Ventricular function and natriuretic peptides in sequentially combined models of hypertension

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Cavallero S, González GE, Seropian IM, Cerrudo CS, Matorra F, Morales C, Hertig CM, Puyó AM, Fernández BE, Gelpi RJ. Ventricular function and natriuretic peptides in sequentially combined models of hypertension. Am J Physiol Heart Circ Physiol 298: H1290–H1299, 2010. First published February 5, 2010; doi:10.1152/ajpheart.00911.2009.—Hemodynamic parameters and natriuretic peptide levels were evaluated in cardiac hypertrophy produced by sequentially applied renovascular (RV) and deoxycorticosterone acetate-salt (DS) models of hypertension. We studied hypertensive rats by RV or DS treatment at 2 and 4 wk, as well as by the combination of 2 wk of each treatment in an inverse sequence: RV 2 wk/DS 2 wk (RV2/DS2) and DS 2 wk/RV 2 wk (DS2/RV2). The in vivo cardiac function, interstitial fibrosis, and synthesis and secretion of types A (ANP) and B (BNP) natriuretic peptides were monitored in hypertensive models compared with their corresponding sham (Sh2, Sh4). There were no differences in relaxation parameters among RV or DS groups and combined treatments. Left ventricular +dP/dtmax increased only in RV4 (P < 0.01 vs. Sh4), and this increase was abolished in RV2/DS2. Interstitial collagen concentration increased after 4 wk in both RV4 and RV2/DS2 groups. Although there were no changes in collagen concentration in either DS2 or DS4 groups, clipping after 2 wk of DS (DS2/RV2) remarkably stimulated interstitial fibrosis (P < 0.01 vs. DS2). Plasma BNP increased in RV treatment at 4 wk (P < 0.001 vs. Sh4), but not in DS. Interestingly, RV applied after the 2 wk of DS treatment induced a marked increase in BNP levels (P < 0.001 vs. Sh4). In this regard, plasma BNP appears to be a reliable indicator of pressure overload. Our results suggest that the second stimulus of mechanical overload in combined models of hypertension determines the evolution of hypertrophy and synthesis and secretion of ANP and BNP.

heart hypertrophy; natriuretic peptides; renovascular hypertension; deoxycorticosterone acetate-salt hypertension

HUMAN AND EXPERIMENTAL HYPERTENSION is associated with a complex cardiac remodeling. The development of left ventricular hypertrophy indicates a higher risk of cardiovascular events and particularly sudden death (9).

Although hypertension at early stages may be considered mainly on the basis of pressure overload, the progression of the disease also involves volume overload that finally leads to cardiac failure (14). The transition from compensatory hypertrophy to heart failure is characterized by a complex interaction between left ventricle (LV) pressure and volume overload, which mediate a spectrum of LV remodeling responses that varies from concentric hypertrophy to eccentric hypertrophy, both being the most defined morphological patterns (5, 6). However, the mechanisms involved in the transition and/or reversibility from one type of overload to another are poorly understood.

At the molecular level, the hypertrophic response is characterized by the reprogramming of gene expression, including upregulation of immediate early genes, natriuretic peptide (NP) genes, and those genes encoding structural proteins (5). NP levels are considered quantitative plasma biomarkers of heart failure, and several studies have correlated the contractile properties of the myocardium under different load conditions with the synthesis and secretion of atrial (ANP) and brain (BNP) NPs (10, 16, 17). A close correlation was found between plasma levels of these peptides and the degree of LV dysfunction (4, 10, 13), but their precise involvement in pressure- and volume-induced cardiac hypertrophy is not well characterized.

To explore the hormonal, functional, and morphological cardiac response elicited by different load conditions imposed on the heart in a sequential mode, we have established rat animal models undergoing the combination in different time sequence of two classical models of hypertension: renovascular, in which pressure overload predominates, and deoxycorticosterone acetate (DOCA)-salt, in which volume overload predominates. We previously reported the synthesis and secretion of natriuretic peptides ANP and BNP and its association with cardiac hypertrophy and suggested that the second applied overload stimulus determines the remodeling and hypertrophic pattern in sequentially combined models (3).

