

**Original Paper****Neuronal Nitric Oxide Synthase-Dependent  
Amelioration of Diastolic Dysfunction in Rats  
with Chronic Renocardiac Syndrome**

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**Key Words**

Diastolic function · Cardiorenal syndrome · Neuronal nitric oxide synthase

**Abstract**

We have recently described the chronic renocardiac syndrome (CRCS) in rats with renal failure, cardiac dysfunction and low nitric oxide (NO) availability by combining subtotal nephrectomy and transient low-dose NO synthase (NOS) inhibition. Cardiac gene expression of the neuronal isoform of NOS (nNOS) was induced. Hence, we studied the role of nNOS, in vivo cardiac function and  $\beta$ -adrenergic response in our CRCS model by micromanometer/conductance catheter. Left ventricular (LV) hemodynamics were studied during administration of dobutamine (dobu), the highly specific irreversible inhibitor of nNOS L-VNIO [L-N5-(1-Imino-3-butenyl)-ornithine], or both at steady state and during preload reduction. Rats with CRCS showed LV systolic dysfunction at baseline, together with prolonged diastolic relaxation and rightward shift of the end-systolic pressure-volume relationships. After L-VNIO infusion, diastolic relaxation of CRCS rats further prolonged. The time constant of active relaxation (*tau*) increased by  $25 \pm 6\%$  from baseline ( $p < 0.05$ ), and the maximal rate of pressure decrease was  $36 \pm 7\%$  slower ( $p < 0.001$ ). These variables did not change in controls. In our CRCS model, nNOS did not seem to affect systolic dysfunction. In summary, in this model of CRCS, blockade of nNOS further worsens diastolic dysfunction and L-VNIO does not influence inherent contractility and the response to dobu stress.

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## Introduction

Advanced chronic kidney disease (CKD) and end-stage renal disease are associated with systolic and diastolic dysfunction and adverse outcome [1, 2]. CKD is also associated with diminished availability of nitric oxide (NO). In turn, basal (i.e. unstressed) cardiac function is importantly regulated by NO [3]. To study the relationship between CKD, NO and cardiac function, we have recently developed a model consisting of 5/6th nephrectomy combined with transient, low-dose, nonselective NO synthase (NOS) inhibition. This caused severe systolic and diastolic dysfunction documented using echocardiography and invasive hemodynamic measurements, and a strong reduction in systemic NO availability [4] persisting weeks after cessation of the NOS inhibitor. This makes it a suitable model to study the chronic renocardiac syndrome (CRCS) [5], characterized by renal impairment and subsequent cardiac failure, together with representative histological changes.

Diastolic dysfunction is an early characteristic of renocardiac disease [6], and neuronal NOS (nNOS) plays a crucial role in diastolic relaxation (lusitropy) [7]. Interestingly, in our model, despite low systemic NO production, gene expression of cardiac nNOS, but not endothelial NOS, was induced [4]. Increased cardiac nNOS expression has been reported in various rodent models of cardiac disease [8–11]. Induction of cardiac nNOS is thought to inhibit  $\beta$ -adrenergic responsiveness and may serve as a protective adaptation against chronic  $\beta$ -adrenergic stimulation. Therefore, cardiac nNOS induction may also protect diastolic function in our model.

The hypothesis of the current study was that inotropy and lusitropy in our CRCS model are relatively preserved by local induction of nNOS. Importantly, all experiments were performed >6 weeks after discontinuation of the nonselective NOS inhibition. In the present study, nNOS was blocked with L-N5-(1-Imino-3-butenyl)-ornithine (L-VNIO), which has a very high specificity (>100 times) for nNOS compared to endothelial NOS [12].

## Methods

The study protocol was approved by the Utrecht University Committee on Animal Experiments and conformed to the Dutch Law on Laboratory Animal Experiments and the US National Institutes of Health guidelines on use of animals for research. Male inbred Lewis rats (Lew/Crl; Charles River, Cologne, Germany), weighing 180–200 g, were housed in a climate-controlled facility with a 12:12-hour light:dark cycle.

