Left ventricular wall stress and sarcoplasmic reticulum Ca\(^{2+}\)-ATPase gene expression in renal hypertensive rats: dose-dependent effects of ACE inhibition and AT\(_1\)-receptor blockade

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Received 19 June 1995; accepted 22 November 1995

Abstract

Background: Cardiac hypertrophy is associated with altered Ca\(^{2+}\) handling and may predispose to the development of LV dysfunction and cardiac failure. At the cellular level, the re-expression of ANF represents a well-established marker of myocyte hypertrophy while the decreased expression of the sarcoplasmatic reticulum (SR) Ca\(^{2+}\)-ATPase is thought to play a crucial role in the alterations of Ca\(^{2+}\) handling and LV function. We assessed the dose-dependent effect of chronic ACE inhibition or AT\(_1\)-receptor blockade on cardiac function in relation to the cardiac expression of the SR Ca\(^{2+}\)-ATPase and ANF.

Methods and Results: Renal hypertensive rats (2K-IC) were treated for 12 weeks with three different doses of the ACE inhibitor benazepril, the AT\(_1\)-receptor antagonist valsartan (each drug 0.3, 3, and 10 mg/kg per day i.p.) or placebo. LV dimensions, hypertrophy and wall stress were determined in vivo by magnetic resonance imaging and the gene expressions of ANF and SR Ca\(^{2+}\)-ATPase were quantified by Northern blot. Low doses of both drugs did not affect blood pressure, hypertrophy, systolic wall stress and the ANF and SR Ca\(^{2+}\)-ATPase gene expression. High doses of each drug reduced systolic blood pressure, wall stress, and LV hypertrophy to a similar extent and to values comparable to normotensive, age-matched rats. In addition, high dose treatment reduced LV end-systolic and end-diastolic volume as compared to untreated 2K-IC animals and normalized the mRNA levels of both ANF and SR Ca\(^{2+}\)-ATPase (as compared to normotensive animals).

Conclusions: We conclude that in this model, high doses of ACE inhibition and AT\(_1\)-receptor blockade are necessary to normalize systolic blood pressure, LV hypertrophy and systolic LV wall stress which, in turn, is associated with restoration of a normal cardiac phenotype with respect to SR Ca\(^{2+}\)-ATPase and ANF and normalization of cardiac function.

Keywords: Sr Ca-ATPase; ACE inhibitors; Angiotensin receptor; Gene expression; Rat, renal hypertensive; Atrial natriuretic factor

1. Introduction

Left ventricular (LV) hypertrophy is an adaptive process which permits maintenance of physiological function under more demanding conditions. The increase in LV wall thickness following chronic pressure overload aims at keeping the wall stress within normal limits, and, therefore, is considered to represent an important and beneficial adaptation. Yet, LV hypertrophy is associated with a number of quantitative and qualitative alterations which, in turn, may favor the development of heart failure and arrhythmias, and may adversely affect survival [1].

Although the mechanical load imposed on the left ventricle seems to be an important determinant of myocardial structure [2,3], clinical and experimental observations suggest that the degree of LV hypertrophy is not only load-dependent [4,5]. Recent data suggest that angiotensin II (Ang II) is a critical factor in mediating cardiac hypertrophy in vivo [6]. There is evidence for a “local” renin-angioten-
sin system in the heart [7], functional Ang II receptors have been identified on cultured myocytes [8], and the autocrine release of Ang II has been shown to mediate stretch-induced hypertrophy of cardiac myocytes in vitro [9]. In addition, angiotensin converting enzyme (ACE) inhibition has been reported to antagonize LV hypertrophy in vivo independently of a blood pressure lowering effect [10,11], supporting the notion that Ang II is involved in the development of LV hypertrophy in response to pressure overload. Whether or not the antihypertrophic properties of ACE inhibitors are solely mediated by a reduction in circulating or tissue levels of Ang II or are, in part, due to inhibition of local bradykinin degradation remains controversial [12–14]. However, improvements in cardiac metabolism and function may be achieved by chronic ACE inhibition without affecting LV hypertrophy and these effects appeared to be related to bradykinin [14]. These findings would indicate that changes in cardiac metabolism and function are dissociated from the events leading to hypertrophy. In this respect, Feldman et al. provided evidence that the hypertrophy gene program that accompanies hypertrophy is dissociated from changes in SR Ca\(^{2+}\)-ATPase gene expression [15]. Several studies have shown that myocardial hypertrophy secondary to pressure overload is associated with reduced ventricular levels of mRNAs encoding SR Ca\(^{2+}\)-ATPase [16–17] while the gene expression of the atrial natriuretic factor (ANF) is upregulated [17]. The reduced expression and functional activity of the SR Ca\(^{2+}\)-ATPase has been implicated in the altered Ca\(^{2+}\) handling of the failing myocardium and may contribute to abnormal excitation-contraction coupling [18].

