt-Induced and 5/6-Subtotal Nephrectomy-Salt-Induced Cardiorenal Disease

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Abstract
Background: Renal failure is associated with adverse cardiac remodeling and sudden cardiac death. The mechanism leading to enhanced arrhythmogenicity in the cardiorenal syndrome is unclear. The aim of this study was to characterize electrophysiological and tissue alterations correlated with enhanced arrhythmogenicity in two distinct mouse models of renal failure.

Methods: Thirty-week-old 129Sv mice received a high-salt diet and deoxycorticosterone acetate (DOCA) for 8 weeks, followed by an additional period of high-salt diet for 27 weeks (DOCA-salt aged model). Adult CD-1 mice were submitted to 5/6-subtotal nephrectomy (SNx) and treated for 11 weeks with a high-salt diet (SNx-salt adult model). Vulnerability to arrhythmia as well as conduction velocities (CVs) of the hearts were determined ex vivo with epicardial mapping. Subsequently, the hearts were characterized for connexin 43 (Cx43) and fibrosis.

Results: DOCA-salt and SNx-salt mice developed renal dysfunction characterized by albuminuria. Heart, lung and kidney weights were increased in DOCA-salt mice. Both DOCA-salt and SNx-salt mice were highly susceptible to ventricular arrhythmias. DOCA-salt mice had a significant decrease in both longitudinal and transversal CV in the left ventricle. Histological analysis revealed a significant reduction in Cx43 expression as well as an increase in interstitial fibrosis in both DOCA-salt and SNx-salt mice. Conclusion: DOCA-salt and SNx-salt treatment
induced renal dysfunction, which resulted in structural and electrical cardiac remodeling and enhanced arrhythmogenicity. The reduced Cx43 expression and increased fibrosis levels in these hearts are likely candidates for the formation of the arrhythmogenic substrate.

Introduction

The cardiorenal syndrome is a condition characterized by the influence of a diseased kidney on the heart and vice versa, which can lead to progression of failure of both organs [1]. Patients with renal failure have an increased mortality risk due to cardiovascular disease [2]. Around 25% of all mortality of dialysis patients is caused by sudden cardiac death, mostly arising from arrhythmias such as ventricular tachycardia or fibrillation [3–5]. The mechanism leading to enhanced arrhythmogenicity in the cardiorenal syndrome is unclear.

Cardiac arrhythmias can be caused by three basic mechanisms: enhanced automaticity, triggered activity or reentry [6]. Reentry-based arrhythmias are responsible for the majority of ventricular arrhythmias and are often observed in dialysis patients [3, 6]. Typical electrophysiological characteristics of reentry are slow impulse conduction, conduction block and a lower effective refractory period. Hearts prone to reentrant arrhythmias typically show reduced levels and a heterogeneous distribution of the gap junction protein connexin 43 (Cx43) as well as the presence of fibrotic tissue [6–8].

The aim of this study was to characterize electrophysiological alterations and cardiac remodeling correlated with enhanced arrhythmogenicity on the background of renal failure. Therefore, two mouse models of renal dysfunction with different etiologies were tested: (1) an aldosterone-induced hypertension model using deoxycorticosterone acetate (DOCA) in combination with a high-salt diet in aged mice (DOCA-salt aged model) and (2) a more severe model using 5/6-subtotal nephrectomy (SNx) in combination with a high-salt diet in adult mice (SNx-salt adult model).

Methods

Ethics Statement and Animal Housing

All the experimental protocols were performed in accordance with the national guidelines and approved by the local Ethics Animal Experimental Committee of the University of Utrecht, The Netherlands (approval No. 2010.II.11.201 and 2012.II.10.154). All animals were housed under standard conditions in a light-, temperature- and humidity-controlled environment.

Animal Models of Renal Dysfunction

DOCA-Salt Aged Mice

Thirty-week-old male 129Sv mice were used (Harlan Laboratories, Horst, The Netherlands). Renal dysfunction was induced in the mice (experimental week 0) by the combination of a DOCA pellet (3.3 mg/day) and a high-salt diet (chow containing 3% NaCl) for a period of 8 weeks (n = 7). At experimental week 8, the DOCA pellet was removed and the high-salt diet withdrawn. At experimental week 11, the high-salt diet (6% NaCl) was continued until termination at experimental week 38 (68-week-old mice). Untreated age-matched mice were used as controls (n = 4).