### *Study Setup, Subtotal Nephrectomy, and in vivo Measurements*

Two groups with similar initial body weights were studied: controls (CON; n = 6) and CRCS (n = 7). CRCS animals were pretreated with N $\omega$ -nitro-L-arginine 20 mg/l (L-NNA; Sigma-Aldrich, St. Louis, Mo., USA) in drinking water and CON with normal (acidified) drinking water for 2 weeks. All animals were fed standard pellet rodent chow (CRM-E; Special Diet Services Ltd., Witham, UK). Then, at week 1, a two-stage subtotal (5/6th) nephrectomy by resection (CRCS) or a sham procedure was performed (CON) [4]. After 1 week of recovery, rats were restarted on L-NNA (CRCS) or normal water (CON), and all rats were fed standard powdered chow (CRM-FG; Special Diet Services Ltd.) supplemented with 6% (w/w) NaCl to accelerate progression of CKD for 7 weeks, as described by Bongartz et al. [4]. This method results in stable CKD in Lewis rats [13]. Normal Lewis rats are resistant to high-salt diets (8%) with respect to blood pressure and development of renal injury [14]. Treatment with L-NNA was stopped after week 8, and rats were followed up until week 15 before in vivo measurements were performed as described previously [4]. Thus, cardiac function data were obtained >6 weeks after discontinuation of the nonselective NOS inhibitor. Systolic blood pressure (SBP) was measured with the tail cuff method, and a 24-hour urine sample was collected for creatinine excretion. Left ventricular (LV) volumes were determined on echocardiographic cine loops acquired in the parasternal long axis view and calculated with the prolate ellipsoid area-length method [15]. After echocardiography, a blood sample was drawn from the tail vein to determine plasma creatinine.

### *Hemodynamic and Pressure-Volume Relationship Studies*

Terminal LV hemodynamic and pressure volume (PV) loop measurements were performed via the closed-chest approach at week 16. After induction with isoflurane, the rats were intubated, placed on a thermo-controlled operating table, and mechanically ventilated at a rate of 65/min with 1.5% isoflurane and 40% oxygen. Buprenorphine (0.015 mg/kg i.m.) was injected for analgesia. The left internal jugular vein was cannulated with a PE-50 catheter for infusion of normal saline at a rate of 8 ml/kg body weight/min. The upper abdomen was opened by a small midline incision to provide access to the inferior vena cava (IVC) between the liver and diaphragm for occlusion studies (see below). A catheter was inserted in the bladder via a suprapubic approach. The right common carotid artery was isolated and a 2-Fr pressure-conductance catheter (SPR-838; Millar Instruments, Houston, Tex., USA) was inserted. The conductance catheter was advanced retrogradely into the LV. The PV signals were registered continuously with Chart-software (ADInstruments, Spechbach, Germany) at a sample rate of 1 kHz. Adequate placement of the catheter was verified by the PV loop signals. After a stabilization period of at least 15 min, baseline steady state as well as short and temporary IVC occlusion (IVCO) hemodynamic studies were performed with the ventilator temporarily switched to continuous positive airway pressure. Steady state and IVCO interventions were repeated at least three times, with recovery periods of >1 min. After baseline measurements, dobutamine (dobu) was infused at a rate of 5 µg/kg/min [8]. When a stable increase of LV pressure (LVP), LV volume, and dP/dt-max (see below) was reached, steady state and IVCO studies were performed as explained above. Subsequently, the dobu infusion was stopped and hemodynamic variables were allowed to return to baseline values. Then, the nNOS inhibitor L-VNIO (Alexis Biochemicals/Axxora, Zandhoven, Belgium) was infused for 30 min up to a total of 0.12 µmol [16], which was followed by a stabilization period of 30 min. Subsequently, steady state and IVCO studies were performed as above. Then, dobu was again infused at a rate of 5 µg/kg/min as described above, followed by at least three steady state and IVCO studies. The infusion rate of the saline was adjusted during L-VNIO and dobu infusion to maintain a total fluid infusion rate of 8 ml/kg body weight/min during the experiment. Finally, dobu was switched off and after a washout period rats were euthanized by exsanguination via the abdominal aorta. Organs were harvested and weighed.

