Endogenous cardiac natriuretic peptides protect the heart in a mouse model of dilated cardiomyopathy and sudden death

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Yasuno S, Usami S, Kuwahara K, Nakanishi M, Arai Y, Kinoshita H, Nakagawa Y, Fujiwara M, Murakami U, Ueshima K, Harada M, Nakao K. Endogenous cardiac natriuretic peptides protect the heart in a mouse model of dilated cardiomyopathy and sudden death. Am J Physiol Heart Circ Physiol 296: H1804–H1810, 2009. First published April 3, 2009; doi:10.1152/ajpheart.01033.2008.—Ventricular myocytes are known to show increased expression of the cardiac hormones atrial and brain natriuretic peptide (ANP and BNP, respectively) in response to pathological stress on the heart, but their function during the progression of nonischemic dilated cardiomyopathy remains unclear. In this study, we crossed a mouse model of dilated cardiomyopathy and sudden death, which we generated by cardioselectively overexpressing a dominant-negative form of the transcriptional repressor neuron-restrictive silencer factor (dnNRSF Tg mice), with mice lacking guanylyl cyclase-A (GC-A), a common receptor for ANP and BNP, to assess the effects of endogenously expressed natriuretic peptides during progression of the cardiomyopathy seen in dnNRSF Tg mice. We found that dnNRSF Tg;GC-A−/− mice were born normally, but then most died within 4 wk. The survival rates among dnNRSF Tg;GC-A+/+ and dnNRSF Tg mice were comparable, but dnNRSF Tg;GC-A−/− mice showed greater systolic dysfunction and a more severe cardiomyopathic phenotype than dnNRSF Tg mice. Collectively, our findings suggest that endogenous ANP/BNP protects the heart against the death and progression of pathological remodeling in a mouse model of dilated cardiomyopathy and sudden death.

Heart failure is a leading cause of mortality and morbidity in the Western world (17). In United States, for example, ~550,000 new cases are diagnosed each year (12). Despite recent progress in both medical and surgical management, heart failure remains an extremely lethal condition associated with a very poor quality of life and a 5-year survival rate of only ~50% (12, 34). Therefore, a better understanding of the molecular mechanisms underlying the progression of heart failure would be highly desirable, since it could serve as the basis for developing novel therapeutic approaches to treating the ailment.

Heart failure is accompanied by dysregulation of myocardial expression of a set of cardiac genes. One of the best-characterized genetic alterations seen in failing ventricles is reactivation of fetal cardiac genes, including those encoding atrial natriuretic peptide (ANP), skeletal α-actin, and β-myosin heavy chain, which are active within the fetal ventricles but quiescent in normal postnatal ventricles (3, 26). Such transcriptional alterations have been shown to correlate with deterioration of cardiac function and, conversely, improvement of cardiac function in response to medical and/or nonmedical interventions is accompanied by normalization of these genetic alterations (1, 2, 15, 17). Thus reprogramming cardiac gene expression appears to modify the pathological process during the progression of heart failure.

ANP is a cardiac hormone usually synthesized in the atrium and released in response to wall stretch. Upon its release, ANP acts at multiple sites to exert diuretic, natriuretic, and vasorelaxant effects (21). These biological properties are shared by brain natriuretic peptide (BNP), which, despite its name, is primarily secreted from the ventricles (18, 23, 31). Moreover, recent evidence indicates that ANP and BNP also act as paracrine factors, exerting antihypertrophic and antiﬁbrotic effects in the heart (5, 9, 14, 24, 29). They exert both their hormonal and paracrine effects through activation of their common receptor, guanylyl cyclase-A receptor (GC-A), also known as natriuretic peptide receptor-A, which is expressed in a variety of tissues, including kidneys, blood vessels, adrenal glands, and heart (22), and is coupled to an increase in the intracellular concentration of cGMP (10). Ventricular expression of both ANP and BNP is upregulated in several pathological conditions of the heart, and their plasma concentrations are markedly elevated in patients with congestive heart failure (CHF). In fact, measurements of plasma ANP and BNP levels are used clinically to assist in the diagnosis of CHF, to assess prognosis, and to determine therapeutic strategy (16, 27, 30, 33). In addition, ANP and BNP are already being used to treat patients with acute heart failure (4, 32).