SNx-Salt Adult Mice

Adult (8-week-old) male CD-1 mice were used (Charles River Laboratories, Sulzfeld, Germany). SNx was performed in two steps. At week –1, the right kidney was surgically removed (uninephrectomy), followed by removal of the poles from the left kidney at week 0. To the SNx-salt group, a high-salt diet (chow containing 6% NaCl) was given from week 1 until sacrifice at week 11 (n = 5), whereas 9 mice underwent only SNx (SNx group).
Renal Function and Arterial Pressure Evaluation

To collect 16-hour urine, mice were placed in metabolic cages with food and water; values are expressed as per 24 h. Albumin was measured with a mouse albumin ELISA kit (Bethyl Laboratories Inc., Montgomery, Tex., USA). Systolic blood pressure and mean arterial pressure were measured using tail-cuff plethysmography and a catheter directly inserted into the femoral artery, respectively. Blood samples were collected by cheek puncture. Plasma urea was determined by DiaSys Urea CT FS (DiaSys Diagnostic Systems, Holzheim, Germany).

Echocardiography and Epicardial Mapping of Langendorff Perfused Hearts

Echocardiography was performed to determine cardiac function using a Vevo 2100 device (VisualSonics Inc., Toronto, Ont., Canada) with an MS550D transducer. Before termination, the mice were anesthetized with 4–5% isoflurane in oxygen and air. Next, the heart was excised and attached to a Langendorff retrograde perfusion setup. The heart was continuously perfused with a carbogen-gassed buffer at 37 °C, composed of (in mM): NaCl 116, KCl 5, MgSO₄ 1.1, NaH₂PO₄ 0.35, NaHCO₃ 27, glucose 10, mannitol 16 and CaCl₂ 1.8. Extracellular electrograms were recorded during stimulation (2 × stimulation threshold) from the center of a 19 × 13 multielectrode grid, both from the left ventricle (LV) and right ventricle (RV), as described previously [9]. The conduction velocity (CV) was determined off-line from the recorded electrograms, as described previously [7], using custom-made software based on MATLAB (The MathWorks Inc., Natick, Mass., USA). Susceptibility to arrhythmia was provoked by programmed electrical stimulation using a standardized protocol published earlier [7].

Immunohistochemistry and Histology

After the epicardial mapping procedure, the hearts were snap frozen in liquid nitrogen. For immunohistochemistry and fibrosis detection, cryosections of the heart (10 μm thickness) were prepared. Immunolabeling was performed to assess the subcellular distribution of Cx43, as described previously [9], using rabbit polyclonal anti-Cx43 (71-0700; Invitrogen, Carlsbad, Calif., USA) and mouse monoclonal anti-N-cadherin (C1821; Sigma-Aldrich, Saint Louis, Mo., USA) antibodies. Cx43 levels are expressed as the area immunolabeled for Cx43 normalized to the control. For cardiac fibrosis detection, cryosections were fixed with 4% paraformaldehyde and stained with Picrosirius red, as described previously [10]. The cryosections were visualized by light microscopy (Nikon Eclipse 80i; Nikon Europe B.V., Amstelveen, The Netherlands) and digitally analyzed using ImageJ software. The percentage of fibrosis was calculated as the area stained by Picrosirius red relative to the total tissue area.

Statistical Analysis

Data are presented as means ± standard errors of the mean and analyzed by Student’s t test or Fisher’s exact test using GraphPad Prism 6 software (La Jolla, Calif., USA). Differences were considered statistically significant if p < 0.05. Animals that did not complete the experiment were excluded from analysis.

Results

Arrhythmogenic Remodeling in DOCA-Salt Aged Mice

Renal Dysfunction, Morphological and Echocardiographic Data

DOCA-salt treatment of aged mice resulted in renal dysfunction confirmed by a significant increase of albumin in the urine (4.8 ± 1.4 vs. 0.1 ± 0.02 mg/24 h, p < 0.05; fig. 1a). DOCA-salt mice developed hypertension, detected by an increase in systolic blood pressure as compared with aged control mice (135 ± 5 vs. 88 ± 8 mm Hg, p < 0.01; fig. 1b). Plasma urea was not elevated or different between the two groups of mice (11.1 ± 1.0 vs. 11.0 ± 0.9 mmol/l, not significant; fig. 1c).