### *Hemodynamic and PV Analysis*

Hemodynamic measurements were analyzed off-line with the PVAN software (PVAN v3.6; Millar Instruments). Volume calibration of the conductance values was done by plotting the mean conductance catheter values for maximal and minimal volume of the baseline steady state loops against the end-diastolic (EDV) and end-systolic volumes determined by echocardiography (see above.) This yielded an equation with a value for slope and y-intercept that was entered in the PVAN program. At least three separate data samples, each consisting of a minimum of five cardiac cycles, were averaged and the following variables were calculated to assess changes in steady state hemodynamics: heart rate (HR; bpm), end-systolic pressure (ESP; mm Hg), end-diastolic pressure (EDP; mm Hg), maximum and minimal rate of LV pressure change (dP/dt-max and dP/dt-min; mm Hg/s), and regression of dP/dt versus pressure during isovolumic relaxation (*tau*; Glantz method; ms), and stroke work (SW; mm Hg × µl).

The data from the occlusion studies were used to calculate load-independent variables of cardiac function. Since the ESP volume relationship (ESPVR) of the rat heart has been shown to be curvilinear [17], we calculated the maximal elastance from the quadratic curve fit of the ESPVR ( $E'_{max}$ ; mm Hg/µl) and its corresponding volume intercept ( $V_0$ ; µl) by employing the following formula embedded in the PVAN software:  $p = a(V - V_0)^2 + b(V - V_0)$ . In addition, the preload recruitable SW relationship (PRSW; SW vs. EDV, mm Hg) and its volume intercept ( $PRSW_0$ , µl) were computed. Preload recruitable SW is determined by the linear regression of SW with the EDV. Changes in both the slope and the volume intercept were considered to assess changes in contractility. The EDP volume relationship (EDPVR) was calculated from the exponential curve fit using the formula  $EDP = C \cdot e^{\beta \cdot EDV}$ , where  $C$  is a constant and  $\beta$  is a measure of chamber stiffness.

### *Immunoblotting*

LV samples (n = 4/group) were resolved by SDS-PAGE, electrotransferred to nitrocellulose membranes and immersed in TBST buffer with 5% milk powder. The blots were incubated with the respective antibodies (overnight) and developed on the Biorad ChemiDocXRS+. Protein levels were normalized against the digitized signals of Ponceau staining (total protein). The following antibodies were used for the Western blots. cGMP-dependent protein kinase G (PKG-1 alpha; Abcam: ab69532, dilution: 1:1,000) and cGMP-dependent 3',5'-cyclic phosphodiesterase [PDE2A; Santa Cruz Biotech (N-20 SC-17227, dilution: 1:1,000)].

**Table 1.** Biometric data

	CON	CRCS
BW, g	428±14	380±24***
Tail cuff SBP, mm Hg	106±6	168±20***
Creatinine clearance, ml/min/100 g BW	0.74±0.15	0.34±0.17***
LV weight, g	0.832±0.056	1.244±0.127***
LV weight/100 g BW, g/g	0.194±0.006	0.328±0.039***
RV weight/100 g BW, g/g	0.049±0.009	0.055±0.015
Lung weight/100 g BW, g/g	0.31±0.02	0.40±0.10**

Values are means ± SD. General characteristics and organ weights of CON and CRCS rats at the time of the hemodynamic experiments are shown. BW = Body weight; RV = right ventricle. \*\* p < 0.01, \*\*\* p < 0.001 vs. CON.

