Effects of Pressure Overload on Extracellular Matrix Expression in the Heart of the Atrial Natriuretic Peptide–Null Mouse

Dajun Wang, Suzanne Oparil, Ji An Feng, Peng Li, Gilbert Perry, Lan Bo Chen, Meiru Dai, Simon W.M. John, Yiu-Fai Chen

Abstract—This study tested the hypothesis that atrial natriuretic peptide has direct antihypertrophic actions on the heart by modulating expression of genes involved in cardiac hypertrophy and extracellular matrix production. Hearts of male, atrial natriuretic peptide–null and control wild-type mice that had been subjected to pressure overload after transverse aortic constriction and control unoperated hearts were weighed and subjected to microarray, Northern blot, and immunohistochemical analyses. Microarray and Northern blot analyses were used to identify genes that are regulated differentially in response to stress in the presence and absence of atrial natriuretic peptide. Immunohistochemical analysis was used to identify and localize expression of the protein products of these genes. Atrial natriuretic peptide–null mice demonstrated cardiac hypertrophy at baseline and an exaggerated hypertrophic response to transverse aortic constriction associated with increased expression of the extracellular matrix molecules periostin, osteopontin, collagen I and III, and thrombospondin, as well as the extracellular matrix regulatory proteins, matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3, and the novel growth factor pleiotrophin compared with wild-type controls. These results support the hypothesis that atrial natriuretic peptide protects against pressure overload–induced cardiac hypertrophy and remodeling by negative modulation of genes involved in extracellular matrix deposition. (Hypertension. 2003;42:655–662.)

Key Words: atrial natriuretic factor • constriction • aorta • pressure overload • hypertrophy, cardiac • extracellular matrix • growth substances

Atrial natriuretic peptide (ANP) inhibits cell growth and proliferation and induces apoptosis in a variety of cell types, including vascular smooth muscle cells (VSMCs) and cardiomyocytes.1–3 Intracardiac ANP might also play an important autocrine/paracrine role in modulating cardiac remodeling under stress conditions and might protect against the development of pathologic cardiac hypertrophy.4–7 Synthesis and release of ANP in the heart are increased under stressful conditions such as pressure and volume overload–induced pathologic cardiac hypertrophy, exercise–induced physiologic cardiac hypertrophy, heart failure, and hypoxic pulmonary hypertension.1,2 Expression of ANP is inversely related to cardiac growth/hypertrophy.5,8–11 Transgenic mice overexpressing ANP have smaller hearts than do wild-type mice, and ANP gene delivery attenuates cardiac hypertrophy in spontaneously hypertensive rats.5,7 Conversely, transgenic mice with homozygous disruption of the pro-ANP gene (Nppa−/− mice) or the natriuretic peptide receptor-A (NPR-A) gene (Npr1−/− mice) exhibit cardiac hypertrophy at baseline that is out of proportion to the modest elevations in blood pressure (BP) observed in these models.8–11 Furthermore, in Npr1−/− mice, pressure overload induced by transverse aortic constriction (TAC) results in a greater (55%) increase in left ventricular (LV) weight than in Npr1+/+ controls.12 β-Adrenergic agonist treatment has been shown to induce the expression of pleiotrophin, a new member of the heparin-binding cytokine/growth factor family,13,14 in Npr1−/− mice, but not in Npr1+/+ controls.14 This provocative finding suggests the possibility that modulation of pleiotrophin expression might contribute to the antihypertrophic effects of ANP.

In contrast to ANP, B-type natriuretic peptide (BNP) appears not to modulate cardiac hypertrophy. Circulating BNP is not increased, and BNP is unable to compensate for the lack of ANP in Nppa−/− mice.15 BNP−/− mice do not develop cardiac hypertrophy or systemic hypertension.16 These findings suggested that ANP deficiency alone is sufficient to generate cardiac hypertrophy, particularly under conditions of hemodynamic stress.

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This study tested the hypothesis that ANP has a counter-regulatory effect that protects against pressure overload–induced cardiac hypertrophy and remodeling. Male Nppa+/− and control wild-type Nppa+/+ mice were subjected to TAC, and the effects of the resultant pressure overload on cardiac hypertrophy and remodeling were examined. Microarray and Northern blot analyses were used to identify genes that are regulated differentially in response to stress in the presence and absence of ANP. Immunohistochemical analysis was used to identify and localize expression of the protein products of these genes.