DOCA-salt mice presented with cardiac hypertrophy, backward failure (indicated by increased lung weight) and enlargement of the kidneys, as shown in figure 1d–f. Secondly, cardiac function was impaired in DOCA-salt mice as assessed by echocardiography, which showed a decrease in fractional shortening (22.5 ± 1.2 vs. 29.9 ± 1.6%, p < 0.01; fig. 1g).
Arrhythmia Induction and CV

Isolated Langendorff perfused DOCA-salt mouse hearts were highly arrhythmogenic. An example of a polymorphic tachyarrhythmia is shown in figure 2a. Arrhythmias were induced in 86% (6/7) of the DOCA-salt hearts as compared with 0% of the control hearts (p < 0.05; fig. 2b). Epicardial activation maps generated during ventricular tachyarrhythmia (VT) showed no signs of reentry activity (fig. 2c).

CVs were obtained from paced epicardial activation maps for the LVs and RVs (fig. 2d). Longitudinal and transverse CVs in the RV were similar between control and DOCA-salt mice. Interestingly, DOCA-salt mice showed a significant decrease in both longitudinal (–32%) and transverse (–22%) CVs in the LV (p < 0.05; fig. 2d).

Cx43 Expression and Fibrosis

Cx43 expression was significantly reduced in DOCA-salt mice compared with control mice as assessed by immunohistochemistry (0.6 ± 0.05 vs. 1.0 ± 0.05, p < 0.01; fig. 3a). Cardiac fibrosis as assessed by Picrosirius red staining was significantly increased (3.7-fold) in DOCA-salt aged mice as compared with control mice of similar age (p < 0.01; fig. 3b).
Fig. 2. Arrhythmias and CV induced in perfused control and DOCA-salt aged mice.  

a Representative epicardial electrogram of a stimulation-induced polymorphic VT in DOCA-salt mice. Asterisks (*) indicate the last 4 burst-paced (60-ms) complexes.  

b Incidence of arrhythmias in control (n = 4) and DOCA-salt (n = 7) mice. *p < 0.05 vs. control.  

c Activation maps from the 4 numbered VT complexes indicated in the electrogram in a. The black isochronal lines of activation are 1 ms apart. Red (colors refer to the online version only) represents the earliest activation time and blue the latest.  

d CV measured by epicardial mapping on the LV (control: n = 4; DOCA-salt: n = 5) and RV (control: n = 4; DOCA-salt: n = 7) in longitudinal (CV_L; left) and transverse (CV_T; right) directions. *p < 0.05, **p < 0.01 vs. control.

Fig. 3. Cx43 expression and fibrosis in isolated control (n = 4) and DOCA-salt (n = 7) aged mouse hearts. **p < 0.01 vs. control.  

a Representative pictures of Cx43 (green) and N-cadherin (red) expression in control and DOCA-salt hearts (top). N-cadherin was used as a marker for intercalated disks. Scale bar = 100 μm. Bottom: quantification of Cx43 immunolabeling.  

b Representative pictures of fibrosis in control and DOCA-salt hearts (top). Scale bar = 100 μm. Bottom: quantification of fibrosis staining. Colors refer to the online version only.

Fig. 4. Urinary albumin, mean arterial pressure, plasma urea and tissue parameters in SNx and SNx-salt adult mice. **p < 0.01 vs. SNx.  

a Albumin measured in 24-hour urine samples (SNx: n = 5; SNx-salt: n = 5).  

b Mean arterial pressure measured using a catheter inserted into the femoral artery (SNx: n = 9; SNx-salt: n = 5).  

c Urea measured in plasma samples (SNx: n = 9; SNx-salt: n = 5).  

d Heart weight-to-body weight ratio (HW/BW).  

e Lung weight-to-body weight ratio (LW/BW).  

f Kidney (remnant) weight-to-body weight ratio (KW/BW).  

d–f SNx: n = 8; SNx-salt: n = 5.

(For figures see next page.)
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Relative Cx43 quantity

Control
DOCA-salt

Picrosirius red

Relative fibrosis (%)

Control
DOCA-salt

Albumin (mg/24 h)

SNx
SNx-salt

Mean arterial pressure (mm Hg)

SNx
SNx-salt

Urea (mmol/l)

SNx
SNx-salt

HW/BW (mg/g)

SNx
SNx-salt

LW/BW (mg/g)

SNx
SNx-salt

KW/BW (mg/g)

SNx
SNx-salt

Color version available online.
Renal Dysfunction and Morphological Data

SNx alone was not sufficient to develop renal dysfunction in adult mice of this strain (data not shown). Therefore, an additional SNx group was created in which salt was given to the food for 10 weeks as an extra renal challenge (SNx-salt).