We recently found that, following myocardial infarction, mice lacking GC-A showed a higher incidence of acute heart failure, more severe left ventricular (LV) remodeling, and greater impairment of LV systolic function than mice expressing GC-A (20). This suggests that endogenous ANP/BNP may protect heart after myocardial infarction, but the role of intrinsic ANP/BNP signaling during the development of nonischemic dilated cardiomyopathy remains unclear. To address that question, in this study, we crossed a transgenic (Tg) mouse cardioselectively overexpressing a dominant-negative form of the transcriptional repressor neuron-restrictive silencer factor (dnNRSF Tg mice), which is a mouse model of dilated cardiomyopathy and sudden death, with mice lacking GC-A (GC-A−/−; see Ref. 11). Almost all of dnNRSF Tg;GC-A−/−
mice died by 4 wk after birth. The survival rates among dnNRSF Tg;GC-A+/− and dnNRSF Tg mice were comparable, but dnNRSF Tg;GC-A−/− mice showed greater systolic dysfunction and a more severe cardiomyopathic phenotype than dnNRSF Tg mice. These findings suggest that endogenous ANP/BNP protects the heart against the sudden death and the progression of pathological remodeling in the mouse model of dilated cardiomyopathy.

MATERIAL AND METHODS

Animals. The animal care and all experimental protocols were reviewed and approved by the Animal Research Committee in the Kyoto University Graduate School of Medicine. GC-A knockout (KO) mice generated as described previously were kindly provided by D. L. Garbers (The University of Texas Southwestern Medical Center) (14). Using methods described previously (11), we established two dnNRSF Tg lines (471 and 474) having different survival rates. In the present study, we used dnNRSF Tg line 474, whose survival rate was ~80% at 20 wk of age. The GC-A KO (GC-A−/−), GC-A heterozygous KO (GC-A−/+), dnNRSF Tg;GC-A−/−, and dnNRSF Tg;GC-A+/− mice used to examine effects on survival were generated by crossing male GC-A−/− and female dnNRSF Tg;GC-A−/− mice. The wild-type (WT), GC-A+/−, and dnNRSF Tg;GC-A−/− mice were used in other experiments were generated by crossing male GC-A+/− and female dnNRSF Tg;GC-A+/− mice. The genetic background of the original GC-A KO and dnNRSF Tg mice was C57BL/6.

Echocardiographic and hemodynamic analyses. After anesthetization by intraperitoneal injection of a 2.5% wt/vol solution (8 μL/g) of tribromoethanol/amylyne hydrate (Avertin), echocardiography was carried out using a Toshiba Power Vision 8000 echocardiographic system equipped with a 12-MHz imaging transducer as described previously (11). For hemodynamic analyses, mice were intubated and the left carotid artery and advanced in the left ventricle to record LV systolic and diastolic pressures, as well as the maximum and minimum rates of LV pressure development (dP/dt) (7).

Histological examination. Hearts were fixed in 10% formalin and prepared for histological analysis as described previously (13).

Quantitative RT-PCR analysis. Using 1- or 50-ng samples of total RNA prepared from ventricles, levels of mRNA encoding mouse ANP and BNP; skeletal α-actin; sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) 2; hyperpolarization-activated cyclic nucleotide-gated potassium channel (HCN) 2 and HCN4, which encode channels that carry the hyperpolarization-activated current; calcium channel, voltage-dependent, T-type, α1H-subunit (CACNA1H), which encodes the α1H T-type Ca2+ channel; GC-A; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were then determined by quantitative real-time PCR following the manufacturer’s protocol (Applied Biosystems, Zaventem, Belgium) as described previously (20). The real-time PCR primers and probes for ANP, BNP, skeletal α-actin, SERCA2, HCN2, HCN4, CACNA1H, GC-A, and GAPDH were all purchased from Applied Biosystems.