In the present work, we tested the hypothesis that the lately applied overload stimulus also determines the evolution of cardiac function in correlation with the NP profile. Thus our goal was to further characterize the temporal progression of left ventricular function and the geometrical patterns of cardiac hypertrophy in combined models of hypertension and its correlation with the NP profile at both the protein and mRNA level.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180–220 g at the beginning of the study were used. They were housed in controlled conditions and were given standard rat chow with free access to drink. Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.
Experimental Animals of the Canadian Council on Animal Care and the institutional guidelines of the University of Buenos Aires for the care and use of research animals. All protocols concerning animal use were approved by the Institutional Animal Care and Research Committee of the University of Buenos Aires.

Protocol for grouping. The experimental protocol is shown in Fig. 1. Animals with renovascular (RV) or DOCA-salt hypertension (DS) were studied at 2 wk (groups RV2 and DS2) and 4 wk of treatment (RV4 and DS4). We also studied the combination of 2 wk of each model in a consecutive fashion but with a different sequence of initial presentation: groups RV2/DS2 and DS2/RV2, which were evaluated at 4 wk.

Sham animals for the six groups were included. All parameters corresponding to sham animals for RV2 and DS2 groups were similar, and they were pooled into one group, sham 2 wk (Sh2). Likewise, parameters corresponding to 4-wk sham animals subjected to one surgery did not differ from those subjected to two surgeries. Therefore, they were also pooled into one group, sham 4 wk (Sh4).

The number of animals studied for each group was as follows: Sh2 (8), Sh4 (9), RV2 (8), RV4 (10), DS2 (8), DS4 (9), RV2/DS2 (7), and DS2/RV2 (7).

One kidney-one clip hypertension. After being anesthetized with ketamine (80 mg/kg)/xylazine (2.5 mg/kg), the left kidney was removed, and a silver clip with a 0.28-mm gap was placed on the right renal artery. For sham-operated rats, flank incisions were made to expose the kidneys and the renal artery without clipping the vessel.

DOCA-salt hypertension. Rats were anesthetized as described above and subjected to left nephrectomy. They received weekly injections of DOCA (30 mg/kg; Sigma, St. Louis, MO) suspended in sesame oil and were supplied with 1% NaCl in the drinking water. Animals subjected to sham surgery received vehicle injections and tap water to drink.

**Combination of RV and DS models.** After 2 wk of clipping, some 1K-1C rats were randomly selected to undergo renal artery declipping. Under anesthesia, the clips were removed carefully by a surgery performed close to the initial incision. Afterward, rats received DOCA and 1% NaCl treatment during two additional weeks. This group was named RV2/DS2. Sham animals were subjected to surgery and vehicle injections and were given tap water to drink.

In the same way, after 2 wk of DOCA-salt treatment, DOCA injections and salt were discontinued in randomly selected animals, which underwent renal artery constriction with a 0.28-mm clip for two additional weeks. This group was named DS2/RV2. Sham animals discontinued vehicle injections and were subjected to sham surgery.

**Hemodynamic and LV function measurement.** At the end of the experimental protocol, rats were anesthetized with ketamine (80 mg/kg)/xylazine (5 mg/kg), and the hemodynamic parameters were measured in closed-chest spontaneously breathing animals by means of a heparinized fluid-filled catheter inserted in the right carotid artery, advanced upstream in the aorta and LV, and connected to a Deltan II pressure transducer (Utah Medical Products, Midvale, UT). After an equilibration period of 10 min, heart rate, systolic and diastolic blood pressure (SBP and DBP, respectively), LV systolic pressure (LVSP), the rate of rise and fall of ventricular pressure (LV ± dP/dt; mmHg/s), and LV end diastolic pressure (LVEDP) were recorded. To evaluate relaxation, we measured the time required for pressure to fall to 50% from the value at which −dP/dtmax occurs (T1/2), the time required for pressure to fall to 63% from the value at which −dP/dtmax occurs (T3/4), and the time constant for pressure decay (τ, τa) (22). Mean arterial pressure (MAP) and LV developed pressure (LVEDP) were calculated.