Although several studies have assessed the effect of ACE inhibitors and/or AT\(_1\) receptor antagonists on LV hypertrophy and expression of ANF [10,11], important issues concerning the role of the renin–angiotensin system on cardiac function remain unanswered. Specifically, little is known about the role of blood pressure reduction and the renin–angiotensin system in affecting the myocardial expression of the SR Ca\(^{2+}\)-ATPase, which is assumed to be characteristic for the failing heart. Given the beneficial effects of ACE inhibitors in experimental and clinical studies, the question arises whether the interference with the renin–angiotensin system is able to alter the hypertrophic phenotype and if so, whether such an effect is associated with restoration of normal cardiac function. Accordingly, the present study was designed to assess the dose-dependent effects of ACE inhibition and AT\(_1\)-receptor blockade comparable with respect to cardiac function, macroscopic LV structure and the cardiac gene expression of ANF and SR Ca\(^{2+}\)-ATPase in a 2K-1C rat model of hypertension. To allow a valid comparison, we applied 3 different doses of either an ACE inhibitor or an AT\(_1\)-receptor antagonist including a low dose not affecting systolic blood pressure. LV dimensions and function were determined in vivo by means of magnetic resonance imaging (MRI).

2. Materials and methods

2.1. Experimental protocol

The experiments were approved by the Kantoni poke Veterinäramt, Basel, Switzerland. Renal hypertension was produced in male Wistar Kyoto rats by placing a silver clip (0.2 mm inner diameter) on the left renal artery under halothane anesthesia (2.5%) in animals with 120 g body weight (2K-1C hypertension). Four weeks later, systolic blood pressure was determined by the tail cuff method. Animals with systolic blood pressure of less than 180 mmHg were excluded from the study. A group of animals did not undergo clipping and served as an age-matched normotensive sham-operated control group. Seven weeks after clipping, animals were randomized into 7 groups (n = 8 each) with similar systolic blood pressure and treated with the ACE inhibitor benazepril (0.3, 3.0, and 10 mg/kg per day i.p.) or the competitive AT\(_1\)-receptor antagonist valsartan (0.3, 3.0, and 10 mg/kg per day i.p.) or with placebo. The doses were chosen on the basis of a previous dose finding study, in which the low dose of each drug showed no effect on blood pressure, and the medium and high doses of benazepril and valsartan were about equally potent in lowering blood pressure. Systolic blood pressure was monitored on a weekly basis during the 12-week treatment period. After the 12-week treatment period, MRI was performed and LV hemodynamic parameters were determined invasively, the hearts were excised, weighed, and rapidly frozen in liquid nitrogen. The tissue was stored at −70°C until the gene expression measurements.

2.2. Substances

Benazepril and valsartan were dissolved in 1 N NaOH. This solution was then neutralized with 2 N HCl to obtain a final pH between 7 and 8. The substances were administered intraperitoneally by minipumps (model 2MLA, Alza Corp., Palo Alto, USA). Untreated animals received only solvent via the minipumps.

