Atrial Natriuretic Peptide Affects Cardiac Remodeling, Function, Heart Failure, and Survival in a Mouse Model of Dilated Cardiomyopathy

Dong Wang, Inna P. Gladysheva, Tai-Hwang M. Fan, Ryan Sullivan, Aiilyan K. Houng, Guy L. Reed

Abstract—Dilated cardiomyopathy is a frequent cause of heart failure and death. Atrial natriuretic peptide (ANP) is a biomarker of dilated cardiomyopathy, but there is controversy whether ANP modulates the development of heart failure. Therefore, we examined whether ANP affects heart failure, cardiac remodeling, function, and survival in a well-characterized, transgenic model of dilated cardiomyopathy. Mice with dilated cardiomyopathy with normal ANP levels survived longer than mice with partial ANP (P<0.01) or full ANP deficiency (P<0.001). In dilated cardiomyopathy mice, ANP protected against the development of heart failure as indicated by reduced lung water, alveolar congestion, pleural effusions, etc. ANP improved systolic function and reduced cardiomegaly. Pathological cardiac remodeling was diminished in mice with normal ANP as indicated by decreased ventricular interstitial and perivascular fibrosis. Mice with dilated cardiomyopathy and normal ANP levels had better systolic function (P<0.001) than mice with dilated cardiomyopathy and ANP deficiency. Dilated cardiomyopathy was associated with diminished cardiac transcripts for NP receptors A and B in mice with normal ANP and ANP deficiency, but transcripts for NP receptor C and C-type natriuretic peptide were selectively altered in mice with dilated cardiomyopathy and ANP deficiency. Taken together, these data indicate that ANP has potent effects in experimental dilated cardiomyopathy that reduce the development of heart failure, prevent pathological remodeling, preserve systolic function, and reduce mortality. Despite the apparent overlap in physiological function between the NPs, these data suggest that the role of ANP in dilated cardiomyopathy and heart failure is not compensated physiologically by other NPs. (Hypertension. 2014;63:514-519.)

Key Words: atrial natriuretic peptide ■ cardiomyopathy, dilated ■ heart failure ■ natriuretic peptides

We assessed the role of ANP in modulating the progression of HF in a well-established transgenic mouse model of DCM characterized by progressive HF and death caused by the specific action of a cardiac-targeted, dominant-negative cAMP response element-binding protein transcription factor.7,8

Methods

Experimental details are found in the online-only Data Supplement.

Results

ANP Affects Mortality in Mice With DCM

To examine whether ANP affects the development and progression of DCM, we monitored the survival of DCM mice with normal ANP levels and ANP deficiency because of gene deletion (Figure 1). By comparing DCM mice with normal ANP levels (DCM^{ANP+/+}), mice with partial ANP deficiency (DCM^{ANP+/−}) and mice with complete ANP deficiency (DCM^{ANP−/−}), mice with complete ANP deficiency of
ANP (DCMANP−/−) had a significantly reduced survival (median survival, 84 days) when compared with DCMANP+/− mice with partial ANP deficiency (P<0.001) or DCM mice with normal ANP levels (P<0.001). These data indicate that mortality in DCM was modulated by ANP levels in a gene-dose–related fashion.

ANP Modulates the Development of HF

If ANP modulates survival in DCM mice, the presence or the absence of ANP may affect the development and progression of HF. Chest radiographs showed that DCMANP−/− mice had cardiomegaly (enlarged heart silhouettes) and mild lung edema without pleural effusions (Figure S1 in the online-only Data Supplement). In contrast, DCMANP+/− mice had cardiomegaly with pronounced bilateral lung edema and pleural effusion (Figure S1). A postmortem analysis of DCMANP−/− mice showed severe lung congestion and pleural effusions. DCMANP+/+ mice had greater lung fluid accumulation as indicated by higher total lung wet weight (P<0.05) and the lung wet weight/body weight ratio (lung wet weight/body weight, P<0.05) than DCMANP+/+ mice (n=12–23 each group; Figure 2A). Lung histology analysis showed that DCMANP−/− mice had more severe congestion in the interstitial and intra-alveolar space (Figure 2B, pink area, asterisks) and spotty hemorrhagic lesions in alveolar capillaries (Figure 2C). The total alveolar area filled with congestion was significantly increased in DCMANP−/− mice when compared with DCMANP+/+ mice (P<0.001; n=3–4 each group; Figure 2D). Renal function may affect salt and water retention associated with pleural effusions and pulmonary edema; creatinine levels were statistically higher in the DCMANP−/− mice (P<0.001) although blood urea nitrogen and creatinine levels were within the normal range in both groups (Figure S2).