All measurements were performed during 10–15 min using a computer with analog-digital conversion.

**Plasma and tissue processing.** At the end of the experiment, blood samples were obtained from the abdominal cava vein, and a solution of 1 mol/l KCl was injected immediately to induce diastolic arrest. The hearts were removed, washed in cold saline solution, blotted, and weighed. For histological examination, both ventricles were stored together without further dissection in phosphate-buffered 10% formaldehyde (pH 7.2). Heart samples assigned for gene expression analysis were carefully dissected into the four chambers, which were weighed individually, frozen in liquid nitrogen, and kept at −70°C. The septum was included with the left chamber.

The lungs were excised, washed in cold saline solution, blotted, and weighed. A lung fragment of 250–500 mg was frozen in liquid nitrogen. These lung specimens were dried to constant weight for 16 h at 85°C, and the wet-to-dry weight ratio was calculated as an indicator of potential edema (1).

**Plasma ANP and BNP RIA.** Plasma ANP extraction and ANP RIA were performed as described by Sarda et al. (20). Anti-human ANP-(99–126) was purchased from Peninsula Laboratories (Belmont, CA) and 125I-labeled ANP from New England Biolabs (Boston, MA) (15). Plasma BNP was measured using a BNP-45 RIA commercial kit (Phoenix Pharmaceuticals, Burlingame, PA).

**Cardiac ANP and BNP gene expression.** Total RNA was extracted from atrial and ventricular chambers with Trizol (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol, and subjected to Northern blot analysis as previously described (3). Scanning values for ANP and BNP mRNA were normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA.

**Intersitial collagen.** Formaldehyde-fixed hearts were sectioned in transversal slices from apex to base. Serial sections (5 μm) from each heart were obtained. Intersitial collagen concentration was evaluated in the LV free wall in Picosiris Red-stained slides. Cardiomyocytes stain yellow while collagen stains red with Picosiris Red staining. At least 50 microscopic fields under 10× magnification were examined for each heart. The percentage of collagen in each assessed region was calculated as the ratio between the sum of areas corre-
sponding to collagen, divided by the sum of areas corresponding to cardiomyocytes plus the areas of collagen tissue (8).

All measurements were performed with image analysis software (Image Pro-Plus 3.0; Media Cybernetics, Silver Spring, MD). Sections were evaluated under blind conditions.

Statistical analysis. All data are expressed as means ± SE. Statistical analysis was performed with GraphPad Instat software by one-way ANOVA. For RV hypertension, Sh2, Sh4, 2 wk and 4 wk, respectively; RV2 and RV4, renovascular hypertension 2 wk and 4 wk, respectively, DS2 and DS4, deoxycorticosterone acetate-salt 2 wk and 4 wk, respectively; BW, body weight; HW, heart weight; LVW, left ventricle weight; RVW, right ventricle weight; LAW, left atria weight; RAW, right atria weight; LuW, lung weight. Indexes are expressed in mg tissue/g BW.