**Methods**

**Animal Preparation**

Nppa−/− mice originally generated in the laboratory of Dr Oliver Smithies5 as well as control Nppa−/+ mice of the 129(C57Bl/6) strain (8 to 10 generations from the cross) were studied. Because the phenotypes of Nppa−/− and Nppa−/+ mice were identical, we examined only Nppa−/− and Nppa−/+ mice.6,8,11 Mice were raised in our resident colony, which was founded with pathogen-free breeding pairs. Genotypes were identified by polymerase chain reaction (PCR) assay of genomic DNA from tail snips after weaning.11,17 All pairs. Genotypes were identified by polymerase chain reaction phenotypes of Nppa strain (8 to 10 generations from the cross) were studied. Because the products of these genes.

**Surgical Procedures**

Ten-week-old, male Nppa−/− and Nppa−/+ mice, weighing 20 to 30 g, were anesthetized with a mixture of ketamine (8 mg/100 g) and xylazine (1.2 mg/100 g) administered intraperitoneally. TAC was performed as described previously by Rockman et al.18 TAC was performed between the left common carotid artery and the brachiocephalic trunk by tying a 7-0 silk suture against a 0.4-mm wire. The wire was then removed after banding the ascending aorta. The pneumothorax was evacuated, and the mouse was allowed to recover from anesthesia. Unoperated mice served as controls.

One, 3, or 7 days after TAC, mice (TAC and control) were anesthetized with ketamine/xylazine, and both common carotid arteries were cannulated with PE-10 cannulas for BP gradient measurements with pressure transducers coupled to a data-acquisition unit (model 100, Biopac) with output to a personal computer, sampling at 100 Hz, and Acknowledge software (Biopac Systems) to ensure a similar degree of afterload in each mouse.

**Tissue Collection**

Mice were humanely killed with an overdose of pentobarbital sodium (Nembutal, 50 mg/mL); the hearts were quickly removed, and the LV, right ventricle (RV), and atria (AT) were carefully dissected, weighed, and immediately frozen in liquid nitrogen for RNA isolation. Lung, kidney, brain, liver, and spleen were also dissected, weighed, and immediately frozen in liquid nitrogen for RNA isolation. Lung, kidney, brain, liver, and spleen were also obtained at the 7-day time point.

**RNA Isolation**

Total RNA was isolated from snap-frozen LV by a modification of the acid guanidinium thiocyanate and phenol/chloroform extraction method, with the appropriate reagent (TRizol, Life Technologies Inc) and following the procedures provided by the manufacturer.

**cDNA Microarray Analysis**

We used a cDNA microarray analysis to delineate the profile of genes whose expression is differentially regulated in the heart after TAC in the presence or absence of ANP. We reasoned that these genes, or a subset thereof, might function in a coordinated manner to mediate pressure overload–induced LV hypertrophy and its negative modulation by ANP. Microarray analysis of cDNA was performed with mouse microarray membranes (GeneFilter GF-400 membranes, Research Genetics) and following the procedures provided by the manufacturer.

Total RNA was extracted from LV, and 5 μg RNA (pooled from 3 mice in the control Nppa−/+, TAC Nppa−/−, control Nppa−/−, and TAC Nppa−/− groups at 7 days after TAC) was reverse-transcribed (Superscript Kit, Research Genetics) and labeled with [3]PdCTP. The 32P-labeled cDNAs were purified and hybridized with the membranes (GeneFilter) for 16 hours. The membrane was washed, and the radioactive signals were recorded on a high-performance screen (Storage Phosphor) and scanned with a scanner system (Cyclone Storage Phosphor System, Packard BioScience). The image data were processed with a software package (Pathways 4 software, Research Genetics) and transferred to another (Microsoft Excel) for further analysis.

Experimental and control samples (control Nppa−/−, TAC Nppa−/−, control Nppa−, and TAC Nppa− groups) were processed simultaneously under identical conditions and hybridized on 4 GeneFilter membranes produced from the same batch. The signal on each spot was normalized against the signals of housekeeping genes printed on the same membrane. The ratio of the normalized values of corresponding spots on membranes with experimental and control samples were calculated as an index of the increase (experimental/control ratio>1) or decrease (experimental/control ratio<1) in mRNA levels under the experimental conditions. Experiments were repeated twice (6 mice from each genotype/treatment group) with different membranes, and the data were averaged. Results for genes of particular interest were verified by Northern blot analysis. Comparisons between genotypes (Nppa−− vs Nppa−+−) and treatment groups (TAC vs control) were carried out.