2.3. Magnetic resonance imaging

2.3.1 Spectrometer setup

MRI experiments were performed similarly to the method described earlier [19]. The study was carried out on a 4.7 Tesla, 30 cm bore Spectrospin BIOSPEC spectrometer (Bruker, Karlsruhe, Germany) equipped with a 15 cm gradient system. The radiofrequency antenna was a birdcage resonator with an inner diameter of 70 mm. Images were acquired by using a multislice spin-echo sequence with an echo-time of 18 ms. The field of view was 50 mm and the spatial resolution in plane was 195 \(\mu\)m. The zero filling technique was applied for image reconstruction (image matrix 256 \(\times\) 256). The MRI data
acquisition was gated to the cardiac cycle using a Physiolgrad SM 785 NMR monitoring system from Bruker (Karlsruhe, Germany). The ECG was recorded using two subcutaneous silver electrodes positioned on the neck and the left side of the sternum. Images were obtained at the end-diastolic and end-systolic time point of the cardiac cycle. Ten to thirteen adjacent slices of 1.5 mm thickness covering the entire left ventricle were recorded in the transversal plane. Each slice was triggered individually to ensure that all slices were recorded exactly at the same time point during the cardiac cycle. The body temperature was maintained at 37 ± 0.3°C by heating the air inside the magnet. The position of transverse sections was adjusted using a coronal scout scan.

2.3.2. Image analysis

The images were quantitatively analyzed by individually counting the pixels in the LV cavity and myocardial wall in each slice, using software provided by Bruker. Myocardial volume and cavity volume were computed from the known distance between the center of the slices using a truncated cone model. A specific gravity of 1.05 g cm⁻³ has been used to convert the volume of the LV wall into mass [20]. The analysis of end-diastolic and end-systolic images yielded the ejection fraction (EF) according to

\[ EF(\%) = \left(1 - \frac{LVV_{dia}}{LVV_{sys}}\right) \times 100 \]

where LVM is LV mass, \( V \) represents either diastolic or systolic values, and the factor 1/1.05 accounts for the conversion of mass to volume.

The average myocardial wall thickness (W) was calculated assuming a spherical shape of the LV:

\[ W(i) = \left(\frac{3}{4\pi}\right)^{1/2} \times \left[\left(\frac{LVV_{sys} + LVM(i)}{1.05}\right)^{1/2} - LVV_{dia}^{1/2}\right] \]

where LVM(i) is LV mass, (i) represents either diastolic or systolic values, and the factor 1/1.05 accounts for the conversion of mass to volume.

Calculations of end-diastolic inner radius \( r_{dia} \) and end-systolic inner radius \( r_{sys} \) were also derived from LV volumes according to

\[ r_{i} = \left(\frac{LVV_{i}}{3/4\pi}\right)^{1/3} \]

assuming a spherical shape of the LV.

The MRI-derived determinations of LV wall thickness and LV cavity dimensions allowed an estimation of the systolic and end-diastolic myocardial wall stress in vivo according to Laplace. Again, a spherical shape of the left ventricle was assumed:

\[ WS(i) = P_{i} \times 2r_{dia} / 4W(i) \]

where \( WS(i) \) is systolic or end-diastolic wall stress (kdyn/cm²) and \( P_{i} \) is systolic blood pressure or LV end-diastolic pressure (kPa).

2.4. Hemodynamic measurements

Systolic blood pressure was determined weekly in conscious animals using the tail cuff method. Following the MRI acquisition, the animals were anaesthetized with a mixture of 2.5% isoflurane/97.5% O₂. The animals breathed spontaneously. Heart rate and LV pressure were measured with an ultraminiaturess catheter pressure transducer (model SPR 249, Millar Instruments Inc., Houston, TX), which was placed in the left ventricle via the right carotid artery. All data were recorded on-line with a 486 DX 50 computer (IBM) and evaluated using commercially available software (Digital Acquisition Analysis and Archive Systems, Po-Ne-Mah Inc., Shorrs, CT, USA). In addition, pressure curves and ECGs were recorded on a 6-channel recorder (Heilige, Freiburg, Germany). After completion of the hemodynamic measurements, 5 ml of blood were taken for determination of plasma Ang II levels. The hearts were excised and the right ventricular wall was trimmed off. Regional heart weights were determined on a balance (model AE 200, Mettler-Toledo, Nänikon, Switzerland). All tissue was quickly frozen in liquid nitrogen and was stored at −70°C.