Table 1. Tissue weight indexes

<table>
<thead>
<tr>
<th>Group</th>
<th>HW/BW</th>
<th>LVW/BW</th>
<th>RVW/BW</th>
<th>LAW/BW</th>
<th>RAW/BW</th>
<th>Lu/W/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh2</td>
<td>2.87±0.04</td>
<td>1.93±0.03</td>
<td>0.549±0.019</td>
<td>0.159±0.012</td>
<td>0.150±0.008</td>
<td>4.99±0.38</td>
</tr>
<tr>
<td>Sh4</td>
<td>2.77±0.11</td>
<td>1.92±0.07</td>
<td>0.500±0.019</td>
<td>0.159±0.019</td>
<td>0.128±0.008</td>
<td>5.33±0.37</td>
</tr>
<tr>
<td>RV2</td>
<td>3.34±0.10a</td>
<td>2.36±0.08a</td>
<td>0.548±0.023</td>
<td>0.168±0.010</td>
<td>0.150±0.005</td>
<td>4.83±0.12</td>
</tr>
<tr>
<td>RV4</td>
<td>3.37±0.08b</td>
<td>2.45±0.07b</td>
<td>0.528±0.009</td>
<td>0.177±0.011</td>
<td>0.142±0.010</td>
<td>4.35±0.16</td>
</tr>
<tr>
<td>RV2/DS2</td>
<td>3.65±0.13b</td>
<td>2.32±0.10b</td>
<td>0.540±0.022</td>
<td>0.169±0.016</td>
<td>0.133±0.012</td>
<td>4.36±0.10</td>
</tr>
<tr>
<td>DS2</td>
<td>3.31±0.11a</td>
<td>2.32±0.05b</td>
<td>0.548±0.022</td>
<td>0.165±0.012</td>
<td>0.120±0.005</td>
<td>4.89±0.23</td>
</tr>
<tr>
<td>DS4</td>
<td>3.49±0.05b</td>
<td>2.67±0.08b</td>
<td>0.390±0.026</td>
<td>0.177±0.012</td>
<td>0.141±0.008</td>
<td>5.39±0.83</td>
</tr>
<tr>
<td>DS2/ RV2</td>
<td>3.24±0.16c</td>
<td>2.34±0.12c</td>
<td>0.533±0.019</td>
<td>0.167±0.013</td>
<td>0.140±0.014</td>
<td>4.76±0.25</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 7–10 rats. Sh2 and Sh4, sham 2 wk and 4 wk, respectively; RV2 and RV4, renovascular hypertension 2 wk and 4 wk, respectively, DS2 and DS4, deoxycorticosterone acetate-salt 2 wk and 4 wk, respectively; BW, body weight; HW, heart weight; LVW, left ventricle weight; RVW, right ventricle weight; LAW, left atria weight; RAW, right atria weight; LuW, lung weight. Indexes are expressed in mg tissue/g BW. 

Table 2. Hemodynamic and ventricular relaxation parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>LVEDP, mmHg</th>
<th>τ, ms</th>
<th>T⁰, ms</th>
<th>T½, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh2</td>
<td>115±3</td>
<td>85±2</td>
<td>95±2</td>
<td>320±14</td>
<td>4.80±1.03</td>
<td>12.03±1.36</td>
<td>9.73±1.21</td>
<td>6.99±0.87</td>
</tr>
<tr>
<td>Sh4</td>
<td>113±4</td>
<td>83±3</td>
<td>93±4</td>
<td>274±10</td>
<td>3.99±0.79</td>
<td>12.60±1.15</td>
<td>10.96±0.96</td>
<td>7.80±0.68</td>
</tr>
<tr>
<td>RV2</td>
<td>148±5</td>
<td>105±5</td>
<td>119±4</td>
<td>278±18</td>
<td>4.70±0.81</td>
<td>13.68±1.07</td>
<td>12.13±0.84</td>
<td>8.59±0.63</td>
</tr>
<tr>
<td>RV4</td>
<td>172±7</td>
<td>124±9</td>
<td>140±6</td>
<td>290±22</td>
<td>5.51±0.80</td>
<td>13.58±1.22</td>
<td>12.25±0.89</td>
<td>8.68±0.65</td>
</tr>
<tr>
<td>RV2/DS2</td>
<td>173±8</td>
<td>114±5</td>
<td>114±5</td>
<td>260±25</td>
<td>5.56±0.50</td>
<td>13.32±1.91</td>
<td>11.46±1.41</td>
<td>8.14±1.02</td>
</tr>
<tr>
<td>DS2</td>
<td>138±4</td>
<td>94±3</td>
<td>108±3</td>
<td>307±30</td>
<td>6.17±1.01</td>
<td>14.70±1.75</td>
<td>12.86±1.40</td>
<td>9.00±1.00</td>
</tr>
<tr>
<td>DS4</td>
<td>161±3</td>
<td>107±5</td>
<td>124±6</td>
<td>285±20</td>
<td>5.60±1.10</td>
<td>13.46±1.47</td>
<td>12.49±1.36</td>
<td>8.77±0.95</td>
</tr>
<tr>
<td>DS2/ RV2</td>
<td>170±6</td>
<td>114±6</td>
<td>133±5</td>
<td>254±15</td>
<td>5.91±1.18</td>
<td>13.28±1.30</td>
<td>12.07±1.11</td>
<td>8.44±0.79</td>
</tr>
</tbody>
</table>

Values are means ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; LVEDP, left ventricular end diastolic pressure; τ, time constant for pressure decay; T⁰ and T½, time required for ventricular pressure to fall to 63% and 50%, respectively, from the value at which –dp/dtmax occurs. 

