Diastolic wall stress and ANG II in cardiac hypertrophy and gene expression induced by volume overload

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Yamakawa, Hiroshi, Takuroh Imamura, Takeshi Matsu, Hisamitsu Onitsuka, Yoko Tsunori, Johji Kato, Kazuo Kitamura, Yasushi Koiwaya, and Tanenao Eto. Diastolic wall stress and ANG II in cardiac hypertrophy and gene expression induced by volume overload. Am J Physiol Heart Circ Physiol 279: H2939–H2946, 2000.—We investigated the effects of diastolic wall stress (WS) and angiotensin II (ANG II) on the left ventricular (LV) hypertrophy (LVH) induced by volume overload and on the gene expression of LV adrenomedullin (AM) and atrial natriuretic peptide (ANP) in volume overload. Diastolic WS was pharmacologically manipulated with (candesartan) or without (calcium channel blocker manidipine) inhibition of ANG II type 1 receptors in aortocaval-shunted rats over 6 wk. Diastolic WS reached a plateau at 2 wk and subsequently declined regardless of further LVH. Although diastolic WS was decreased to a similar extent by both compounds, candesartan blunted LVH over 6 wk, whereas manidipine blunted LVH at 2 wk but not after 4 wk. Levels of AM and ANP gene expression increased as LVH developed but were completely suppressed by candesartan over 6 wk. ANP expression level was also attenuated by manidipine over 6 wk, whereas AM expression level was suppressed at 2 wk but not after 4 wk by manidipine. We concluded that diastolic WS and ANG II might be potent stimuli for the LVH and LV AM and ANP gene expression in volume overload and that diastolic WS could be relatively involved in the early LVH and in the gene expression of ANP rather than of AM.

aortocaval shunt; adrenomedullin; atrial natriuretic peptide; angiotensin II type 1 receptor antagonist; calcium channel blocker; left ventricular hypertrophy

INCREASED HEMODYNAMIC LOAD produces different types of left ventricular (LV) hypertrophy (LVH) through different processes of adaptation (10). Pressure overload increases systolic tension, resulting in myocardial fiber thickening and concentric hypertrophy in an effort to normalize systolic wall stress (WS) (10). Similarly, diastolic WS is also believed to stimulate eccentric hypertrophy in response to volume overload (6, 9). However, diastolic WS is not normalized in volume overload-induced LVH associated with aortic regurgitation (14) and aortocaval (AC) shunt in the rat models (2, 5). In the AC shunt model, LV end-diastolic pressure (LVEDP) rapidly increases after the imposition of acute volume overload, peaks at an early phase, and declines thereafter. Despite the decrease in LVEDP, LVH continues to develop (2). These findings suggest that the contribution of a load-dependent factor to the development of LVH decreases and that of a load-independent factor alternatively increases during volume overload imposed by AC shunt. However, the mechanisms of the further progression of LVH in volume overload after the decrease in LVEDP and the lowering effect of LVEDP on the suppression of LVH in volume overload remain unclear.

The cardiac renin-angiotensin system (RAS) is activated in experimental models with pressure and volume overload (16, 28). In fact, load-independent factors, such as angiotensin II (ANG II) and endothelin 1, also play important roles in LVH (15, 24), as do load-dependent factors (18, 25) in vitro and in vivo. However, whether or not the cardiac hypertrophy caused by pressure overload is prevented by an ANG II type 1 (AT1) receptor antagonist in vivo has remained controversial (3, 32). Harada et al. (11) reported that acute pressure overload induces LVH in AT1a receptor knockout mice as well as in wild-type mice. They suggested that the acute hypertrophic response can be induced by pressure overload per se without AT1 signaling (11). In contrast, they also reported that LV remodeling after myocardial infarction was less remarkable in AT1a receptor knockout mice than in wild-type mice (12). Taken together, these findings imply that the magnitude of the role of mechanical stress and/or RAS differs between pressure and volume load-induced LVH. The influence of RAS on LVH seems to be more critical in volume than in pressure overload.