2.5. Angiotensin II plasma levels

Blood was withdrawn into tubes containing 50 μl of an inhibitor solution [2% ethanol, 2.5 mM phenanathol, 6.2 mM EDTA, 10 μM MK 422, and 5 μM CGP 44099 (an inhibitor of rat renin [21]) to avoid degradation of angiotensin. The tubes were kept in ice-water before and immediately after sampling. The blood was centrifuged without delay at 4000 rpm for 5 min and the plasma immediately transferred into polypropylene tubes and snap-frozen on dry ice. The samples were stored at −80°C until analysis. Ang II concentrations were measured after extraction from plasma on phenyl-silica gel cartridges (BondElut, Analyticchem, Harbor City, CA) inserted into a vacuum device (Vac Elut, Analyticchem). The column was conditioned with 1 ml ethanol and then 1 ml water. Subsequently, 1 ml of cold plasma was rapidly passed through the cartridge followed by a wash with 3 ml water. Adsorbed angiotensins were eluted with 0.5 ml methanol into conical polypropylene tubes coated with albumin buffer (1 M Tris buffer pH 7.5, 5 g/1 BSA, 0.2 g/l NaN₃, heat inactivated for 3 h at 58°C). The methanol was lyophilized and RIA buffer was added. Ang II was measured by radioimmunoassay using a specific rabbit antiserum against Ang II. The interassay variation is approximately 20%, the intra-assay variation varied between 8 and 13%.

2.6. Gene expression

2.6.1. RNA preparation

About 200 mg of frozen LV tissue was dismembrated in a Mikro-Dismembrator II (B. Braun Melsungen Inc., Mel-
sungen, Germany). Total cellular RNA was isolated from the dismembrated frozen tissue by the method of acid guanidinium thiocyanate/phenol/chloroform extraction [22] and stored in DEPC-treated water at -20°C. The integrity of the RNA was checked by agarose gel electrophoresis and ethidium bromide staining. RNA concentration was evaluated in triplicate by absorbance at 260 nm, using a DU-65 spectrophotometer (Beckmann Instruments Ltd., Munich, Germany).

2.6.2. Northern blot analysis

10 μg of total RNA was prepared and subjected to electrophoresis according to Rosen et al. [23], transferred to a nylon membrane (Amersham Buchler Ltd., Braunschweig, Germany) by overnight capillary blotting and fixed to the membrane by UV irradiation. The blot membrane was successively hybridized with a 1.2 kb rat SR Ca2+ -ATPase cDNA fragment (EcoRI/EcoRI) [24], a 0.58 kb rat atrial natriuretic factor (ANF) cDNA fragment (PstI/PstI) [25], and a 1.2 kb chicken β-actin cDNA fragment (EcoRI/HindIII) [26]. To obtain radioactive labelled cDNA probes, the inserts were cut out from the plasmid vectors by using the appropriate restriction enzymes and separated from the vector DNA on a low melting point Sea plaque agarose gel (Biozym, Hameln, Germany). The cDNA fragments were labelled with [α-32P]dCTP (Amersham Buchler Ltd., Braunschweig, Germany) by using a Multiprime DNA labelling kit according to the supplier’s recommendation. For cDNA hybridization, the blot membrane was prehybridized for about 4 h at 65°C in hybridization buffer containing 2 X SSC (0.3 M NaCl, 0.03 M Na-citrate pH 7.0), 10 X Denhard’s solution (0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll), 0.1% SDS, 0.1% pyrophosphate, 2 mM EDTA, 2.5% dextran sulfate and 50 μg/ml sonicated, denatured E. coli DNA (Sigma Ltd., Deisenhofen, Germany). Hybridization was performed in fresh hybridization solution at 65°C for at least 16 h completed with the respective radioactive labelled cDNA probe to a concentration of about 1 X 106 cpm/ml. After hybridization, the membrane was successively washed in 2 X SSC/0.1% SDS, 0.5 X SSC/0.1% SDS, and 0.1 X SSC/0.1% SDS for 30 min at 60°C, respectively. After the washing procedure, the membranes were kept moist between plastic foil and exposed at -80°C to X-OMAT AR X-ray films (Kodak Inc., Stuttgart, Germany) with Quanta III intensifying screens (Siemens, Stuttgart, Germany). Relative amounts of each RNA species were evaluated by densitometric scanning (Usonal Densitometer, Molecular Dynamics Ltd., Krefeld, Germany) of the band densities obtained within the linear response range of the X-ray film. The β-actin hybridization signal was used as an internal standard to confirm equal loading and transfer of total RNA.