**Table 2.** Cardiac hemodynamics and PVR

	CON (n = 6)		CRCS (n = 7)			
	baseline	L-VNIO	baseline	dobu	L-VNIO	L-VNIO + dobu
E'max	1.12±0.28	1.08±0.23	1.85±1.30	2.48±0.61*	1.12±0.48	2.71±1.70 <sup>††</sup>
V <sub>0</sub>	-12±14	66±86	226±137 <sup>##</sup>	124±67	163±108	62±143
PRSW	124±37	82±37	124±28	177±30**	118±31	142±40 <sup>††</sup>
PRSW <sub>0</sub>	172±134	-46±254	383±138 <sup>#</sup>	283±98 (0.058)	356±106	244±121 <sup>‡</sup>
EDPVR-C	0.60±0.21	0.63±0.34	1.23±1.38	0.64±0.50	3.40±3.42	1.27±0.62
EDPVR-β	0.004±0.001	0.005±0.003	0.003±0.004	0.002±0.001	0.001±0.002	0.000±0.000

Values are means ± SD. PVRs were assessed invasively under sequential infusions of dobu, the selective nNOS inhibitor L-VNIO and their combination. Since L-VNIO irreversibly inhibits nNOS, a first dobu infusion was performed after which a washout period was observed. Then, L-VNIO was infused, measurements were performed and a second dobu infusion was started to assess effects of the combination. # p < 0.05, ## p < 0.01 vs. CON rats at baseline; \* p < 0.05, \*\* p < 0.01 vs. CRCS at baseline; † p < 0.05, †† p < 0.01, ††† p < 0.001 vs. CRCS dobu; ‡ p < 0.05, †† p < 0.01, ††† p < 0.001 vs. CRCS L-VNIO.