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Similarly, both parameters increased in DS2 and DS4 groups compared with Sh2 and Sh4. However, 2 wk of clipping applied after DS treatment (DS2/RV2) affected LVSP and LVDP more than the single DS treatment applied during 4 wk compared with the values at 2 wk (Fig. 2, B and D).

LV +dP/dt$_{max}$ significantly increased in clipped animals at 4 wk. This increase was abolished when DS treatment was applied during the last 2 wk in the combined group RV2/DS2 (Fig. 2E). On the other hand, LV +dP/dt$_{max}$ did not change either in groups treated with DOCA for 2 and 4 wk or the combined group DS2/RV2 (Fig. 2F).

Left ventricular diastolic function. The in vivo LV diastolic function was assessed through its two components, LVEDP and LV relaxation times (τ, T$_{63}$, and T$_{1/2}$) (Table 2). No changes in either LVEDP or relaxation times were found among RV or DS groups and the combined treatments.

Interstitial fibrosis. The development of interstitial fibrosis in LV was monitored by staining of collagen content in the LV free wall (Fig. 3). No fibrosis was observed in the RV group until 4 wk of treatment. Both RV4 and RV2/DS2 groups showed similar increased levels of collagen (Fig. 3A). There were no changes in collagen concentration in either DS2 or DS4 groups. Interestingly, clipping after 2 wk of DS treatment (DS2/RV2 group) remarkably stimulated interstitial fibrosis (Fig. 3B).

Plasma ANP and BNP levels. Circulating ANP levels were significantly elevated in RV and DS groups compared with their respective sham controls at 2 and 4 wk (Fig. 4, A and B).
ANP levels in RV2 and RV4 did not differ from values determined in DS2 and DS4 groups. There were no significant differences in ANP levels among RV and DS models after 2 or 4 wk, although values at 4 wk tended to be higher. The combined RV2/DS2 group showed a similar ANP concentration to RV4 (Fig. 4A). Interestingly, the 2-wk DS group subjected to renal artery clipping (DS2/RV2) exhibited a threefold increase in ANP levels compared with DS or RV at 4 wk, representing the higher ANP response among groups (Fig. 4B) (3).

Circulating BNP levels were found significantly increased in the 4-wk RV group and at a relatively lower level in the combined RV2/DS2 treatment (Fig. 4C).

Despite the lack of a progressive increase in plasma BNP levels in DS2 and DS4 groups, the treatment of the DS2 group by renal artery clipping (DS2/RV2) led to the highest BNP levels at 4 wk (Fig. 4D). This result is coincident with the observed increase in ANP level in the DS2/RV2 group.

Cardiac expression of NP mRNA. The expression level of the corresponding ANP and BNP mRNAs was evaluated in LV tissue (Fig. 5, A–D). There was a progressive increase in LV ANP mRNA levels in RV2 and RV4 groups compared with respective Sh2 and Sh4, which did not reach statistical significance. A significant twofold increase in ANP expression level was found in 2-wk RV rats subjected to DS treatment (RV2/DS2) (Fig. 5A).

ANP mRNA expression showed a significant time-dependent increase in DS2 and DS4 groups. ANP mRNA levels in the combined treatment DS2/RV2 were similar to those of DS4 (Fig. 5B).

LV BNP mRNA expression slightly increased only in RV groups at 2 and 4 wk (Fig. 5C). There was a trend toward a
higher BNP expression level in DS groups (Fig. 5D). No significant changes were observed in either combined model.

We did not observe increased expression of ANP and BNP mRNA in left and right atrial tissue in any of the single treated groups nor in the combined models (data not shown).