Renal dysfunction was clearly present in SNx-salt compared with SNx mice, as indicated by a significant increase in albumin in the urine (186 ± 25 vs. 8 ± 6 mg/24 h, p < 0.01; fig. 4a). The mean arterial pressure tended to be higher in SNx-salt mice (97 ± 10 vs. 82 ± 4 mm Hg, p = 0.16; fig. 4b). Plasma urea was elevated in both the SNx-salt and the SNx group (21 ± 2 vs. 22 ± 2 mmol/l, not significant; fig. 4c). Furthermore, there was a tendency toward increased heart, lung and kidney weights in the SNx-salt group (fig. 4d-f).

Fig. 5. Arrhythmias and CVs induced in perfused SNx and SNx-salt adult mice. 

a Representative epicardial electrogram of a stimulation-induced polymorphic VT in SNx-salt mice. Asterisks (*) indicate the last 3 burst-paced (30-ms) complexes. 

b Incidence of arrhythmias in SNx (n = 8) and SNx-salt mice (n = 4). 

c Activation maps from the 4 numbered VT complexes indicated in the electrogram in a. The black isochronal lines of activation are 1 ms apart. Red represents the earliest activation time and blue the latest. 

d CV measured by epicardial mapping on the LV (SNx: n = 8; SNx-salt: n = 3) and RV (SNx: n = 8; SNx-salt: n = 2) in longitudinal (CV_L; left) and transverse (CV_T; right) directions. Colors refer to the online version only.

Arrhythmogenic Remodeling in SNx-Salt Adult Mice

Renal Dysfunction and Morphological Data

SNx alone was not sufficient to develop renal dysfunction in adult mice of this strain (data not shown). Therefore, an additional SNx group was created in which salt was given to the food for 10 weeks as an extra renal challenge (SNx-salt).

Renal dysfunction was clearly present in SNx-salt compared with SNx mice, as indicated by a significant increase in albumin in the urine (186 ± 25 vs. 8 ± 6 mg/24 h, p < 0.01; fig. 4a). The mean arterial pressure tended to be higher in SNx-salt mice (97 ± 10 vs. 82 ± 4 mm Hg, p = 0.16; fig. 4b). Plasma urea was elevated in both the SNx-salt and the SNx group (21 ± 2 vs. 22 ± 2 mmol/l, not significant; fig. 4c). Furthermore, there was a tendency toward increased heart, lung and kidney weights in the SNx-salt group (fig. 4d-f).
Arrhythmia Induction and CV

SNx-salt hearts were highly susceptible to polymorphic ventricular arrhythmias (fig. 5a). In the SNx-salt group, 75% of the hearts showed arrhythmias, as compared with 25% of the SNx hearts (not significant; fig. 5b). The activation maps obtained during the VT showed no signs of reentry activity (fig. 5c). Epicardial CVs were similar in SNx and SNx-salt hearts (fig. 5d).

Cx43 Expression and Fibrosis

Cx43 expression was significantly decreased upon administration of salt to SNx mice (0.6 ± 0.08 vs. 1.0 ± 0.06, p < 0.01; fig. 6a). Additionally, SNx-salt hearts presented with significantly higher levels (1.4-fold) of cardiac fibrosis than SNx hearts (p < 0.05; fig. 6b).

Discussion

In this study, the relation between cardiac remodeling and arrhythmogenicity was investigated in two different mouse models of renal dysfunction: aged mice subjected to DOCA and salt (DOCA-salt) and adult mice subjected to SNx and salt (SNx-salt). The main findings of this study are the following:
(1) The cardiorenal syndrome was present in both mouse models; renal failure was established with albuminuria in both mouse models, although DOCA-salt, but not SNx-salt, resulted in hypertension, cardiac hypertrophy and decreased cardiac contractility.

(2) Both models presented with a high incidence of arrhythmias accompanied by increased interstitial fibrosis and decreased Cx43 expression in the heart.

**Induction of the Cardiorenal Syndrome in Mice**

Mice are known to be very resistant to induction of renal failure [11, 12], usually requiring removal of large parts of the kidneys, therefore leaving little renal tissue for analysis. In order to retain both kidneys, we opted for the DOCA-salt mouse model and combined it with aging as an alternative trigger to the commonly used uninephrectomy [13], as aging increases the susceptibility for development of hypertension as well as renal and cardiac failure [14–16]. In our model, treating mice with DOCA-salt caused hypertension and albuminuria, confirming renal dysfunction. Besides renal injury, the combination of DOCA (which mimics aldosterone) and a high-salt diet induced cardiac remodeling as evidenced by hypertrophy, decreased fractional shortening, cardiac fibrosis and reduced Cx43 expression. Several studies suggested that the development of cardiac hypertrophy and fibrosis in the DOCA-salt model is at least partly independent of the extent of hypertension [17–19]. Therefore, renal dysfunction but not hypertension may be the key factor causing cardiac remodeling.