**Northern Blot Analysis**

Based on the results of microarray analysis, total RNA isolated from LV at 1, 3, or 7 days after TAC was subjected to Northern blot analysis for mRNAs of interest, as described previously.11,17 with aP-labeled selective cDNA probes for ANP and BNP (courtesy of Drs R. Wiegand and H.E. Tolunay, respectively, Monsanto, St Louis, Mo) and cDNA probes for periostin, collagen I and III, osteopontin, and plieotrophin that had been generated in our laboratory by reverse transcription-PCR with mouse heart RNA as the template, as previously described.19 18S rRNA was used as a control to account for variation in RNA loading.

**Morphology and Immunohistochemical Analysis**

At the 7-day time point, hearts from separate sets of mice were fixed with freshly prepared 4% paraformaldehyde, paraffin-embedded, and sectioned for morphological and immunohistochemical examination.

**Statistical Analysis**

Results are expressed as mean±SEM. Our primary statistical test was ANOVA. Because the body weights of Nppa−/− and Nppa−/+ mice were different, the effects of TAC on relative tissue weights were determined by ANCOVA, with body weight as the covariate. Differences in mean values due to main effects (genotype and TAC) and interactions between these main effects were tested, with P<0.05 considered statistically significant.

**Results**

**TAC Gradient and Adjusted Tissue Weight**

The pressure gradients across the TAC were similar in both genotypes (Figure 1A; TAC Nppa−/− mice: right carotid mean arterial pressure [MAP]=113.2±10.8 mm Hg and left carotid MAP=89.8±10.9 mm Hg, n=9; TAC Nppa−/− mice: right carotid MAP=117.4±10.2 mm Hg and left carotid MAP=95.9±9.9 mm Hg, n=7). The low MAP gradient across the TAC observed in this study compared with the...
published literature\(^{9,18}\) was related to the choice of anesthetic (ketamine/xylazine). This was replaced by tribromoethanol in subsequent experiments, with a resulting increase in the observed TAC gradient to 52.7 ± 6.5 mm Hg, \(n=8\), in TAC Nppa\(^{-/-}\) mice and 55.3 ± 6.8 mm Hg, \(n=6\), in TAC Nppa\(^{-/-}\) mice.

Whole-heart weight and the individual chamber weights were significantly greater in Nppa\(^{-/-}\) than in Nppa\(^{+/+}\) mice at baseline (Figure 1B and Table 1). Both genotypes demonstrated cardiac hypertrophy (LV, RV, and AT), as evidenced by increased heart and individual chamber weights as early as 3 days after TAC (Figure 1B and Table 1). Pressure overload–induced cardiac hypertrophy was exaggerated in Nppa\(^{+/+}\) mice compared with the wild type. Nppa\(^{-/-}\) mice tended to be heavier than the wild type under both control and 7-day TAC conditions, and organ weights tended to be greater in Nppa\(^{-/-}\) mice (Table 1). Interestingly, lungs of TAC Nppa\(^{-/-}\) mice were significantly heavier than in the other groups, likely reflecting incipient heart failure at 7 days after TAC.

**ANP and BNP mRNA Expression in the Heart**

Northern blot analysis demonstrated significant increases in steady-state levels of ANP mRNA in LV of TAC Nppa\(^{+/+}\) mice at 3 and 7 days after TAC compared with control, unoperated Nppa\(^{+/+}\) mice (Figure 2A). Intact ANP mRNA was not detectable in Nppa\(^{-/-}\) mice.

To study whether an enhancement of BNP expression would compensate for the loss of ANP in Nppa\(^{-/-}\) mice, we measured steady-state BNP mRNA in LV of control, unoperated and TAC mice. BNP peptide levels were not measured because of the unavailability of a selective antibody against mouse BNP. In control unoperated mice, steady-state BNP mRNA levels in the LV were not different between Nppa\(^{+/+}\) (1.19 ± 0.10, \(n=15\)) and Nppa\(^{-/-}\) (1.00 ± 0.03, \(n=15\)) mice. LV BNP mRNA increased in both genotypes after TAC (Figure 2B). BNP mRNA peaked sooner (1 day after TAC) in Nppa\(^{-/-}\) mice than in Nppa\(^{+/+}\) mice (3 days after TAC). There was no difference in LV BNP mRNA levels between genotypes at 3 and 7 days after TAC.