ANP Levels Affect Progression of Left Ventricular Dysfunction

The increased lung congestion or pulmonary edema found in DCMANP−/− mice may signify severe left ventricle (LV) dysfunction. Therefore, cardiac function was assessed by echocardiography. When compared with DCMANP+/+ mice, DCMANP−/− mice showed greater LV dilation in both diastole (P<0.001) and systole (P<0.001; Figure 3A–3C), suggesting that DCMANP−/− mice had worse heart dilatation. There was no significant difference between DCMANP−/− and DCMANP+/+ mice in myocardial measures, such as thickness of the interventricular septum or posterior wall (P>0.05; Figure 3D and 3E). However, DCMANP−/− mice had marked decreases in LV systolic function as assessed by ejection fraction (P<0.001) and fractional shortening (P<0.001) when compared with DCMANP+/+ mice (Figure 3F and 3G).

ANP Affects Cardiac Remodeling

Autopsy and histological studies showed that DCMANP−/− mice had more severe heart enlargement than DCMANP+/+ littermate controls (Figure 4A). In comparison with DCMANP+/+ mice, left atrial enlargement was more marked in DCMANP−/− mice. Heart weight (P<0.01) and heart weight:body weight ratio was significantly greater in DCMANP−/− mice than in DCMANP+/+ mice (P<0.05; n=12–23 each group; Figure 4B). Because eccentric hypertrophy may be a feature of DCM, we examined whether ANP deficiency affected cardiomyocyte hypertrophy by wheat germ agglutinin staining.7 The cardiomyocyte cross-sectional area was significantly increased in DCMANP−/− versus DCMANP+/+ mice (P<0.001; Figure 4C and 4D).

Fibrosis often plays a critical role in pathological remodeling in cardiomyopathy. When compared with DCMANP+/+ mice, DCMANP−/− mouse hearts had more extensive interstitial fibrosis between bundles of myocytes (Figure 4E and 4F; P<0.001). DCMANP−/− mouse hearts showed areas consistent with fibrotic scarring in the LV (Figure 4E, arrow head), which have been associated with focal cardiomyocyte degeneration. Perivascular fibrosis was also more marked in DCMANP−/− versus DCMANP+/+ hearts (Figure 4G and 4H; P<0.001). There was extensive perivascular fibrosis in the cardiac veins and in different sized arteries (conductive arteries, prearterioles, and arterioles) in the heart of DCMANP−/− mice (Figure 4I). In addition, DCMANP−/− mice hearts had
more severe atrial fibrosis, especially of the left atrium, than DCM ANP+/+ mice (Figure 4A).

**Effects of ANP Deficiency on NP Cardiac Gene Expression and cGMP Levels**

We have previously shown that ANP levels were increased in DCM ANP+/+ mice in comparison with mice without DCM.\(^4\) As expected there were no detectable transcripts for ANP in DCM ANP−/− mice (Figure 5A). There was a trend toward increased BNP expression in DCM ANP−/− mice, but the expression levels were not statistically different from DCM ANP+/+ mice (Figure 5B; \(P<0.05\)). Cardiac transcripts for C-type natriuretic peptide (CNP) were significantly lower in DCM ANP−/− mice than in DCM ANP+/+ mice (Figure 5C; \(P<0.01\)) although both showed a trend to lower expression than wild-type (WT) mice. Cardiac expression of the NP receptors (NPR) A and B was significantly decreased in both DCM ANP+/+ and DCM ANP−/− mice when compared with WT mice (NPRA, \(P<0.01\) versus WT; NPRB, \(P<0.05\) versus WT; \(n=5\) each group), but they did not differ from each other (\(P>0.05\); \(n=5\) each group; Figure 5D and 5E). Similarly, cardiac expression of NPRC was lower in DCM ANP+/+ mice (\(P<0.01\) versus WT) but not in DCM ANP−/− mice (\(P>0.05\) versus WT; Figure 5F). However, cardiac levels of NPRC were 2× higher in DCM ANP+/+ mice versus DCM ANP−/− mice (\(P<0.01\); \(n=5\) each group; Figure 5F). There was no significant difference between DCM ANP+/+ mice and DCM ANP−/− in plasma levels of angiotensin II, renin activity, or aldosterone (Figures S3–S5); however, levels of cortisol were 33% higher in DCM ANP−/− mice (Figure S6; \(P<0.05\)). Blood levels of cGMP were significantly higher in DCM ANP+/+ versus DCM ANP−/− mice (Figure S7; \(P<0.01\)) consistent with higher cardiac transcripts of ANP.