**Relationship between functional and hormonal expression profile.** To achieve an integrative understanding of the functional and hormonal cardiac response to different overload challenges, we analyzed the relationship between ventricular functional parameters and NP plasma levels and their mRNA expression in the LV. Some hormonal data were reported by us in a previous work (3).

We chose LVSP and +dP/dt\text{max} as representative parameters for the evaluation of systolic properties of the myocardium. LVSP was correlated to plasma ANP and BNP levels in RV groups (Fig. 6, A, C, and D). This correlation is maintained in the DS group, despite a lack of significance in the upraising levels of LVSP and ANP in the combined group DS2/RV2 (Fig. 6B). LVSP had a significant correlation with ANP mRNA levels in DS groups, including the combined DS2/RV2 (Fig. 6F). An overall correlation between LVSP and BNP mRNA levels was significantly achieved in both RV and DS groups (Fig. 6, G and H).

+dP/dt\text{max} was related to both circulating ANP and BNP levels in RV groups (Fig. 7, A and C), but only to BNP plasma levels in DS (Fig. 7D). In contrast, this systolic parameter was related to ventricular expression of both ANP and BNP mRNA in DS groups (Fig. 7, F and H), but not in RV (Fig. 7, E and G).

**DISCUSSION**

In the present study, we have examined the influence of a second applied stimulus of mechanical overload during the progression of cardiac hypertrophy. We show that the second applied stimulus determines the evolution of the pathophysiological pattern of cardiac hypertrophy and ventricular synthesis and secretion of ANP and BNP. Nevertheless, the second stimulus determines the evolution of LV functional behavior only in initially pressure-overloaded groups. Moreover, increased plasma BNP levels and the development of myocardial fibrosis closely parallel the instauration of pressure overload. No changes in diastolic function were found either in single models of hypertension or in the combined forms.

The adaptation of the cardiovascular system to hypertension is structurally and functionally heterogeneous, resulting from the interaction between pressure and volume overload that can mediate variable left ventricular geometric patterns of hypertrophy (5, 6, 14). However, there are no reports in the literature that combine pressure and volume overload in different sequences of time to resemble the natural evolution of ventricular function in hypertension. In a previous study, we characterized the morphometric parameters of cardiomyocyte hypertrophy in single and combined models of hypertension and described that the lately applied stimulus of overload determines the cardiomyocyte hypertrophic pattern in the combined models (3).

**Functional parameters.** We show here that both RV and DS models developed a progressive increase in blood pressure with no changes in heart rate. SBP, DBP, and MAP showed a similar behavior. The combined treatments after the corresponding switches did not substantially modify blood pressure in single treated groups. A similar behavior was observed in single RV and DS groups for LVSP and LVDP parameters. These results suggest that functional parameters related to left ventricular systolic function are more related to the global process of cardiac hypertrophy (without difference between concentric or eccentric hypertrophic pattern) than to the type of cardiac overload. The contractile properties evaluated through
$+dP/dt_{\text{max}}$ showed a differential behavior, being enhanced in RV at 4 wk, but remained unchanged in DS groups.

However, in the combined models, the temporal sequence of overload determined the evolution of cardiac dysfunction. Accordingly, RV treatment after 2 wk of DS (DS2/RV2) improved LVSP and LVDP parameters compared with the DS2 group. In addition, DS treatment applied after 2 wk of RV (RV2/DS2 group) resulted in similar contractile properties to those of RV2. This means that the contractility increase elicited by 4 wk of sustained RV (RV4) is blunted when the stimulus switches to DS (RV2/DS2).

**Cardiac fibrosis.** Interstitial collagen concentration increased in RV groups after 4 wk of treatment and in the combined groups independently of the sequence of RV treatment. Therefore, pres-
sure overload and the resulting concentric hypertrophic process are accompanied by fibrosis development.