LVH is characterized by an increase in cell size and is accompanied by changes in the expression of several genes. Adrenomedullin (AM) and atrial natriuretic peptide (ANP) are potent vasoactive peptides that play roles in cardiovascular homeostasis and circulate in the blood, and their genes are expressed in the heart (4, 27). Cardiac AM and ANP gene expression levels in-
crease in volume overload produced by an AC shunt (13, 22) and in postmyocardial infarction (17), suggesting that AM and ANP also play important roles in volume overload-induced LVH. We confirmed that the plasma levels of AM and ANP are increased during the acute phase of the volume overload model and that the plasma concentrations of AM and ANP increase according to different profiles (13). The former correlates with plasma renin activity and the latter correlates with LVEDP (13).

Accordingly, we surmised that ANG II, in addition to LVEDP or diastolic WS, might be a potent stimulus for AC shunt-induced LVH, that pharmacological manipulation of LVEDP with or without inhibition of AT1 receptor signaling might attenuate LVH by different means, and that LV AM and ANP gene expression might be differentially regulated as an autocrine or paracrine hormone in this model. To test these hypotheses, we investigated whether LVH in the AC shunt rat model is suppressed in a different manner by the AT1 receptor antagonist candesartan and the calcium channel blocker manidipine over 6 wk, evaluated the ventricular expression level of AM and ANP mRNA during volume overload, and examined whether or not the gene expression is affected by candesartan or manidipine.

METHODS

Animals and experimental protocols. Eight-week-old male Wistar rats (280–310 g), obtained from Charles River (Atsugi, Japan), were housed in a room at a controlled temperature under 12 h of light and 12 h of darkness. After an acclimatization period of at least 3 days, we divided the animals into five groups as follows: sham operation (n = 21), AC shunt with vehicle (AC + V; n = 20), AC shunt with low-dose candesartan cilexetil (AC + L; 0.1 mg·kg⁻¹·day⁻¹, n = 20), AC shunt with high-dose candesartan cilexetil (AC + H; 1 mg·kg⁻¹·day⁻¹, n = 20), and AC shunt with manidipine (AC + M; 10 mg·kg⁻¹·day⁻¹, n = 20). Drug administration commenced 3 days before surgery and continued for 2, 4, and 6 wk thereafter. The AC shunt was constructed using 18-gauge disposable needles as described by Garcia and Diebold (8). Sham-operated animals serving as controls underwent the same procedure but without the aorta and inferior vena cava being punctured. To minimize operative stress, all procedures were completed within 15 min. Drugs were suspended in 5% gum arabic and were administered daily through the stomach by gastric gavage. Rats were euthanized 2, 4, and 6 wk after the operation. All manipulations and care of the animals proceeded according to the guidelines for Institutional Animal Care and Use of Laboratory Animals of Miyazaki Medical College.

Hemodynamic measurements and echocardiographic evaluation. Rats were anesthetized by an intraperitoneal administration of pentobarbital sodium (50 mg/kg). Heart rate (HR), systolic aortic blood pressure (SBP), LVEDP, and mean right atrial pressure (RAP) were measured using a Statham pressure transducer (model P231D, Gould, Saddle Brook, NJ) inserted into the ascending aorta and LV through the right carotid artery and by another catheter placed in the right atrium through the right jugular vein. An echocardiograph (model USI-738; Aloka) equipped with a 7.5-MHz transducer obtained two-dimensional short-axis views of the LV at the level of the papillary muscle. We determined the LV end-diastolic dimension (LVEDD) and LV posterior wall thickness by echocardiography. The LV diastolic WS was routinely calculated according to the following formula: diastolic WS = LVEDP × r + 2r(LV wall thickness), where r is radius of the LV.

Measurement of LV ANG II concentrations. The LV ANG II concentrations in the sham and AC + V groups were determined by extraction using florisil adsorption, elution with acetone-hydrochloric acid, and radioimmunoassay.