**Discussion**

Chronic HF is increasing in prevalence. Both ANP and BNP are potent biomarkers for predicting mortality in patients with chronic HF.\(^10\) High levels of ANP are correlated with progressive HF, but it remains unclear whether ANP is protective...

Figure 3. Echocardiographic findings in DCM ANP+/+ and DCM ANP−/− mice. A, Representative M-mode images. B, Left ventricular internal dimension in diastole (LVIDd). C, left ventricular internal dimension in systole (LVIDs). D, Interventricular septal thickness in diastole (IVSd). E, Left ventricular posterior wall thickness in diastole (LVPWd). F, Ejection fraction (EF %). G, Fractional shortening (FS %). **P<0.001, ***P>0.05, \(n=9\) each group. ANP indicates atrial natriuretic peptide; and DCM, dilated cardiomyopathy.

Figure 4. Pathological cardiac remodeling in DCM ANP−/− mice. A, Representative longitudinal sections of hearts from DCM ANP+/+ and DCM ANP−/− mice (12 weeks old). Severe 4-chamber dilation was frequently observed in DCM ANP+/+ mice. Scale bars, 2 mm. B, Heart weight (HW), body weight (BW), and HW/BW ratio (\(n=12–23\) in each group). C and D, Representative wheat germ agglutinin stain (20×) and quantitation of cardiomyocyte cross-sectional area. Results are averages of 100 to 150 cardiomyocytes from 4 to 6 mice each group. E and G, Representative Masson trichrome stain showing interstitial fibrosis (IF) and perivascular fibrosis (PF). F and H, Quantitative analysis of IF (% of total area) and PF (PF, ratio to lumen area). A total of 12 to 15 fields (40×) or 12 to 15 arteries (40×) from left ventricle per heart were measured. Results are means of averages of 3 to 6 mice for each group, I, Intense PF involving cardiac veins and various sizes of coronary arteries. C, E, G, and I, Scale bars, 100 μm. Dashed lines represent normal reference value from wild-type mice. **P<0.001, ***P>0.05. ANP indicates atrial natriuretic peptide; and DCM, dilated cardiomyopathy.
or merely serves as a biomarker in HF, particularly in conditions such as DCM. The paradox of high ANP levels and progressive HF has been attributed to (1) incomplete processing of pro-ANP to the active form; (2) the inability of current immunologic assays to distinguish between functional ANP and dysfunctional or nonfunctional ANP-related peptides; (3) NPRA/NPRB downregulation or clearance; (4) diminished kidney responsiveness and other mechanisms. Experimental data indicate that ANP plays a key role in modulating the hypertrophic response of cardiomyocytes in normal hearts to increased pressure overload (eg, transverse aortic constriction) and in volume overload (aortocaval fistula). Human studies have largely focused on short-term treatment of patients with impaired systolic function and show that brief ANP infusions do improve natriuresis in some but not all in subjects; however, there are no data that they modify cardiac structure, function, or survival. Thus, to our knowledge these are the first data to show that ANP plays a key role in the progression of preexisting DCM by modifying systolic function, cardiac fibrosis, HF, and mortality.