**cDNA Microarray Analysis**

Of >5000 genes (~1200 known genes) examined, expression of 24 was elevated >2-fold and expression of 30 was reduced to <0.5 in LV of control, unoperated Nppa\(^{-/-}\) mice compared with control, unoperated Nppa\(^{+/+}\) mice. Expression of 95 genes was elevated >2-fold and expression of 20 was reduced to <0.5 in LV of TAC Nppa\(^{-/-}\) mice at 7 days after TAC compared with control, unoperated Nppa\(^{-/-}\) mice. Expression of 80 genes was elevated >2-fold and expression of 10 was reduced to <0.5 in 7-day TAC Nppa\(^{-/-}\) compared with control, unoperated Nppa\(^{-/-}\) mice. Importantly, most of the identifiable genes whose expression was increased in LV after TAC, particularly in the Nppa\(^{-/-}\) genotype, encoded extracellular matrix (ECM) molecules (eg, periostin increased 12-fold in TAC Nppa\(^{-/-}\) mice and 43-fold in TAC Nppa\(^{+/+}\) mice; procollagen increased 3-fold in TAC Nppa\(^{+/+}\) mice and 5-fold in TAC Nppa\(^{-/-}\) mice; and thrombospondin increased 2-fold in TAC Nppa\(^{-/-}\) mice and 3-fold in TAC Nppa\(^{+/+}\) mice, compared with their respective control, unoperated groups of mice) or enzymes/ enzyme inhibitors that regulate expression of ECM proteins (eg, matrix metalloproteinase-2 [MMP-2] increased 2-fold in TAC Nppa\(^{-/-}\) mice and 3-fold in TAC Nppa\(^{+/+}\) mice, and tissue inhibitor of metalloproteinase-3 [TIMP-3] increased 2-fold in TAC Nppa\(^{-/-}\) mice and 5-fold in TAC Nppa\(^{+/+}\) mice, compared with their respective control, unoperated groups of mice). Calmodulin was also overex-

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**Figure 1.** A, MAP gradient across the TAC in ketamine/xylazine–anesthetized, male Nppa\(^{-/-}\) and Nppa\(^{+/+}\) mice 1 week after TAC. B, Whole-heart weights of TAC and control mice at 1, 3, and 7 days after surgery. Adjusted tissue weights were determined by ANCOVA with body weight as a covariate. *\(P<0.05\) vs respective unoperated mice; #\(P<0.05\) vs respective Nppa\(^{-/-}\) mice.

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**Table 1.** Effects of 1-Week TAC on Body Weights and Adjusted Tissue Weights of Nppa\(^{+/+}\) and Nppa\(^{-/-}\) Mice

<table>
<thead>
<tr>
<th>Genotype and Treatment</th>
<th>BW, g</th>
<th>Heart, mg</th>
<th>LV, mg</th>
<th>RV, mg</th>
<th>Atria, mg</th>
<th>Lung, mg</th>
<th>Kidney, mg</th>
<th>Brain, mg</th>
<th>Liver, mg</th>
<th>Spleen, mg</th>
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<tr>
<td>Control</td>
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<tr>
<td>Nppa(^{+/+})</td>
<td>(10)</td>
<td>22.4±0.8</td>
<td>115±2</td>
<td>84±1</td>
<td>24±1</td>
<td>8±0.2</td>
<td>150±1</td>
<td>303±13</td>
<td>494±24</td>
<td>1125±28</td>
</tr>
<tr>
<td>Nppa(^{-/-})</td>
<td>(7)</td>
<td>28.4±1.2</td>
<td>173±3</td>
<td>118±3*</td>
<td>43±2*</td>
<td>11±0.2*</td>
<td>160±4</td>
<td>399±13</td>
<td>519±4</td>
<td>1140±47</td>
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<tr>
<td>TAC</td>
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<td></td>
</tr>
<tr>
<td>Nppa(^{+/+})</td>
<td>(10)</td>
<td>23.9±0.4</td>
<td>137±4</td>
<td>106±3</td>
<td>20±1</td>
<td>10±0.2</td>
<td>168±6</td>
<td>282±5</td>
<td>464±9</td>
<td>994±10</td>
</tr>
<tr>
<td>Nppa(^{-/-})</td>
<td>(10)</td>
<td>27.1±1.3</td>
<td>266±7</td>
<td>205±6</td>
<td>37±1</td>
<td>24±1.2</td>
<td>207±3</td>
<td>386±22</td>
<td>486±4</td>
<td>1050±56</td>
</tr>
</tbody>
</table>

Results are mean ± SEM. BW indicates body weight; LV, left ventricle; RV, right ventricle. (n) indicates the number of mice per group. The adjusted tissue weights were determined by ANCOVA with BW as a covariate.