Statistical analysis. Data are presented as means ± SE. ANOVA was used to make multiple group comparisons. If ANOVA showed a significant difference (P < 0.05), a post hoc Fisher least-significant difference test was used to identify which group differences accounted for the significant P value. Survival rate was analyzed using the

Fig. 1. Kaplan-Meier analysis of survival after birth among guanylyl cyclase-A (GC-A)-sufficient (GC-A+/+) mice, GC-A heterozygous knockout (GC-A−/+), mice, mice lacking GC-A (GC-A−/−), mice overexpressing a dominant-negative form of the transcriptional repressor neuron-restrictive silencer factor (dnNRSF Tg line 474 was used in this study. *P < 0.05 vs. dnNRSF Tg;GC-A−/−. n. No. of mice.

Fig. 2. Expression of GC-A mRNA and heart weight (HW)-to-body weight (BW) ratios (mg/g) in wild-type, GC-A−/−, dnNRSF Tg, and dnNRSF-Tg; GC-A−/− mice. A and B: expression of GC-A mRNA of the heart (A) and the kidney (B) in wild-type, GC-A−/−, dnNRSF Tg, and dnNRSF-Tg;GC-A−/− mice. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. *P < 0.05. C: HW-to-BW ratios (mg/g) in wild-type, GC-A−/−, dnNRSF Tg, and dnNRSF-Tg;GC-A−/− mice. Note that the ratio is significantly increased in dnNRSF Tg;GC-A−/− mice. *P < 0.05.
Kaplan-Meier method with the log-rank test. Values of $P < 0.05$ were considered significant.

RESULTS

Loss of GC-A is perinatally lethal in dnNRSF Tg mice. To determine the effects of the increased cardiac expression of ANP/BNP during the development and progression of dilated cardiomyopathy leading to sudden death, we generated dnNRSF Tg mice having a GC-A-null background by crossing dnNRSF Tg (line 474) with GC-A$^{-/-}$ mice (14). The resultant dnNRSF Tg;GC-A$^{-/-}$ mice were born at a rate similar to GC-A$^{-/-}$ mice. Moreover, the heart weight-to-body weight ratios in postnatal day 2 did not differ significantly between the two genotypes (data not shown). Both dnNRSF Tg mice and dnNRSF Tg mice with a heterozygous GC-A background (dnNRSF Tg;GC-A$^{-/-}$) grew normally until about 3 wk of age and started to die at ~4 wk of age (Fig. 1) (11). By contrast, ~80% of dnNRSF Tg;GC-A$^{-/-}$ mice died by 4 wk of age (by the time they were weaned), suggesting that GC-A is crucial for the survival of mice with dilated cardiomyopathy and lethal arrhythmia.

Diminished GC-A expression leads to deterioration of cardiac function in dnNRSF Tg mice. The perinatal lethality of the dnNRSF Tg;GC-A$^{-/-}$ genotype made it difficult to assess cardiac function in these mice. We therefore used dnNRSF Tg;GC-A$^{-/-}$ mice, which expressed ~50% less GC-A mRNA than dnNRSF Tg mice, to evaluate the functional contribution of GC-A to the dnNRSF Tg heart (Fig. 2, A and B) (25). We initially compared the heart weight-to-body weight ratios in 8-wk-old WT, GC-A$^{+/+}$, dnNRSF Tg, and dnNRSF Tg;GC-A$^{-/-}$ mice. At that age, the cardiac structure and function of dnNRSF Tg mice were not yet disturbed (11). As shown in Fig. 2C, the heart weight-to-body weight ratios did not significantly differ among WT, dnNRSF Tg, and GC-A$^{+/+}$ mice but was significantly higher in dnNRSF Tg;GC-A$^{-/-}$ mice than in the other three groups. Moreover, subsequent echocardiography revealed dnNRSF Tg;GC-A$^{-/-}$ mice to have enlarged LV systolic and diastolic dimensions, increased LV mass, and reduced systolic function compared with dnNRSF Tg mice (Table 1). The hemodynamic parameters obtained through intracardiac catheterization showed significantly reduced LV systolic pressure and impaired dP/dt (Table 1). Thus impairment of GC-A signaling appears to degrade cardiac function in dnNRSF Tg mice.