2.7. Statistical analysis

All data are given as mean ± s.e.m. Statistical analysis was done using the SPSS software for windows (SPSS Inc., Chicago, IL, USA). Statistical differences between groups were calculated by ANOVA followed by the Student-Newman-Keul test. Correlations were assessed by linear regression analysis. A P value < 0.05 was considered significant.

3. Results

3.1. Systolic blood pressure and cardiac hypertrophy

Seven weeks after clipping of the left renal artery, all groups showed a similar elevation of systolic blood pressure (Fig. 1A and B). In untreated 2K-1C animals systolic blood pressure remained essentially at the same level throughout the study. The smallest dose of benazepril or valsartan (0.3 mg/kg per day) did not significantly affect

| Table 1 | Animal weight (BW), post mortem regional heart weights (LVW/BW, LV weight/body weight ratio; RVW/BW, right ventricular weight/body weight ratio), plasma angiotensin II (Ang II) levels, LV end-diastolic pressure (LVEDP), and diastolic wall stress (WSd) after 12 weeks treatment with the ACE inhibitor benazepril (B, 0.3, 3.0, and 10.0 mg/kg per day) or the AT1-receptor antagonist valsartan (V, 0.3, 3.0, and 10.0 mg/kg per day) as compared to WKY animals and to untreated 2K-IC hypertensive animals |
|---------|-------------------------------------------------|----------------|----------------|----------------|----------------|----------------|
|         | BW (g)                                          | LVW/BW (mg/g)  | RVW/BW (mg/g)  | Ang II (fmol/ml) | LVEDP (mmHg)   | WSd (kdyn/cm²) |
| WKY animals | 320±8                                        | 2.20±0.10 *     | 0.52±0.02 *    | 6±1             | 7±1 *          | 12.2±2         |
| 2K-IC animals (untreated) | 323±13                                   | 3.80±0.24       | 0.74±0.08      | 155.7±33.2      | 13±1           | 17.2±1.0       |
| B:       | 0.3 mg                                         | 316±15          | 3.68±0.28      | 0.71±0.05       | 117.2±56.2     | 12±1.5         | 16.0±1.9       |
|          | 3.0 mg                                         | 345±9           | 2.44±0.10 *    | 0.64±0.09       | 20.3±3.2 *     | 10±0.6         | 14.1±1.7       |
|          | 10.0 mg                                        | 329±14          | 2.14±0.04 *    | 0.50±0.05       | 5.0±0.5 *      | 8±0.9 *        | 11.1±2.5       |
| V:       | 0.3 mg                                         | 360±13          | 3.36±0.13      | 0.64±0.04       | 158.1±28.9     | 16±3.0         | 23.3±4.5       |
|          | 3.0 mg                                         | 389±11          | 2.46±0.09 *    | 0.54±0.04 *     | 247.9±40.4     | 9±1.1          | 14.0±1.8       |
|          | 10.0 mg                                        | 337±19          | 2.15±0.06 *    | 0.50±0.01 *     | 229.1±28.3     | 7±0.9 *        | 10.7±1.3 *     |

* P < 0.05 vs untreated 2K-IC animals.
systolic blood pressure. The medium (3.0 mg/kg per day) and high (10 mg/kg per day) doses of both substances showed dose-dependent and sustained reductions in systolic blood pressure which were comparable to systolic blood pressure in age-matched, normotensive, sham-operated rats (124 ± 5 mmHg). Benazepril showed a slightly more pronounced reduction in blood pressure compared with valsartan indicating that the doses were not exactly equally effective.