ANP has an antihypertrophic effect on cardiomyocytes both in vitro and in vivo. We found that ANP deficiency significantly affected cardiac remodeling. DCMANP−/− mice hearts were greater in mass and in mass normalized to body weight. There was an increase in cardiomyocyte cross-sectional area in DCMANP−/− versus DCMANP+/+ mice. In contrast to models of pressure overload, the increased cardiomyocyte hypertrophy in this study was not associated with protective or compensatory effects because DCMANP−/− mice showed greater impairment of systolic function, with lower ejection fractions, and greater ventricular dilation, with larger LV internal diameter in diastole and LV internal diameter in systole. Moreover, DCMANP−/− mice did not show typical hypertrophic increases in the interventricular septum or posterior wall when compared with DCMANP+/+ mice. Thus, the increased cardiac mass and worsened systolic function may be because of the fact that DCMANP−/− mice developed more extensive interstitial and perivascular fibrosis in the ventricles and atria than that found in DCMANP+/+ mice or in ANP−/− mice (data not shown). ANP inhibits cardiac fibroblast-mediated collagen synthesis and may protect hearts from pathological remodeling. As such, ANP deficiency may have additive or synergistic effects on promoting fibrosis in the setting of a preexisting cardiomyopathy, such as DCM. Although plasma renin activity, angiotensin II, and aldosterone levels were not significantly different between DCMANP−/− and DCMANP+/+ mice, ANP may still affect aldosterone and angiotensin II–induced fibrosis at the tissue level. The finding of elevated cortisol levels in the DCMANP−/− mice underscores the potential pathogenic role of enhanced mineralocorticoid receptor activity in fibrosis. Extensive interstitial fibrosis can cause ventricular stiffness, abnormal electric behavior of the myocardium, and fatal arrhythmias. There is also a clear link between interstitial fibrosis and ventricular dysfunction. DCMANP−/− hearts also showed extensive fibrosis in the left atrium, which is also associated with HF. There were areas of microscopic scar formation noted in DCMANP−/− hearts that may indicate cardiomyocyte loss. There was also extensive perivascular fibrosis of coronary vessels, particularly small coronary arteries and arterioles in DCMANP−/− mice (Figure 4I). Severe perivascular fibrosis can lead to structural remodeling and luminal narrowing of intramural coronary arteries and arterioles, which may be associated with impaired coronary blood flow. In patients with DCM, coronary microvascular dysfunction has been associated with advanced HF and increased risk of death.

In addition to acting on cardiac tissue through autocrine and paracrine mechanisms, ANP enhances natriuresis and modulates renal function. Although ANP-deficient mice have normal blood pressures under normal salt diets, they may develop hypertension under high-salt diets mediated by angiotensin II. Because these mice were not on high-salt diets, and there were not significant differences in angiotensin II levels between the groups, this may not have contributed to the adverse outcomes in DCMANP−/− mice. In the present study, no physiologically significant differences were observed between DCMANP−/− versus DCMANP+/+ mice in indices of renal function, sodium, or electrolyte levels. Still DCMANP−/− mice showed objective evidence of more severe HF as indicated by chest radiography, increased lung fluid, and extensive alveolar congestion.

We analyzed cardiac transcripts to determine how ANP deficiency in DCM affected other key members of the NP system (ANP, BNP, CNP and their receptors NPRA, NPRB, and NPRC) which are thought to be regulated by LV wall stress in HF. Transcript analyses of DCMANP+/+ hearts showed that ANP levels were significantly increased, BNP levels trended toward elevation, and CNP levels were unchanged to decreased when compared with WT controls. In contrast, in DCMANP−/− mice, transcripts for ANP were undetectable, there was a trend toward increased BNP transcripts, and CNP transcripts were significantly diminished. The NPs exert their biological effects through specific receptors, NPRA (ANP and BNP) and NPRB (CNP), that increase intracellular cGMP. NPRA downregulation occurs in HF and impairs the beneficial effects of NPs. We found that NPRA and NPRB were significantly and comparably downregulated in DCMANP+/+ mice and DCMANP−/− mice versus WT littermates. Unexpectedly, NPRC