RNA isolation and Northern blotting. The total RNA isolated from rat LV tissues using TRIzol (Life Technologies) according to the manufacturer’s protocol was resolved by electrophoresis on 1.0% agarose gels and transferred to nylon membranes (Immobilon probe, Millipore). The membranes were hybridized with 32P-labeled cDNA probes and then washed with 2× and 1× saline-sodium citrate. Radioactive signals on blots were quantified using an image analyzer (BAS2000, Fuji Film). Results were normalized to signals from GAPDH mRNA.

Statistical analysis. All results are expressed as means ± SE. Differences among the five groups were evaluated by the one-way analysis of variance followed by Scheffe’s test. An unpaired t-test was used to compare LV ANG II concentrations between the AC + V and sham groups in each time point. Differences were considered significant at P < 0.05.

RESULTS

The changes in HR, mean RAP, and SBP are shown in Table 1. SBP did not differ between the AC + L and AC + V groups but was significantly decreased in the AC + H and AC + M groups compared with the AC + V group over 6 wk. SBP between the AC + H and AC + M groups did not differ over 6 wk. Changes in LVEDP and diastolic WS over 6 wk are shown in Figs. 1 and 2, respectively. Both LVEDP and diastolic WS in all groups peaked at 2 wk and then gradually declined. However, diastolic WS in the AC + V group remained high compared with that in sham-operated rats at 6 wk (Fig. 2). Diastolic WS and LVEDP did not significantly change among the AC + L, AC + H, and AC + M groups over 6 wk. Thus the changes in SBP and LVEDP were hemodynamically very similar between the AC + H and AC + M groups. These findings suggest that diastolic WS is directly related to LVEDP independently of the inhibition of AT1 signaling. The values of LV weight-to-body weight ratio (LV/BW; Fig. 3) and LVEDD (Fig. 4) in volume-overloaded rats were significantly increased at all times between 2 and 6 wk compared with those in the sham-operated rats (2.64 ± 0.09 mg/g and 8.80 ± 0.22 mm in volume-overloaded rats vs. 1.80 ± 0.02 mg/g and 6.71 ± 0.07 mm in sham-operated rats at 2 wk, respectively, P < 0.01; 2.95 ± 0.09 mg/g and 9.40 ± 0.13 mm in volume-overloaded rats vs. 1.79 ± 0.03 mg/g and 7.00 ± 0.19 mm in sham-operated rats at 4 wk, respectively, P < 0.01; 3.00 ± 0.10 mg/g and 10.52 ± 0.33 mm in volume-overloaded rats vs. 1.83 ± 0.03 mg/g and 7.28 ± 0.07 mm in sham-operated rats at 6 wk, respectively, P < 0.01). Candesartan (AC + L and AC + H groups) significantly decreased LV/BW (P < 0.01) and LVEDD (P < 0.01) over 6 wk compared with vehicle. In contrast, LV/BW and LVEDD were significantly decreased by manidipine at 2 wk (P < 0.05 in LV/BW and
P < 0.01 in LVEDD) compared with vehicle, but no significant decrease was evident after 4 wk. Thus although similar hemodynamic effects on hypertrophied myocardium were sustained over 6 wk in the AC + H and AC + M groups, both compounds attenuated LVH at 2 wk, and only candesartan blunted LVH after 4 wk. The LV/BW and diastolic WS values significantly correlated (Fig. 5). The LV/BW and LVEDP values also