The second model of renal failure was based on SNx in CD-1 mice. Although it is known that the susceptibility to developing renal failure is dependent on the strain of mouse used [11, 12], a study has shown that both CD-1 mice and 129S3 mice (a substrain of 129Sv) developed renal failure after SNx [20]. In our mouse model, however, SNx alone was not sufficient to result in hypertension or renal dysfunction, requiring the addition of another trigger. Therefore, we used a combination of SNx with a high-salt diet. The follow-up time of 11 weeks was limited by the progressive worsening of the clinical condition of the animals. The addition of a high-salt diet did not induce significant hypertension, but as the mean arterial pressure was determined under anesthesia, blood pressure might have been underestimated. Although no significant differences were found between SNx and SNx-salt mice with respect to heart, lung or kidney weights, SNx-salt mice were more susceptible to arrhythmias than SNx mice. The low percentage of arrhythmias in the SNx group suggests that these mice were in an early stage of cardiac remodeling, which was further enhanced in the SNx-salt group.

Both mouse models used in this study showed renal dysfunction and cardiac remodeling. In addition, they were highly susceptible to arrhythmias; therefore, they at least phenotypically reflect the cardiorenal syndrome in patients. Furthermore, the presence of similar and severe arrhythmias in both models without significant hypertension, left ventricular hypertrophy and uremia in the SNx-salt model suggests that hypertension, left ventricular hypertrophy and uremia per se are presumably not prerequisites for arrhythmias in the cardiorenal syndrome. More subtle changes within the heart are probably required, as discussed below.

**Development of the Arrhythmogenic Substrate in the Cardiorenal Syndrome**

Both models of the cardiorenal syndrome exhibited high levels of arrhythmias. The high susceptibility to cardiac arrhythmias in our mouse models was accompanied by cardiac remodeling with reduced Cx43 expression and increased fibrosis. Previous studies have shown that reduced or abnormal Cx43 expression and/or increased fibrosis are strongly associated with arrhythmias in patients [21–23] as well as in dog [24, 25] and mouse models of cardiac remodeling and failure [7, 21, 26]. Furthermore, in a mouse model of cardiac pressure overload, the involvement of abnormal Cx43 expression in the arrhythmogenic substrate was associated with dispersion in CV, rather than conduction slowing, suggesting a
triggered activity mechanism [21]. In the LV of DOCA-salt aged hearts, CV was slowed both along and parallel to the fiber orientation, which may have contributed to the arrhythmogenic substrate. In SNx-salt hearts, however, arrhythmogeneity was also high, with comparable polymorphic VTs and activation patterns, albeit without conduction slowing. It seems therefore that conduction slowing is not a prerequisite for arrhythmias in this model. Triggered activity as an arrhythmia mechanism was shown earlier in a chronic kidney disease rat model [27]. This is well supported by the high-resolution epicardial activation mapping in our study, which did not show evident signs of reentry, making a triggered activity a very likely mechanism for the arrhythmias.

Clinical Implications

Our data using cardiorenal syndrome mouse models show that under conditions of excess mineralocorticoid hormone and high salt, or upon the combination of SNx and high salt, there is an increased susceptibility to arrhythmias. Previously, we demonstrated that chronic treatment of aged arrhythmogenic mice with the aldosterone antagonist eplerenone significantly decreased cardiac fibrosis, restored normal levels of Cx43 in the heart and, most importantly, reduced the amount of arrhythmias [8]. A similar result was obtained in the context of pressure-overloaded mouse hearts treated with spironolactone, which normalized Cx43 expression, reduced fibrosis and restored normal impulse conduction [28]. Inhibiting the aldosterone pathway may therefore be a therapeutic avenue to suppress the development of the arrhythmogenic substrate in cardiorenal syndrome patients.

Conclusion

In conclusion, our data provide evidence that renal dysfunction in the high-salt DOCA and SNx models causes pronounced structural and electrical cardiac remodeling and a markedly enhanced susceptibility to arrhythmias. The reduced Cx43 expression and increased fibrosis levels in these hearts are likely candidates for the formation of the arrhythmogenic substrate.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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