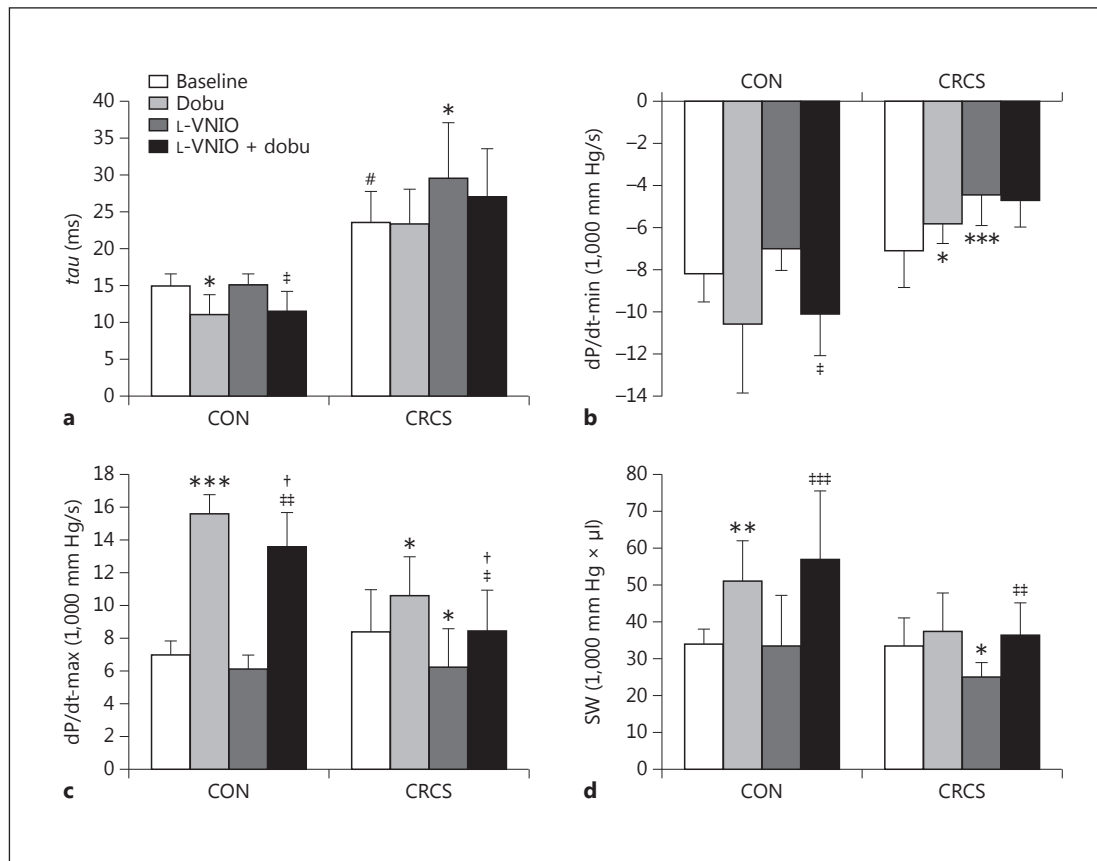
### Statistics

Data are shown as means ± SD and were analyzed and graphed using SigmaPlot 11.0 (Systat Software, Inc., San Jose, Calif., USA). Student's t test or Mann-Whitney rank sum test was used for group comparisons at baseline. For hemodynamic studies, a two-way repeated measures ANOVA was performed. The differences in variables between baseline and L-VNIO in CON rats during IVCO were assessed by paired t test. Data that were not normally distributed were log-transformed or ranked to achieve normality. A p value < 0.05 was considered statistically significant.

## Results

### General Characteristics of Rats with CRCS

Rats with CRCS had a lower body weight, higher tail cuff SBP and a lower creatinine clearance than CON rats (table 1). CRCS rats showed marked LV hypertrophy and increased lung weight. Both absolute LV weight and LV weight expressed as g/g body weight were significantly higher in CRCS animals.



**Fig. 1.** The in vivo hemodynamic response of **a** time constant of pressure decay (*tau*), **b** maximal rate of pressure decline ( $dP/dt\text{-min}$ ), **c** maximal rate of pressure increase ( $dP/dt\text{-max}$ ) and **d** SW in CON rats (CON) and rats with CRCS during a baseline period (white bars) and in response to sequential infusions of dobu and/or L-VNIO. Since L-VNIO irreversibly inhibits nNOS, a first dobu infusion was performed (light grey bars) after which a washout period was observed. Then, L-VNIO was infused (grey bars), measurements were performed and a second dobu infusion was started to assess the effects of the combination (black bars). The response to dobu of  $dP/dt\text{-max}$  and SW was blunted in CRCS rats. Acute nNOS blockade induces a decrease, but with subsequent  $\beta$ -adrenergic stimulation both parameters increased significantly. Data are shown as means  $\pm$  SD. #  $p < 0.001$  vs. CON at baseline; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. baseline; †  $p < 0.05$  vs. dobu; ‡  $p < 0.05$ , ‡‡  $p < 0.01$  vs. L-VNIO, ‡‡‡  $p < 0.001$  vs. L-VNIO.

*Both Systolic and Diastolic Dysfunction Occur in Rats with CRCS*

Invasive measurements of LV hemodynamics and PV relationships indicate that rats with CRCS exhibit marked diastolic as well as systolic dysfunction. With regard to diastolic function, the linear constant (C) and chamber stiffness constant ( $\beta$ ) of the exponential curve fit for the EDPVR were not significantly different between groups or within groups when comparing interventions (data not shown). Functionally, the time constant *tau* was significantly prolonged in CRCS rats ( $p < 0.001$ ; fig. 1a), indicating reduced active diastolic relaxation. Under baseline conditions,  $dP/dt\text{-min}$  was not different (fig. 1b).