*\(P<0.05\) vs respective Nppa\(^{+/+}\) groups; #\(P<0.05\) vs respective control unoperated groups.
pressed (>2-fold increase in both genotypes) after TAC. Expression of the aforementioned genes was not significantly different (<2-fold) between control, unoperated Nppa\(^{+/+}\) and Nppa\(^{-/-}\) groups by microarray analysis. Complete microarray analysis data are posted on the web page at http://info.dom.uab.edu/hypertension.

Northern blot analysis was carried out to confirm the results of microarray analysis and to test whether expression of other ECM molecules known to be involved in cardiac hypertrophy (e.g., collagen I and III and osteopontin) were regulated differentially in response to pressure overload in the presence and absence of ANP. Expression of periostin was increased significantly in Nppa\(^{-/-}\) mice by 3 days (13-fold) after TAC and reached 17-fold elevation compared with control, unoperated Nppa\(^{+/+}\) mice by 7 days after TAC (Figure 3A). This was the most dramatic response to pressure overload of any gene studied. A similar pattern was seen for collagen I and III and osteopontin (Figures 3B through 3D). Expression of the ECM molecules also increased in wild-type mice after TAC, but the increments were less robust than in Nppa\(^{-/-}\) mice. At baseline, steady-state mRNA levels of periostin, collagen III, and osteopontin were significantly increased in LV of control, unoperated Nppa\(^{-/-}\) mice compared with the control, unoperated Nppa\(^{+/+}\) mice (Figure 4).

Pleiotrophin, a novel ECM- and cell surface–associated growth factor, has recently been demonstrated in the heart of Npr1\(^{-/-}\) mice after \(\beta\)-adrenergic agonist treatment.\(^{14}\) To test the hypothesis that pleiotrophin might be involved in the development of the exaggerated cardiac hypertrophy seen in Nppa\(^{-/-}\) mice, we measured steady-state pleiotrophin mRNA levels in TAC and control mice. Northern blot analysis demonstrated significant increases in pleiotrophin expression in LV of Nppa\(^{-/-}\) mice at 3 and 7 days after TAC compared with all other treatment groups (Figure 5). There was no significant change in this parameter in Nppa\(^{+/+}\) hearts after TAC.
Nppa does not compensate for disrupted ANP expression in the same in both genotypes and that TAC increased LV BNP in cardiac hypertrophy and remodeling, at least in part by These observations suggest that ANP plays an important role in compensating for increased BNP expression in not sufficient to replace the function of ANP in the current model.

Remodeling of the cardiac interstitium is a major determinant of pathologic hypertrophy, leading to cardiac dysfunction and heart failure. Increased cardiac ECM deposition is due to a combination of increased biosynthesis and decreased degradation of ECM proteins, likely related to reduced MMP activity. MMPs are tightly regulated at several levels, including transcription, secretion, and conversion of the zymogen to the active protease, in part through the action of endogenous protease inhibitors (TIMPs). Altered expression and activity of various MMPs and TIMPs have been detected during cardiac remodeling induced by a variety of stimuli. More specifically, expression of MMP-1, -2, -9, and -13 and of TIMP-1, -2, and -4 have been correlated with the development of cardiac hypertrophy and failure in rodent heart. Our microarray analysis gave preliminary evidence of increased expression of MMP-2 and TIMP-3 in the mouse (Nppa+/+Nppa−/−) LV after TAC. Further study is needed to examine in depth the roles of various components of these complex ECM regulatory mechanisms in mediating the exaggerated cardiac hypertrophy in Nppa−/− mice.

The most dramatic response to pressure overload in the current study was the huge (43-fold by microarray analysis; 17-fold by Northern blot analysis) increase in periostin expression in the LV of the Nppa−/− mouse. This response was approximately 3-fold greater than that seen in the Nppa+/+ mouse. Periostin protein (by immunohistochemical analysis) was localized in the LV interstitium and within fibroblasts and coronary artery SMCs of Nppa+/+ mice after TAC (7 days); it was barely detectable in Nppa−/− mice, even after TAC. Periostin (90-kDa protein), also previously known as osteoblast-specific factor-2, is a novel cell-adhesion protein secreted by osteoblasts and osteoblast-like cell lines. Periostin is expressed in bone and to a lesser extent in lung and kidney and has recently been described in the infarcted region of rat heart, in the embryonic and fetal mouse heart, and in the valves of the adult mouse heart. We propose that periostin expression is negatively modulated by ANP and that in the absence of ANP (Nppa−/− mouse), increased expression of periostin contributes to cardiac hypertrophy and remodeling, particularly in the setting of pressure overload stress.