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**Figure 5.** Changes in natriuretic peptides (NPs) and NP receptors (NPR) in wild-type (WT), DCMANP+/+, and DCMANP−/− mice. **A–C,** Relative cardiac expression of atrial NP (ANP), B-type NP (BNP), and C-type NP (CNP). **D–F,** Relative cardiac expression of NPRA, NPRB, and NPRC. Transcripts are means of averages of triplicate measurements in 5 mice in each group assessed by quantitative reverse transcriptase polymerase chain reaction. **P<0.001,** **P<0.01,** and **P<0.05.**

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levels were higher in DCM\textsuperscript{ANP$^{-/-}$} mice than in DCM\textsuperscript{ANP+/+} mice. NPRC is considered a physiological clearance receptor for all 3 NPs,\textsuperscript{7} which suggests that NP clearance may be accelerated and contributed to functionally reduced level of NPs in DCM\textsuperscript{ANP$^{-/-}$} mice although definitive data are lacking. Taken together, the changes in transcript expression do not reflect an obvious pattern of coordination between members of the NP system to compensate for the loss of ANP activity. This failure of compensation is further supported by the finding that plasma cGMP levels were lower in DCM\textsuperscript{ANP$^{-/-}$} mice when compared with DCM\textsuperscript{ANP+/+} mice (Figure 5H). Although it has been difficult to assign unique molecular functions to ANP and BNP at a cellular level, DCM\textsuperscript{ANP$^{-/-}$} mice showed severe structural and functional impairments, indicating that ANP plays an essential role that was not compensated by the activity or function of other members of the NP system.

CNP transcripts were particularly diminished in DCM\textsuperscript{ANP$^{-/-}$} mice. This may reflect the direct effect of ANP deficiency because ANP enhances the production and secretion of CNP in cell culture.\textsuperscript{23} CNP is not stored in granules, and activity seems dependent on transcriptional regulation.\textsuperscript{21} CNP potently inhibits cardiac fibroblast proliferation and collagen synthesis and has been shown to play a significant role in modulating fibrosis in vivo.\textsuperscript{27,28} CNP also regulates the coronary circulation or microcirculation,\textsuperscript{29} which suggests that it may affect myocardial blood supply in DCM\textsuperscript{ANP$^{-/-}$} mice. Thus, low CNP levels may also have affected cardiac function and remodeling in the DCM\textsuperscript{ANP$^{-/-}$} mice.\textsuperscript{27}

In conclusion, these data suggest that ANP affects cardiac remodeling, function, HF progression, and survival in the setting of DCM. Surprisingly, ANP deficiency is not adequately complemented by changes in the activity of other components of the NP system. Further studies are necessary to understand the functional inter-relationships of the NP and NPRs and how they act coordinately in processes, such as cardiomyopathy.

**Perspectives**

ANP is a biomarker for predicting mortality in patients with chronic HF. Still is unclear whether ANP functionally modulates the development of HF, especially in the setting of DCM. DCM mice with partial or complete ANP deficiency have a gene-dose–dependent accelerated mortality. ANP deficiency is associated with cardiac dilatation, hypertrophy, fibrosis, and impaired heart function. These data suggest that in addition to its value as a biomarker, ANP affects the progression of cardiac fibrosis, systolic dysfunction, HF, and death in DCM.

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**Disclosures**

None.

**References**

11. Ibebuogu UN, Gladysheva IP, Houng AK, Reed GL. Decompensated heart failure is associated with reduced corin levels and decreased cleavage of pro-atrial natriuretic peptide. *Circ Heart Fail.* 2011;4:114–120.
24. Tsutamoto T, Kamonari T, Morigami N, Sugimoto Y, Yamaoka O, Kinoshita M. Possibility of downregulation of atrial natriuretic peptide...
receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure. Circulation. 1993;87:70–75.

What Is New?

• In experimental dilated cardiomyopathy we found the following:
  1. Partial or complete atrial natriuretic peptide (ANP) deficiency accelerates mortality.
  2. ANP deficiency worsens heart function and worsens heart failure.
• ANP deficiency adversely affects cardiac remodeling by increasing both interstitial and perivascular fibrosis.
• ANP deficiency affects cardiac transcript expression of other NPs and receptors but is not functionally compensated by other members of the NP family.