Reducing GC-A promotes cardiac pathology in dnNRSF Tg mice. Histological analysis revealed additional effects of diminished GC-A expression on the structure of the dnNRSF Tg heart. The left ventricles were dilated to a greater extent in 8-wk-old dnNRSF Tg;GC-A$^{-/-}$ mice than in dnNRSF Tg or GC-A$^{+/+}$ mice (Fig. 3A). In addition, microscopic examination showed fibrosis to be more extensive in dnNRSF Tg;GC-A$^{-/-}$ mice than dnNRSF Tg mice, suggesting that endogenous ANP/BNP acts via GC-A to attenuate cardiac fibrosis in dnNRSF Tg hearts (Fig. 3B).

Finally, we assessed the mRNA expression of ANP, BNP, skeletal $\alpha$-actin, and SERCA2, four marker genes used to evaluate cardiac pathology, and the mRNA expression of HCN2, HCN4, and CACNA1H, which we previously reported to be upregulated in dnNRSF Tg hearts (11). In that earlier study, we also observed that cardiac expression of ANP, BNP, and skeletal $\alpha$-actin mRNA is upregulated in 8-wk-old dnNRSF Tg mice but that expression of SERCA2 mRNA is similar in 8-wk-old WT and dnNRSF Tg mice (11). In the present study, we found that the levels of ANP mRNA in dnNRSF Tg;GC-A$^{-/-}$ mice were even higher than in dnNRSF Tg hearts, whereas the levels of BNP mRNA were similar in dnNRSF Tg;GC-A$^{-/-}$ and dnNRSF Tg hearts (Fig. 4). Moreover, levels of skeletal $\alpha$-actin mRNA were higher, whereas those of SERCA2 mRNA were significantly lower in dnNRSF Tg;GC-A$^{-/-}$ hearts than dnNRSF Tg hearts (Fig. 4). Taken together, these findings are consistent with the notion that reducing GC-A expression promotes pathological remodeling of dnNRSF Tg hearts. The cardiac expression of HCN2, HCN4, and CACNA1H mRNA did not significantly differ in dnNRSF Tg;GC-A$^{-/-}$ and dnNRSF Tg;GC-A$^{-/-}$ mice (Fig. 5).

DISCUSSION

Although it is well recognized that ventricular expression of both ANP and BNP is upregulated in hearts affected by diluted