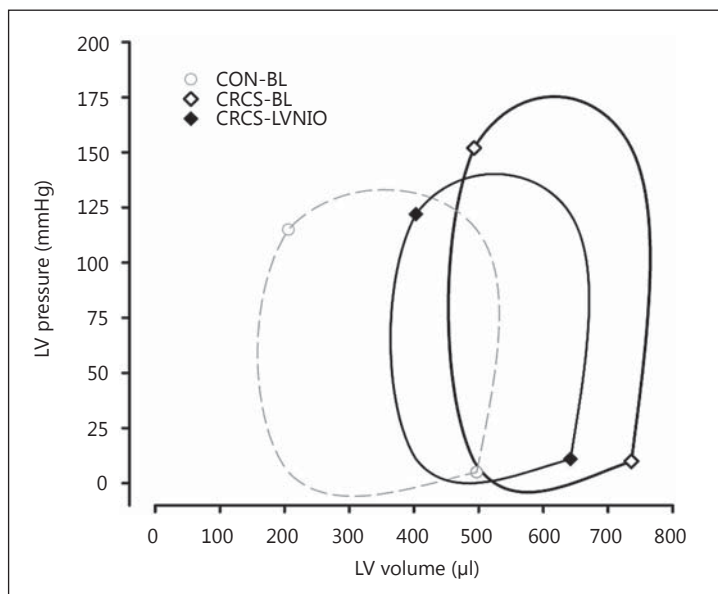
Concerning systolic function, ejection fraction (EF) and cardiac output (CO) were significantly lower in CRCS rats, but the rate of maximal LV pressure increase ( $dP/dt\text{-max}$ ) and SW were comparable to CON (fig. 1c, d). Since the ESPV relationship of the rat heart shows curvilinear contractility dependency [17], we calculated the quadratic curve fit of the ESPVR ( $E'\text{max}$ ). This was not significantly different, but its volume intercept (the volume where

**Table 3.** Percent changes in variables of LV hemodynamics between different interventions

	CONs (n = 6)			CRCS (n = 7)		
	dobu vs. BL	L-VNIO vs. BL	L-VNIO + dobu vs. L-VNIO	dobu vs. BL	L-VNIO vs. BL	L-VNIO + dobu vs. L-VNIO
dP/dt-max	+127±13	-11±7	+126±22	+32±12	-24±9	+40±12
dP/dt-min	+32±18	-13±8	+46±14	-13±10	-36±7	+7±6
<i>Tau</i>	-24±9	+2±5	-24±5	-1±5	+25±6	-8±4
SW	+51±31	+0±48	+80±61	+15±36	-21±29	+49±23
EF	+34±12	-4±21	+47±27	+47±37	+17±37	+39±26
CO	+40±35	-0±33	+60±63	+41±40	-16±19	+49±23

Values are means ± SD. Percent changes in hemodynamic variables under different conditions in CON and CRCS rats. *Tau* = Time constant of diastolic pressure decay.

**Fig. 2.** Steady state PV loops graphed from mean data (group averages) in CRCS; thick line, diamonds) before (BL) and after L-VNIO infusion (L-VNIO). The PV loop from CON rats at BL is included for reference (CON-BL; dashed line, light grey).



pressure is 0) was at a higher value in CRCS rats ( $p = 0.001$ ; table 2). This rightward shift indicates reduced contractility, as similar pressures are reached at higher volumes. A similar pattern was observed for preload recruitable SW (table 2). These results are in agreement with our previous findings in this model [18] and show that CRCS rats suffer from combined diastolic and systolic dysfunction.

#### *Diastolic Function Does Not Improve in CRCS with DoBU Stimulation*

Changes in diastolic relaxation during  $\beta$ -adrenergic stimulation were strikingly different in CON and CRCS rats. In CON rats,  $\beta$ -adrenergic stimulation caused a significant reduction in *tau* and a faster decline of LV pressure (dP/dt-min), indicating improved diastolic relaxation (fig. 1a, b; table 3). In CRCS rats, the rate of pressure decline was significantly slower and *tau* did not shorten with dobu stimulation, indicating worsened diastolic relaxation.