What Is Relevant?

• Beyond its role in natriuresis and vascular dilation, ANP seems to affect cardiac function, remodeling, heart failure development, and survival in dilated cardiomyopathy

Summary

In addition to its value as a biomarker, ANP seems to play a critical, irreplaceable role in modulating cardiac function, myocardial fibrosis, heart failure, and survival in experimental dilated cardiomyopathy.
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ANP AFFECTS CARDIAC REMODELING, FUNCTION, HEART FAILURE AND SURVIVAL IN A MOUSE MODEL OF DILATED CARDIOMYOPATHY

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ONLINE SUPPLEMENT

Methods

Mice. Congenic ANP-deficient (ANP<sup>+/−</sup>)<sup>1</sup> and wild-type (WT, ANP<sup>+/+</sup>) mice on a C57BL6 background were obtained from Jackson Laboratory (Bar Harbor, ME). DCM<sup>ANP−/−</sup> and DCM<sup>ANP+/+</sup> were generated by backcrossing with DCM mice on a C57BL6 background<sup>2</sup>. Twelve week old male mice were used for the whole study. Mice were housed in the same cage racks in AAALAC accredited facilities and fed a normal salt chow (0.4% NaCl, Harlan Teklad). Experiments were approved by the Animal Care and Use Committees of University of Tennessee and Georgia Regents University.

Real-time polymerase chain reaction (RT-PCR). Total RNA was extracted from whole hearts using the RNeasy<sup>®</sup> Mini Kit (Qiagen). First strand cDNA synthesis was performed with 1 µg of total RNA (Transcriptor First Strand cDNA Synthesis Kit, Roche). Quantitative real-time PCR (qRT-PCR) was performed using the LightCycler<sup>®</sup> 480 System following the manufacturer’s protocol. Specific primers were: cacagatctgtgattgtaag and cctcatcttacctaggcatc for ANP (NM_008725.2); tcctcagagggctaca and gctttggaggtgatta for BNP (AB039044.1); gagcgtcttggtggttagt and tcagtcagctacgctc for CNP (NM_010933.4); tggagacacagttcagagc and cgaagaacagtggatctgag for NPRA (NM_008727.5); tgaagcaagcaccactt and aggggcccagagatac for NPRB (NM_173788.3); tgtggagacagggctca and gctttggaaccttcagaa for NPRC (NM_008728.2). PCR was performed at: 95°C for 5 min, followed by 40 cycles of 95°C (10 s), 60°C (30 s), and 72°C (10 s). PCR products were confirmed by melting curve analysis using the Lightcycler Software 4.0 and samples normalized to a β-actin control. Experiments were performed in triplicate and the qRT-PCR was subjected to log transformation as recommended to achieve a normal distribution<sup>2</sup>.

Cardiac fibrosis and hypertrophy Analysis. Mouse hearts were fixed in 10% formalin and embedded in paraffin. Longitudinal sections (5 µm thick) of the heart were stained with Masson’s trichrome to analyze cardiac fibrosis. Slides were scanned by Aperio ScanScope CS2 scanner (Aperio) and images were taken using ImageScope software (MAN-0001, revision G). Interstitial fibrosis and perivascular fibrosis were analyzed by ImagePro Plus 6.2 software (Media Cybernetics, Bethesda, MD). Quantification of fibrosis was performed blindly on 12-15 fields (40X) or 12-15 arteries (40X) of left ventricle per heart. Interstitial fibrosis was measured as the percentage of total total area examined occupied by interstitial collagen. Perivascular fibrosis was calculated as the ratio of the area of fibrosis surrounding the vessel to the total vessel lumen area. Mouse hearts weights were normalized to body weight (HW/BW) and cardiomyocyte cross-sectional area were used as indices of cardiac hypertrophy. To measure myocyte cross-sectional area, the sections were stained with FITC-conjugated WGA (Sigma) (myocyte membranes) and DAPI (nuclei) and scanned slide images were taken as mentioned above. Measurements were taken from the outline of 100-150 myocytes with a clear cross-cut nucleus in 4-6 mice from each group.