Sub-antihypertensive doses of benazepril and valsartan were characterized by a lack of effect on RV and LV hypertrophy as indicated by RV and LV weights (Table 1). Three and ten mg/kg per day of both compounds dose-dependently induced a regression of LV hypertrophy. The highest dose of each drug resulted in RV and LV weights comparable to normotensive, age-matched sham-operated animals.

3.2. MRI results

Both end-systolic and end-diastolic volume were restored to normal (i.e. similar to normotensive animals) by medium and high dose valsartan and benazepril and significantly reduced as compared to untreated 2K-1C animals and in animals with low dose treatment of benazepril or valsartan (Fig. 2).

Similarly, the ejection fraction was significantly reduced in untreated 2K-1C animals as compared to the groups treated with high dose valsartan and benazepril in which ejection fraction was preserved as compared to normotensive sham-operated animals (Fig. 2).

The systolic blood pressure in combination with the MRI derived LV chamber dimensions and wall thickness permitted an in vivo estimation of systolic wall stress. In untreated 2K-1C animals systolic (Fig. 2) and diastolic wall stress (Table 1) was significantly increased as compared to normotensive rats. High dose treatment but not subhypotensive doses of each drug resulted in a significant reduction and normalization of systolic and diastolic wall stress. There was a close, linear and positive relationship between systolic wall stress (and systolic blood pressure respectively) and LV mass (Fig. 3).

3.3. Plasma Ang II levels

Untreated 2K 1C animals were characterized by high Ang II plasma levels (Table 1). Animals treated with the receptor antagonist valsartan were not significantly different from controls. Three and ten mg/kg benazepril dose dependently reduced plasma Ang II levels.

3.4. Gene expression data

LV gene expression of ANF and SR Ca²⁺-ATPase normalized to β-actin mRNA was determined by Northern blot analysis in 5–6 animals per group. Each treated group was compared to the untreated 2K-1C group loaded on the same gel. Neither benazepril nor valsartan had any influence on gene expression when given in sub-antihypertensive doses as compared to untreated 2K-1C animals, which showed a high expression of ANF along with a depression of the SR Ca²⁺-ATPase when compared with sham-operated WKY rats (Fig. 4). The medium dose of both treatments significantly decreased ANF gene expression (Fig. 4A); however, there was no concomitant increase in SR Ca²⁺-ATPase gene expression (Fig. 4B). Only the highest dose of both drugs normalized the depressed SR Ca²⁺-ATPase expression (Fig. 4B). ANF expression showed an excellent linear correlation to LV hypertrophy (be-
nazepril/valsartan-treated animals: $r = 0.85/0.85$, respectively) and systolic wall stress (benazepril/valsartan-treated animals: $r = 0.80/0.85$, respectively) (Fig. 5). The regression lines were not significantly different for benazepril and valsartan-treated animals. In contrast, SR Ca$^{2+}$-ATPase expression did not show a linear relationship to LV hypertrophy and systolic wall stress in the analysis of all animals (Fig. 5). However, there was a significant relationship between systolic wall stress and SR Ca$^{2+}$-ATPase expression in valsartan-treated animals ($r = 0.55$). SR Ca$^{2+}$-ATPase mRNA levels varied widely in animals with normal systolic wall stress. In benazepril-treated animals, SR Ca$^{2+}$-ATPase mRNA levels were in the normal range only in rats with normal plasma Ang II levels (Fig. 5 left, open symbols).

4. Discussion

The results of the present study demonstrate that in this rat model of 2K-1C hypertension and pressure overload, (1) only hemodynamically effective doses of ACE inhibition and AT$_1$-receptor blockade caused regression of LV hypertrophy and normalized LV systolic wall stress and steady-state levels of mRNA for ANF and SR Ca$^{2+}$-ATPase, (2) the beneficial effects of both interventions on LV hypertrophy, dimensions, wall stress and ANF mRNA levels were closely and linearly correlated to their blood pressure and wall stress lowering effect and (3) in contrast to ANF, normalization of LV wall stress was necessary but not sufficient to restore SR Ca$^{2+}$-ATPase mRNA levels in this model, raising the possibility that load-independent

![Fig. 2](image-url)
mechanism(s) might be involved in the regulation of cardiac SR Ca\(^{2+}\)-ATPase expression.