Table 1. Echocardiographic and hemodynamic analysis of 8-wk-old mice

<table>
<thead>
<tr>
<th>Echocardiographic data</th>
<th>GCA$^{+/+}$</th>
<th>GCA$^{-/-}$</th>
<th>dnNRSF Tg;GC-A$^{+/+}$</th>
<th>dnNRSF Tg;GC-A$^{-/-}$</th>
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<tbody>
<tr>
<td>Echocardiographic data</td>
<td>n = 6</td>
<td>n = 4</td>
<td>n = 6</td>
<td>n = 4</td>
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<tr>
<td>HR, beats/min</td>
<td>367.0 ± 4.0</td>
<td>378.3 ± 14.3</td>
<td>330.5 ± 5.5</td>
<td>416.0 ± 44.2</td>
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<td>LVDD, mm</td>
<td>4.02 ± 0.11</td>
<td>4.43 ± 0.21</td>
<td>4.00 ± 0.29</td>
<td>4.90 ± 0.19†</td>
</tr>
<tr>
<td>LVDS, mm</td>
<td>2.76 ± 0.14</td>
<td>2.90 ± 0.19</td>
<td>2.87 ± 0.20</td>
<td>4.26 ± 0.15†‡</td>
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<tr>
<td>IVST, mm</td>
<td>0.66 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>0.65 ± 0.03</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.67 ± 0.04</td>
<td>0.68 ± 0.03</td>
<td>0.69 ± 0.03</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>FS, %</td>
<td>32.8 ± 1.9</td>
<td>34.0 ± 4.5</td>
<td>27.9 ± 1.5</td>
<td>12.8 ± 1.5§‡</td>
</tr>
<tr>
<td>EF, %</td>
<td>69.7 ± 2.6</td>
<td>70.0 ± 5.2</td>
<td>67.2 ± 2.7</td>
<td>33.5 ± 5.4†‡</td>
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<tr>
<td>LVM, mg</td>
<td>90.2 ± 8.1</td>
<td>104.2 ± 1.8</td>
<td>89.3 ± 3.0</td>
<td>116.6 ± 11.7†§</td>
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<tr>
<td>Hemodynamic data</td>
<td>n = 4</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>dP/dt, mmHg/s</td>
<td>4.865 ± 201</td>
<td>5.005 ± 283</td>
<td>4.757 ± 325</td>
<td>3.747 ± 202†‡</td>
</tr>
<tr>
<td>dP/dt, mmHg/s</td>
<td>-4.935 ± 218</td>
<td>-5.150 ± 579</td>
<td>-4.756 ± 237</td>
<td>-3.465 ± 308†‡</td>
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<tr>
<td>HR, min$^{-1}$</td>
<td>49.8 ± 26.9</td>
<td>48.7 ± 25.8</td>
<td>533 ± 29.4</td>
<td>596 ± 33.5</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>98.9 ± 4.5</td>
<td>102.7 ± 5.5</td>
<td>95.1 ± 3.6</td>
<td>83.5 ± 3.9‡</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>2.25 ± 0.56</td>
<td>3.15 ± 0.38</td>
<td>2.36 ± 0.69</td>
<td>2.73 ± 0.85</td>
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</table>

Values are means ± SE; n, no. of mice. GC-A$^{-/+}$, guanylyl cyclase-A (GC-A)-sufficient; GC-A$^{-/-}$, GC-A heterozygous knockout mice; dnNRSF Tg mice overexpressing a dominant-negative form of the transcriptional repressor neuron-restrictive silencer factor; HR, heart rate; LVDD, left ventricular end-diastolic dimension; LVDS, left ventricular end-systolic dimension; IVST, interventricular septal thickness; PWT, posterior wall thickness; FS, fractional shortening; EF, ejection fraction; LVM, left ventricular mass; dP/dt, first derivative of pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure. $P < 0.05$ vs. control wild-type mice (†), vs. dnNRSF Tg mice (‡), and vs. GC-A$^{+/+}$ mice (§).
Fig. 3. Histological analysis. A: hematoxylin and eosin staining low-magnification photomicrographs showing the histology of wild-type, GC-A+/−, dnNRSF Tg, and dnNRSF-Tg;GC-A+/− ventricles at 8 wk of age. Scale bars are 2.5 mm. B: Masson’s trichrome staining showing fibrosis in sections from the left ventricles of wild-type, GC-A+/−, dnNRSF Tg, and dnNRSF-Tg;GC-A+/− mice at 8 wk of age. Scale bars are 40 μm.
cardiomyopathy (24), the effects of endogenous ANP/BNP during the development and progression of the ailment were not known. In the present study, we crossed dnNRSF Tg mice, a mouse model of dilated cardiomyopathy leading to sudden death, with GC-A−/−, dnNRSF Tg, and dnNRSF-Tg;GC-A−/− mice. Almost all dnNRSF Tg;GC-A−/− mice died within 4 wk after birth, whereas dnNRSF Tg;GC-A+/− mice lived at least 6 wk; dnNRSF Tg;GC-A−/+ mice showed cardiomyopathic phenotypes that were more severe than dnNRSF Tg;GC-A−/−. The results indicate that an insufficiency of GC-A accelerates the progression from latent to more evident cardiomyopathy in dnNRSF Tg mice, suggesting endogenous ANP/BNP exerts a protective effect against the progression of pathological cardiac remodeling and sudden death.