Lung Edema and Lung Water Retention Analysis. Chest radiographs were taken with VetRay Technology DVR by Sedecal at 40 kVp, 2.0 mAs, and 160 mA (Buffalo Grove, Illinois) and images
were processed using IDEXX-PACSTM Imaging Software version 3.7 (Westbrook, Maine). For lung histology analysis, formalin-fixed lung sections were stained by hematoxylin and eosin. Images (12-15 random fields from each mouse) were analyzed using ImagePro Plus software (Media Cybernetics, Bethesda, MD) to determine the percent of total alveolar area with congestion in each field (40X). Lung edema was also assessed by lung weight/body weight ratios. Right and left lungs were excised and rapidly weighed and then total lung weight was calculated. Data are presented as the ratio of right+left lung wet weight/ body weight.

**Echocardiography.** Transthoracic echocardiograms were performed by an echocardiographer blinded to the mice’s genotype using a Vevo 2100 Imaging System (VisualSonic Inc., Toronto, Canada) as we previously described, with some modifications. Hair from the ventral thorax was removed by chemical depilatory (Nair) one day before the echocardiographic studies. Briefly, mice were sedated with 1.5% inhaled isoflurane. Two-dimensional and M-mode images of the LV were obtained from the parasternal long-axis and short axis acoustic windows. The 2D-guided M-mode recordings were analyzed using Vevo software; left ventricular internal dimension in diastole (LVIDd), left ventricular internal dimension in systole (LVIDs), interventricular septal wall thickness (IVS) and left ventricular posterior wall thickness (LVPW) were measured on at least 3 cardiac cycles and averaged for each mouse. All measurements were performed using the leading-edge-to-leading-edge convention. The fractional shortening (FS, %) and ejection fraction (EF, %) were calculated according to standard equations.

**Assays for aldosterone, cortisol, angiotensin II, renin activity and cGMP in plasma.** Plasma angiotensin II, cGMP, aldosterone and cortisol levels were measured by immunoassays as recommended by the manufacturer (Phoenix Pharmaceuticals, Inc., Assay Designs, Inc., Ann Arbor, MI and Abcam Inc., Cambridge, MA ). Renin activities in plasma samples were measured by fluorescence resonance energy transfer using a SensoLyte 520 Mouse Renin Assay Kit (AnaSpec, CA) according to the manufacturer's adjusted protocol.

**Statistical Analysis.** Survival was analyzed by the Kaplan-Meier method and comparison of two groups was assessed by log-rank test. Other statistical analyses were performed using nonparametric methods (unless otherwise indicated). Comparisons between >2 groups were performed by a 1-way ANOVA. Differences were considered to be significant if the 2-tailed \( p < 0.05 \). The number of animals (n) is indicated in the figure legends or results. All values were expressed as mean \pm SEM.
References


5. Research Animal Resources UoM. Reference values for laboratory animals. 2013.
Figure S1. Representative chest X-ray images showing prominent cardiac enlargement, lung congestion and pleural effusion in a DCM$^{ANP-/}$ mouse as compared with a DCM$^{ANP+/}$ mouse.
Figure S2. Effect of ANP deficiency on renal function in DCM. A) plasma creatinine and B) BUN levels in mice compared to normal reported values (upper limits, dotted line) \(^4,5\). N=8 and 8  
***p<0.001, NS p>0.05.
Figure S3. Effects of ANP deficiency on angiotensin II levels in DCM. Plasma levels were measured in both groups as described in Methods. N=8 and 8, NS, p>0.05.
Figure S4. Effects of ANP deficiency on plasma renin activity. Plasma renin activity was measured as described in Methods. N=8 and 7, NS p>0.05.
Figure S5. Effects of ANP deficiency on aldosterone levels in DCM. Plasma aldosterone levels were measured in both groups as described in Methods. N=8 and 8, NSp>0.05.
**Figure S6.** Effect of ANP deficiency on cortisol levels in DCM. Random cortisol levels were measured in both groups as described in Methods. N=13 and 8, *p<0.05.
Figure S7. Effects of ANP deficiency on cGMP levels in DCM. Plasma cGMP levels were measured in both groups as described in Methods. N=8 and 8, **p<0.01.