4.1. Treatment effects on LV hypertrophy, dimensions and wall stress

End-systolic and end-diastolic volumes were substantially increased and ejection fraction was decreased in untreated 2K-1C hypertensive animals. The development of right ventricular hypertrophy supports the notion that the development of LV hypertrophy did not compensate for the increased afterload imposed by the high systemic blood pressure. This conclusion is also supported by measurements of systolic wall stress which was significantly elevated (+65%) in untreated 2K-1C hypertensive animals as compared to normotensive WKY animals, suggesting that the adaption to pressure overload by increased LV wall thickness was not sufficient to normalize the systolic wall stress. Both treatments resulted in similar effects on LV hypertrophy, dimensions and wall stress at all three doses used. Importantly, non-antihypertensive doses of both the ACE inhibitor and the AT\(_1\)-receptor antagonist were ineffective. LV weights after 12-week treatment showed a close correlation to systolic blood pressure or systolic wall stress regardless of the treatment (r = 0.80 and 0.77, respectively). These results suggest that the antihypertrophic effect of both interventions is load-dependent.

The present study was not designed to differentiate peripheral unloading effects of the therapeutic intervention from direct myocardial effects of these agents. Recent in vivo studies, however, provided convincing evidence that the cardiac renin–angiotensin system plays a crucial role in the development of LV hypertrophy by pressure overload, acting via the AT\(_1\) receptor [27–30]. Notably, hemodynamically effective doses of ACE inhibitor or AT\(_1\)-receptor antagonist were used in these studies; even so, these interventions did not necessarily result in effective unloading of the left ventricle because of proximal aortic banding [31]. In this respect, Sadoshima et al. [9] suggested that the autocrine release of Ang II mediates the stretch-induced hypertrophy of cardiac myocytes. Thus, locally released Ang II may be the critical initial mediator of load-induced cardiac hypertrophy, working through the AT\(_1\) receptor [9].

At the first glance, the lack of effect of low dose ACE inhibition appears to be in contrast to some reports in the literature that describe a load-independent effect of sub-an-
tihypertensive doses of ACE inhibitors on LV hypertrophy [10–12]. Linz et al. [11,12] reported that sub-antihypertensive doses of ramipril prevented the development of LV hypertrophy following aortic banding. Moreover, the sub-antihypertensive doses of this ACE inhibitor exerted antihypertrophic effects which were not related to Ang II but rather to bradykinin, since the antihypertrophic effect was blocked by a bradykinin antagonist [11,12]. Thus, compared to AT$_1$-receptor blockade, one would expect a stronger effect of ACE inhibition on LV hypertrophy in relation to the reduction in blood pressure. Indeed, in a model of abdominal aortic constriction, the AT$_1$-receptor antagonist losartan had a smaller effect on prevention and regression of LV hypertrophy compared to the ACE inhibitor ramipril, despite being administered at a 300-fold higher dose and having a stronger effect on blood pressure [31] compared to the ACE inhibitor. Differences in the experimental design may, in part, account for the discrepant results of the present study compared to the aforementioned studies by Linz and co-workers [11,12,31]. The present investigation assessed the effects of chronic ACE inhibition and AT$_1$-receptor blockade on regression of LV hypertrophy whereas most of the former studies elucidated the potential of ACE inhibitors or AT$_1$-receptor antagonists to prevent the development of hypertrophy. Conceivably, in contrast to the development phase of cardiac hypertrophy, the renin-angiotensin system may not contribute to the maintenance of pressure-induced cardiac hypertrophy, similar to observations recently reported for volume overload-induced cardiac hypertrophy [32]. If so, regression of LV hypertrophy may not occur in response to sub-antihypertensive doses of an ACE inhibitor or AT$_1$-receptor antagonist [31] unless a concomitant reduction in systolic blood pressure and wall stress is achieved. In this respect, we have recently reported that regression of reactive hypertrophy and improvement of survival post myocardial infarction in the rat was only observed with high, hemodynamically effective doses of an ACE inhibitor [33].