Similarly, expression of the ECM/adhesion molecule osteopontin was increased in TAC mice and was expressed differentially in the presence and absence of ANP. This finding is consistent with previous observations that osteopontin levels are increased in hypertrophic and decompensated hearts and that osteopontin production in cardiac fibroblasts is stimulated by angiotensin II. Osteopontin is a noncollagenous ECM protein that was first isolated from bone matrix and is found in a variety of cell types, including cardiac myocytes, endothelial cells, and fibroblasts. Osteopontin is heavil...
In this study, robust increases in mRNA levels of collagen I and III were seen in LV of \( \text{Nppa}^{+/2} \) mice after TAC; increases were much more modest in the \( \text{Nppa}^{1/1} \) mouse (Figures 3B and 3C). Total collagen protein, assessed by Masson’s trichrome staining, was much more abundant in the LV of TAC \( \text{Nppa}^{-/-} \) mice than in the other treatment groups (Figure 6B). These findings are consistent with previous observations that ANP can inhibit collagen expression in cardiac fibroblasts in vitro and support the hypothesis that deletion of the ANP gene results in exaggerated increases in ECM expression in response to pressure overload stress; they are also consistent with the concept that increased ECM deposition contributes to exaggerated cardiac hypertrophy and remodeling in this setting.

Increased expression of mRNA for pleiotrophin (also known as osteoblast-specific factor-1), a member of the heparin-binding cytokine/growth factor family, was...
also demonstrated in LV of Nppa−/− mice after TAC in this study. Pleiotrophin is a secreted ECM- and cell surface–associated growth factor (136 amino acids, ≈18 kDa) that plays an important role in cell differentiation, proliferation, and angiogenesis during development or injury repair.2 It exhibits mitogenic and angiogenic properties for fibroblasts, epithelial cells, and endothelial cells and influences the vascular supply and expansion of tumors.13,36 Activation of both mitogen-activated protein kinase and phosphoinositide-3 kinase signaling pathways is involved in pleiotrophin-induced angiogenesis.37 The role of the enhancement of pleiotrophin expression in the development of cardiac hypertrophy in this model needs further investigation.

A number of hormones, growth factors, and cytokines play critical roles in cardiac hypertrophy and remodeling, leading to heart failure. ANP reduces aldosterone secretion both by directly inhibiting its synthetic pathway and by preventing the agonist effect of angiotensin II.38 Mineralocorticoid receptors are highly expressed in the heart, and aldosterone has been postulated to act as a mediator of cardiac hypertrophy and remodeling. Recent studies in rats demonstrated that administration of angiotensin II or aldosterone induces myocardial and coronary vascular injury, including perivascular inflammation, arterial fibrinoid necrosis, and focal infarctions associated with the expression of inflammatory mediators (e.g., cyclooxygenase-2, macrophage chemoattractant protein-1, osteopontin, intercellular adhesion molecule-1, and tumor necrosis factor-α).39,40 Treatment with the mineralocorticoid receptor antagonists eplerenone or spironolactone attenuates proinflammatory molecule expression in the heart and subsequent vascular and myocardial damage.39,40 These studies suggest that aldosterone contributes to pathologic cardiac remodeling in vivo. However, the molecular mechanisms of aldosterone-induced cardiac remodeling have not been fully revealed.

**Perspectives**

These results provide strong evidence that ANP protects against pathologic cardiac hypertrophy induced by pressure overload, at least in part by negative modulation of genes involved in ECM deposition, including periostin, osteopontin, thrombospondin, and collagen I and III, as well as MMP-2, TIMP-3, and the novel growth factor pleiotrophin. Whether ANP exerts its antihypertrophic/ECM-inhibiting effects by direct actions at the cardiac level or through some indirect action, eg, inhibition of aldosterone secretion, is unclear and requires further study in in vitro systems. Similarly, the contribution of cardiomyocyte hypertrophy to the cardiac enlargement seen in the pressure-overloaded heart in the absence of ANP remains to be assessed. The mouse with homozygous deletion of the ANP gene provides a valuable and convenient model for study of the signaling mechanisms involved in ECM deposition in the hypertrophied heart.

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**References**


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