We did not observe interstitial fibrosis in the LV of DOCA-salt rats. Other reports described cardiac fibrosis after 4 wk of DOCA-salt treatment in Wistar rats (2, 7). It has been suggested that a chronic increase of circulating mineralocorticoid level may stimulate collagen synthesis, but, while aldosterone stimulates not only perivascular but also interstitial fibrosis, DOCA mainly stimulates perivascular fibrosis (21).

NP profile. It is widely accepted that cardiac natriuretic peptides ANP and BNP are useful biochemical markers of cardiac hypertrophy and heart failure (10), but it is unclear whether these peptides exert a regulatory influence on these processes when pressure and volume overload are combined.

Increased levels of circulating BNP accompanied the development of LV concentric hypertrophy and fibrosis. Sustained pressure overload during 4 wk in the RV group as well as RV treatment applied after 2 wk of DS treatment were able to elicit…
an increase in plasma levels of BNP. Moreover, BNP levels were not substantially altered by DOCA-salt treatment and also were reduced after the switch from RV to DS treatment. Taken together, these results strongly suggest that BNP may better serve as an indicator for pressure than for volume overload.

In contrast, plasma ANP levels exhibited a time-dependent increase to a similar extent in both DS and RV models. Then, plasma ANP levels seem to indicate the presence of cardiac hypertrophy instead of cardiac overload. Although the RV to DS switch did not alter ANP levels, the DS to RV switch induced an increase of plasma ANP and BNP levels.

A similar behavior was observed for left ventricular ANP and BNP expression. BNP synthesis increased in single RV models but not in DS models. Moreover, ANP synthesis tended to increase in RV2, RV4, and DS2 groups and was significantly elevated in the DS4 group and both combined models. In agreement with plasma peptide levels, ANP synthesis seems to be related to a global hypertrophic process while BNP synthesis is related to pressure overload. Accordingly, Sakata et al. (19) reported that ANP gene expression in the LV is closely related to hypertrophy, whereas BNP gene expression is not enhanced in the initial adaptive LV hypertrophy, but augments in association with LV fibrosis and ANP II-dependent mal-adaptive hypertrophy (19).

Plasma ANP and BNP levels were markedly elevated in the combined group DS2/RV2, but this increase was not accompanied by a proportional stimulation of ANP and BNP ventricular synthesis. This increase was not a result of augmented atrial tissue synthesis, given that we did not observe changes in ANP and BNP mRNA expression in both atria. Raised plasma levels of both NPs could be the result of a reduced clearance rate by NPR-C receptors (11, 12). In our previous study, renal NPR-C mRNA levels were unaltered by RV treatment, but a decreased expression was described in single DS treatment applied for 4 wk or in combination with RV, independent of the sequence of induction (3). Thus the marked increase in plasma ANP and BNP levels found in the DS2/RV2 group (in the presence of a similar stimulation of cardiac ANP and BNP synthesis, when compared with DS4 and RV2/DS2 groups) is not mediated by a differential decrease in expression of renal clearance receptors because all DS-treated groups exhibited a nearly 50% expression level compared with sham.

LVSP exhibited a positive correlation with plasma ANP and BNP levels and with left ventricular ANP and BNP expression in all experimental groups. The combined DS2/RV2 group, which showed a disproportionate increase of ANP, was out of this rule. Thus endocrine and paracrine cardiac function is proportionally related to ventricular systolic function.

Left ventricular +dP/dt\textsubscript{max} levels are positively correlated with plasma ANP and BNP in RV groups, but only with plasma BNP in DS groups. In addition, left ventricular +dP/dt\textsubscript{max} levels are positively correlated with ANP and BNP mRNA expression in DS groups, but not in RV groups. These results suggest that the pressure overload-mediated increase in LVDP may stimulate the intrinsic mechanisms that induce NP secretion. On the other hand, when volume overload increases LVDP, the genetic pathways to synthesize NP secretion may be stimulated.

To our knowledge, this is the first report to evaluate the impact of different types of cardiac overload induced in different time sequence. Our results suggest that ANP and BNP behave as markers for volume and pressure overload, respectively. Moreover, the lately applied stimulus for hypertrophy determines the behavior of ventricular function in relationship with the NP profile.

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DISCLOSURES

The authors have no potential conflicts of interest.

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