### Table 1. Hemodynamic changes at 2, 4, and 6 wk

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>AC + V</th>
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<td>HR, beats/min</td>
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<td>2 wk</td>
<td>427 ± 13</td>
<td>436 ± 8</td>
<td>391 ± 8</td>
<td>397 ± 14</td>
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<td>4 wk</td>
<td>425 ± 14</td>
<td>429 ± 24</td>
<td>411 ± 8</td>
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<td>6 wk</td>
<td>404 ± 6</td>
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<td>358 ± 6</td>
<td>343 ± 6</td>
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<td>RAP, mmHg</td>
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<td>2 wk</td>
<td>0.0 ± 0.1</td>
<td>0.9 ± 0.6</td>
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<td>4 wk</td>
<td>0.3 ± 0.2</td>
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<td>6 wk</td>
<td>0.3 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.3</td>
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<td>SBP, mmHg</td>
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<tr>
<td>2 wk</td>
<td>153 ± 5.6</td>
<td>144 ± 3.2</td>
<td>133 ± 4.2</td>
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<td>4 wk</td>
<td>145 ± 4.3</td>
<td>140 ± 8.8</td>
<td>132 ± 4.2</td>
<td>122 ± 3.5</td>
<td>116 ± 3.6</td>
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<td>6 wk</td>
<td>144 ± 3.6</td>
<td>137 ± 3.0</td>
<td>136 ± 6.1</td>
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Values are means ± SE; n = 6–7 rats/group. HR, heart rate; RAP, right atrial pressure; SBP, systolic blood pressure; AC + V, aortocaval (AC) shunt with vehicle; AC + L, AC shunt with low-dose candesartan (0.1 mg·kg⁻¹·day⁻¹); AC + H, AC shunt with high-dose candesartan (1 mg·kg⁻¹·day⁻¹); AC + M, AC shunt with manidipine (10 mg·kg⁻¹·day⁻¹). aP, 0.05 vs. sham, bP, 0.01 vs. sham, cP, 0.05 vs. AC + V group, dP < 0.01 vs. AC + V group, *P < 0.05 vs. AC + L group, and fP < 0.01 vs. the AC + V group.
significantly correlated but those of SBP and LV/BW did not. The LV ANG II concentrations in the AC + V group were significantly higher ($P < 0.05$) than those in the sham group at 2 and 6 wk, but the difference was not significant at 4 wk (Table 2).

The expression levels of LV AM and ANP mRNA increased as LVH advanced (Figs. 6 and 7, respectively). The expression levels of AM and ANP mRNA in volume-overloaded rats were increased 1.9- and 8.4-fold, respectively, at 6 wk compared with those in sham-operated rats. More ANP mRNA (2.3- to 4.5-fold) than AM mRNA was expressed at all times over 6 wk. The expression levels of AM mRNA (Fig. 6) and ANP mRNA (Fig. 7) in the AC + H group over 6 wk were suppressed to near-basal levels in sham-operated rats regardless of incomplete suppression of LVH (Fig. 3). In contrast, the ANP mRNA expression level in the AC + M group was also attenuated to some extent over 6 wk (Fig. 7), whereas AM mRNA expression level in the AC + M group was suppressed at 2 wk but not after 4 wk (Fig. 6).

**DISCUSSION**

**Role of diastolic WS and ANG II in volume overload-induced LVH.** The present study demonstrated a positive correlation between diastolic WS and LVH, indicating that diastolic WS is an important factor in volume overload-induced LVH. Diastolic WS and LVEDP reached a plateau at 2 wk and subsequently