In healthy hearts, ANP is primarily secreted from the atrium, whereas BNP is primarily secreted from the ventricle, although small amounts of BNP are secreted from the atrium (18, 21, 23, 31). Ventricular expression of both ANP and BNP is upregulated under such pathological conditions as cardiac hypertrophy and heart failure, which makes plasma ANP/BNP levels a good prognostic indicator of clinical severity in a variety of cardiac diseases (21). Moreover, because improvement of cardiac function in response to medical and/or nonmedical therapy is accompanied by reductions in plasma ANP/BNP levels, they can serve as objective indicators with which to monitor the efficacy of therapy (30). As hormones, ANP and BNP exert diuretic, natriuretic, and vasorelaxant effects and counteract the effects of the renin-angiotensin-aldosterone and sympathetic nervous systems (21, 24, 25). In addition, they also act as paracrine factors, exerting antihypertrophic and antifibrotic effects in the heart (5, 9, 29). For these reasons, ANP and BNP are already being used clinically in patients with acute heart failure (4, 32). The roles played by endogenous ANP/BNP in the pathophysiology of heart failure had nonetheless remained unresolved. However, we recently showed that endogenous ANP/BNP is protective against acute heart failure and cardiac remodeling following experimental myocardial infarction in...
that increased blood pressure accelerates the progression of cardiac dysfunction and die as a result of lethal arrhythmias sometime later (11). The perinatal lethality of the dnNRSF Tg;GC-A−/− genotype would seem to indicate that endogenous ANP/BNP is able to protect dnNRSF Tg mice from sudden cardiac death, perhaps by exerting an antiarrhythmic effect. That said, we were unable to confirm that dnNRSF Tg;GC-A−/− mice die from lethal ventricular arrhythmias because of the technical difficulty of continuously collecting electrocardiography from perinatal mice. Blood pressures are elevated in GC-A−/− mice (14), which raises the possibility that increased blood pressure accelerates the progression of cardiac dysfunction in dnNRSF Tg;GC-A−/− mice. There is also a possibility that an as yet unidentified fundamental alteration caused by the GC-A-null background may have affected the phenotype. On the other hand, the idea that ANP/BNP exerts an antiarrhythmic effect is consistent with findings of an earlier report showing that older (12 mo of age) GC-A−/− mice have an increased susceptibility to ventricular arrhythmias (8). It also suggests the potential usefulness of ANP/BNP in the treatment of cardiomyopathies with a high susceptibility to arrhythmias. Indeed, ANP reportedly exerts a protective effect against arrhythmias induced by ischemia-reperfusion in dogs (28) and against those induced by proarrhythmic drugs in rabbits (6). The mechanism by which ANP/BNP might prevent arrhythmias remains unknown, although the recent report that sildenafil, a specific phosphodiesterase type 5 inhibitor, reduces the severity of arrhythmias during ischemia in dogs (19) suggests the effect is mediated by increasing cGMP levels via activation of GC-A. All of these data are suggestive of the therapeutic potential of ANP/BNP for the prevention of malignant arrhythmias in patients with heart failure or myocardial ischemia.

In conclusion, we have demonstrated that endogenous cardiac natriuretic peptides are able to markedly slow adverse cardiac remodeling during the progression of nonischemic cardiomyopathy toward sudden cardiac death. ANP and BNP are already being used to treat patients with acute heart failure. It is our hope that these findings begin to form the basis for novel and improved approaches to the treatment of patients with chronic heart failure and a high susceptibility to sudden cardiac death.

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