In both groups, dobu infusion induced an increase in HR. Although the response of dP/dt-max and SW to dobu stress appeared blunted in CRCS rats (fig. 1c, d), EF and CO increased



**Table 4.** LV hemodynamics

	CON (n = 6)				CRCS (n = 7)			
	baseline	dobu	L-VNIO	L-VNIO + dobu	baseline	dobu	L-VNIO	L-VNIO + dobu
HR, bpm	372±17	391±12**	343±19***	364±16 <sup>†††,‡</sup>	328±38 <sup>#</sup>	340±29	273±34***	291±32 <sup>†††,‡</sup>
ESP, mm Hg	115±11	113±20	104±13	118±22	152±26 <sup>##</sup>	126±23	122±28*	117±16
EDP, mm Hg	5±1	6±1	7±1*	7±2 <sup>†</sup>	10±7	10±7	11±6	10±5
EDV, µl	497±39	498±111	561±77	555±116	736±122 <sup>###</sup>	658±104	642±132	634±134
Stroke volume, µl	334±39	443±95*	359±105	504±129 <sup>‡</sup>	282±51	379±121*	278±49	395±136 <sup>‡</sup>
CO, ml/min	125±19	173±35*	124±39	185±54 <sup>‡‡</sup>	92±20 <sup>#</sup>	127±33**	75±88*	113±29 <sup>‡‡</sup>
EF, %	63±4	84±5***	61±13	86±8 <sup>‡‡‡</sup>	38±8 <sup>###</sup>	55±13**	43±13	59±17 <sup>‡‡</sup>

Values are means ± SD. Hemodynamic responses of the LV were assessed invasively under sequential infusions of dobu, the selective nNOS inhibitor L-VNIO and their combination. Since L-VNIO irreversibly inhibits nNOS, a first dobu infusion was performed after which a washout period was observed. Then, L-VNIO was infused, measurements were performed and a second dobu infusion was started to assess effects of the combination. <sup>#</sup> p < 0.05, <sup>##</sup> p < 0.01, <sup>###</sup> p < 0.001 vs. CON rats at baseline; \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs. CRCS at baseline; <sup>†</sup> p < 0.05, <sup>†††</sup> p < 0.001 vs. CRCS dobu; <sup>‡</sup> p < 0.05, <sup>‡‡</sup> p < 0.01, <sup>‡‡‡</sup> p < 0.001 vs. CRCS L-VNIO.

to a similar degree as in CON (table 4). Assessment of PV relations under dobu stimulation during preload reduction was difficult in CON rats. The increased contractility likely led to a hyperdynamic ventricle causing impingement of the catheter as ESP sharply increased during preload reduction. This problem has also been encountered by others [19]. The artefact distorted the PV loops making calculation of load-independent variables impossible in all but a few experiments in CON rats.

In CRCS rats, PV relationships (fig. 2) during preload reduction could be analyzed during dobu. This showed that the slopes of the indices of contractility became steeper, without a change in the volume intercept. Thus, in contrast to the disparate effects on diastolic relaxation, dobu stress increased systolic variables to a similar degree in both groups.

*nNOS Blockade Further Worsens Diastolic Function in Rats with CRCS*

Except for bradycardia, blockade of nNOS had no significant effects on cardiac hemodynamics in CON rats (table 4). Effects of L-VNIO on diastolic function in CRCS rats were pronounced, with the rate of pressure decrease (dP/dt-min) becoming 36 ± 7% slower and the time constant of relaxation, *tau*, increasing by 25 ± 6% (table 3; fig. 1). CO was slightly lower due to the pronounced bradycardia. EF remained unchanged (table 4). Both dP/dt-max and SW decreased under nNOS blockade versus baseline in CRCS (fig. 1 c, d), but preload-independent measures of contractility were not affected (table 4).

*The β-Adrenergic Response Is Similar during nNOS Blockade in Both Groups*

Similar to the first dobu challenge in CRCS without nNOS blockade, diastolic variables (dP/dt-min and *tau*) did not change in CRCS during nNOS blockade. In CON rats, dobu improved variables of systolic and diastolic function during steady state measurements similarly with or without concurrent nNOS blockade (fig. 1; tables 3, 4).

In CRCS, the response of dP/dt-max to dobu was still blunted (fig. 1). However, SW increased significantly by β-adrenergic stimulation after nNOS blockade. Assessment of indices of contractility during preload reduction showed that both E' max and PRSW increased significantly. The volume intercept of both relationships was lower, but this only reached statistical significance for the intercept of the PRSW (PRSW<sub>0</sub>; table 2).