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declined, whereas LV remodeling progressed and diastolic WS was not normalized over 6 wk. Similar results have been published by Brower et al. (2), who demonstrated that a sustained increase in LVEDP, which peaked at 3 wk post-AC shunt, was followed by a decrease in LVEDP regardless of further LV dilatation. SBP decreased in the AC1H and AC1M groups but not in the AC1L group, whereas LVEDP and diastolic WS were similarly reduced in the AC1L, AC1H, and AC1M groups compared with the AC1V group. The LVH at 2 wk was similarly attenuated among the three groups compared with the AC1V group. However, LVH after 4 wk was blunted in the AC1H and AC1L groups but not in the AC1M group. These findings indicate that the reduction in SBP does not affect the attenuation of LVH, that the initial phase of LVH was regressed by the reduction in LVEDP, and that the late phase of LVH was attenuated by the reduction in LVEDP associated with AT1 receptor inhibition. Furthermore, LV ANG II concentrations in volume-overloaded rats were higher than those in sham rats at 2 and 6 wk. Our results also support those of Iwai and co-workers (16), who demonstrated that candesartan suppressed AM and ANP mRNA expression to near-basal levels in the sham group over 6 wk and that manidipine also suppressed ANP mRNA expression level to some extent over 6 wk, whereas it suppressed the AM mRNA expression level at 2 wk but not after 4 wk. This suggests that LVEDP and ANG II can modulate both the AM and ANP mRNA expression level. However, the influence of mechanical stress is more critical on the expression of ANP than of AM mRNA. ANG II is a potent stimulator of ANP and AM synthesis (7, 19, 20). We (29) and Sadoshima et al. (26) showed that the stretch-induced increase in the mRNA expression of AM and ANP in cultured myocytes is significantly inhibited by AT1 receptor antagonist. Furthermore, we observed that the AM mRNA level was not normalized as well as LV remodeling progressed and diastolic WS was not normalized over 6 wk. Similar results have been published by Brower et al. (2), who demonstrated that a sustained increase in LVEDP, which peaked at 3 wk post-AC shunt, was followed by a decrease in LVEDP regardless of further LV dilatation. SBP decreased in the AC1H and AC1M groups but not in the AC1L group, whereas LVEDP and diastolic WS were similarly reduced in the AC1L, AC1H, and AC1M groups compared with the AC1V group. The LVH at 2 wk was similarly attenuated among the three groups compared with the AC1V group. However, LVH after 4 wk was blunted in the AC1H and AC1L groups but not in the AC1M group. These findings indicate that the reduction in SBP does not affect the attenuation of LVH, that the initial phase of LVH was regressed by the reduction in LVEDP, and that the late phase of LVH was attenuated by the reduction in LVEDP associated with AT1 receptor inhibition. Furthermore, LV ANG II concentrations in volume-overloaded rats were higher than those in sham rats at 2 and 6 wk. Our results also support those of Iwai and co-workers (16), who demonstrated that candesartan suppressed AM and ANP mRNA expression to near-basal levels in the sham group over 6 wk and that manidipine also suppressed ANP mRNA expression level to some extent over 6 wk, whereas it suppressed the AM mRNA expression level at 2 wk but not after 4 wk. This suggests that LVEDP and ANG II can modulate both the AM and ANP mRNA expression level. However, the influence of mechanical stress is more critical on the expression of ANP than of AM mRNA. ANG II is a potent stimulator of ANP and AM synthesis (7, 19, 20). We (29) and Sadoshima et al. (26) showed that the stretch-induced increase in the mRNA expression of AM and ANP in cultured myocytes is significantly inhibited by AT1 receptor antagonist. Furthermore, we observed that the AM mRNA level in

Taken together, these results suggest that AC shunt-induced eccentric hypertrophy is partly regulated by the RAS in addition to the mechanical stress of LVEDP and that a load-dependent factor is involved during the early, rather than the late, phase of LVH. We speculate that the specific contribution of RAS to developing LVH in volume overload can relatively increase as an alternative factor to mechanical stress after LVEDP decreases.

Liu et al. (21) found, in a rat model of myocardial infarction, that AT2 antagonist alone did not affect LV remodeling, whereas AT1 antagonist attenuated it, and that this effect was blocked by concomitant use of the AT2 antagonist. These results suggest that the effect of candesartan might be due to not only a blockade of the AT1 receptor but also activation of the AT2 receptor in our model. However, because we did not use an AT2 receptor antagonist in this study, we could not determine whether or not blocking the AT1 receptor or allowing unopposed AT2-mediated action is responsible for the reduced LVH. Other load-independent factors, such as endothelin, cytokines, and growth factors, might also be involved in modulating volume overload-induced LVH in this model.
creased by ANG II stimulation in myocytes is completely abolished by AT₁ receptor antagonist but not by AT₂ receptor antagonist (29). However, the role of AT₂ signaling in AM gene expression has not yet been completely elucidated. Taken together, we speculate that LV AM and ANP transcription are regulated by both volume per se and as an autocrine and/or paracrine response to the locally activated RAS in the AC shunt-induced volume-overloaded model, whereas the influence of volume overload per se seems to be more critical on the LV ANP transcription. Our results agree with those reported by Ogawa et al. (23), who stated that ANP expression is related to both load-dependent and -independent components in the aortic banding rat model and that the former is more important than the latter in regulating ventricular ANP mRNA expression.

The possibility of direct or indirect effects of the calcium channel blocker cannot be excluded. Blocking the Ca²⁺ influx could influence the expression of AM mRNA, and the concomitant activation of the cardiac RAS by manidipine may also have effects. Nevertheless, the discrepancy between the suppression of ventricular AM and ANP gene expression induced by manidipine indicates that the influence of the RAS and mechanical stress on AM and ANP gene expression differs in the cardiac hypertrophy induced by volume overload.

We also demonstrated that the levels of AM and ANP gene expression are increased as LVH increases. The gene expression levels of AM and ANP were completely suppressed, whereas LVH was partly attenuated by candesartan in this study. We showed that ANG II stimulates AM expression in cultured cardiac myocytes.

Fig. 6. Relative levels of mRNA for LV adrenomedullin (AM) in sham, AC + V, AC + L, AC + H, and AC + M groups 2, 4, and 6 wk after operation. All mRNA levels are normalized for GAPDH mRNA. The expression level of AM mRNA in the AC + V group at 2 wk was significantly augmented with a gradual increase over 6 wk. Augmentation of AM mRNA expression level was suppressed in the AC + L and AC + H groups to near-basal levels in sham-operated rats. AM mRNA expression level in the AC + M group decreased at 2 wk but was not changed after 4 wk compared with that in the AC + V group (n = 6–7 rats/group). Values are means ± SE. *P < 0.01 vs. sham; **P < 0.05 vs. sham; #P < 0.01 vs. the AC + V group; ##P < 0.05 vs. the AC + V group; †P < 0.01 vs. the AC + M group; ††P < 0.05 vs. the AC + M group.

Fig. 7. Relative levels of mRNA for LV atrial natriuretic peptide (ANP) in sham, AC + V, AC + L, AC + H, and AC + M groups 2, 4, and 6 wk after operation. All mRNA levels are normalized for GAPDH mRNA. ANP mRNA expression level in the AC + V group at 2 wk was significantly augmented with further increases over 6 wk. Augmentation of ANP mRNA expression level was suppressed in the AC + L and AC + H groups to near-basal levels in sham-operated rats. ANP mRNA expression level in the AC + M group significantly decreased at 2 and 4 wk. Although not significant, ANP gene expression level at 6 wk tended to decrease (n = 6–7 rats/group) compared with that in the AC + V group. Values are means ± SE. *P < 0.01 vs. sham; **P < 0.05 vs. sham; #P < 0.01 vs. the AC + V group; ##P < 0.05 vs. the AC + V group; †P < 0.01 vs. the AC + M group; ††P < 0.05 vs. the AC + M group.
and cardiac fibroblasts and that the secreted AM inhibits the hypertrophy of these cells in an autocrine or paracrine manner (30, 31). The present findings suggest that both AM and ANP are partly involved in the development of volume overload-induced LVH in vivo. However, we could not confirm a direct relationship between the expression of these genes and LVH in this study. Further investigations are required to elicit the pathophysiological contribution of AM and ANP to LVH or cardiac function in volume overload.

In conclusion, the AT<sub>1</sub> receptor antagonist candesartan effectively suppressed volume overload-induced LVH, whereas the calcium channel blocker manidipine decreased diastolic WS and reduced the early phase of LVH but could not suppress progressive LVH in volume overload. Both diastolic WS and ANG II might be potent stimuli for the LVH in this model. Diastolic WS might be relatively involved in the early phase of LVH, whereas the RAS could be involved in the late, as well as the early, phase of LVH. An increase in AM and ANP gene expression is associated with the development of AC shunt-induced LVH. The augmented levels of LV AM and ANP gene expression were completely suppressed by candesartan. LV ANP expression level was also attenuated by manidipine, whereas the LV AM expression level was suppressed by manidipine in the early, but not in the late, phase of volume overload. The expression levels of LV AM and ANP mRNA during volume overload are modulated by both diastolic WS and ANG II, but the influence of diastolic WS seems to be more critical on the gene expression of ANP than of AM.

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