*The Ventricular cGMP-Dependent Protein Kinase Levels Were Similar in Both Groups*

Immunoblotting failed to show differences in ventricular PDE2A or PKG-1 alpha content between CRCS and CON rats (n = 4/group; data not shown).

**Discussion**

The most important finding of this study is that diastolic variables of pressure decay were further worsened by inhibition of nNOS by L-VNIO infusion in CRCS rats. nNOS blockade had no effect on diastolic function in CON rats, and no effect on inherent contractility and the response to dobu stress in either CRCS or CON rats. Active diastolic relaxation is already impaired at baseline in CRCS rats with a prolonged *tau*. This is consistent with findings by others in rodent models of CKD [6]. Combined with the LV hypertrophy, dilatation and higher EDP, this shows that diastolic dysfunction is an important pathophysiological feature in this model of CRCS. Diastolic dysfunction is also common in CKD patients, and it can worsen congestion and progress to diastolic heart failure (HF) [20, 21]. It may occur early in the disease, and in one study, CKD-associated mortality was found to be higher in patients with diastolic HF than in those with systolic HF [22].

Data from experimental studies suggest that under basal conditions, nNOS inhibits cardiac contractility and promotes relaxation [23–26]. In various rodent models of cardiac disease, nNOS gene expression and activity were increased [8–11]. Subsequent increased production of NO by cardiac nNOS may serve as a protective mechanism guarding the heart from sustained  $\beta$ -adrenergic overstimulation. However, studies intervening with nNOS activity have not shown consistent results on the effect on diastolic relaxation in cardiac disease states. For example, one study in nNOS<sup>-/-</sup> mice showed a similarly depressed diastolic function compared to wild-type mice after myocardial infarction. Others reported a worsening of diastolic function in nNOS<sup>-/-</sup> mice [27–29]. In healthy rats, nNOS inhibition by L-VNIO worsened dP/dt-min and *tau*, but it did not affect these parameters in rats with HF due to previous myocardial infarction [8]. Conversely, mice overexpressing nNOS that were subjected to transverse aortic constriction (a model of LV pressure overload) showed delayed progression towards HF with sustained EF and better diastolic function than wild-type mice subjected to transverse aortic constriction [30]. To our knowledge, we are the first to show that inhibition of myocardial nNOS worsens diastolic dysfunction in rats with CRCS. Passive diastolic properties were unaffected. This suggests that the previously observed upregulation of nNOS gene expression [18] is a beneficial adaptation that supports compromised diastolic function. NO may support cGMP-dependent relaxation [33]; however, we observed no differences in ventricular PDE2A or PKG-1 alpha content between CRCS and CON rats. However, this does not exclude compartmentalized changes of protein kinases in CRCS [34, 35].

Systolic function is generally maintained in rat models of CKD by subtotal nephrectomy [6]. Similar to our previous observations, LV systolic function was markedly reduced in animals with CRCS in the present study, assessed by echocardiography and invasive hemodynamic measurements [4, 18]. Reduced cardiac  $\beta$ -adrenergic responsiveness has been documented in human and experimental CKD and was found to be secondary to cardiac adrenoceptor desensitization [31, 32]. However, these studies were performed in vitro and with isoprenaline. In our study, we showed in an in vivo acute setup that dobu increased contractility similarly in CRCS and CON rats.

In sum, in this model of CRCS, we have previously documented cardiac nNOS gene induction, and in this study, we showed that the nNOS-derived NO supports cardiac relaxation or lusitropy in vivo. Our results underscore the role of NO in diastolic function and the importance of adequate NO availability to maintain cardiac function. Although basal inherent



contractility is reduced in CRCS rats, the  $\beta$ -adrenergic response appears intact and nNOS-derived NO does not influence this response. Moreover, protein levels of cGMP-dependent kinases were not affected by CRCS in whole ventricle lysates. Further studies on how ventricular nNOS is compartmentalized and supports diastolic relaxation in CRCS are needed.

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