4.2. Treatment effects on LV gene expression of ANF and SR Ca$^{2+}$-ATPase

Long-term ACE inhibition and AT$_1$-receptor antagonism reduced myocardial gene expression of ANF to normal values as compared to age-matched, normotensive animals and restored SR Ca$^{2+}$-ATPase gene expression. However, ANF and SR Ca$^{2+}$-ATPase responded in a divergent fashion to these treatments. Both agents dose-dependently reduced LV ANF gene expression. In fact, this effect of both drugs was highly correlated to the decrease in LV systolic wall stress (Fig. 4A) and to the reduction in LV hypertrophy in a linear manner. Under physiological conditions, LV expression of ANF is very low in adult ventricular myocardium; however, it is re-expressed upon induction of pressure or volume overload [34,35]. It has been shown that stretch is the principle stimulus for the induction and release of ANF [36]; indeed, ANF represents a well established marker of hypertrophy [37]. In the

![Graph](image-url)
The present study, LV ANF expression was closely correlated to the degree of LV hypertrophy and the LV systolic wall stress, consistent with previous experimental and clinical studies [38]. This strong linear correlation between wall stress and ANF gene expression in animals irrespective of dose and agent (benazepril or valsartan) suggests that afterload is an important determinant of ANF expression in our model.

The SR Ca\(^{2+}\)-ATPase mRNA levels were significantly decreased in untreated 2K1C hypertensive rats as compared to sham-operated WKY animals, consistent with previous observations in animals with severe LV hypertrophy due to suprarenal aortic banding [16] or LV hypertrophy and failure due to proximal aortic banding [15]. Notably, the present model of renal hypertension is characterized by elevated LV wall stress (uncompensated by LV hypertrophy) and deterioration of LV function as indicated by a decrease in ejection fraction. Interestingly, only the highest dose of both agents was able to restore SR Ca\(^{2+}\)-ATPase mRNA levels and to preserve ejection fraction. The medium dose of both treatments, though limiting the increase in LV dimensions, systolic wall stress and ANF mRNA levels, did not affect SR Ca\(^{2+}\)-ATPase expression. Thus, a linear relationship between LV hypertrophy, wall stress and gene expression was not observed for the SR Ca\(^{2+}\)-ATPase. As depicted in Fig. 4B, normalization of LV systolic wall stress was not necessarily associated with increased expression of SR Ca\(^{2+}\)-ATPase. Analysis of individual plasma Ang II levels in animals treated with the ACE inhibitor revealed that increased gene expression of SR Ca\(^{2+}\)-ATPase occurred only in animals with normalised LV systolic wall stress and plasma Ang II level within the normal range of normotensive WKY rats (5 fmol/ml [39]). Consistent with previous studies plasma levels of Ang II tend to increase during AT\(_1\) receptor blockade [40]. Presumably, only the highest dose of the AT\(_1\) receptor antagonist was able to completely neutralize Ang II at the receptor and, therefore, affected SR Ca\(^{2+}\)-ATPase gene expression. However, since blood was drawn following catheter instrumentation, anesthesia and/or surgical stress may have affected plasma angiotensin II levels; moreover, due to the long treatment period, animals were studied 19 weeks after clipping, at a time when the elevated plasma angiotensin II levels in this model may have, to variable degree, returned to baseline. These caveats need to be kept in mind in interpreting plasma angiotensin II levels in animals treated with the ACE inhibitor and a AT\(_1\) antagonist were equally effective in exerting regression of LV hypertrophy, prevention of progressive LV chamber dilation and normalization of LV systolic wall stress. The close, positive correlation between LV mass and systolic wall stress indicates that the beneficial effects of both treatment regimens in this model were primarily load-dependent, although the regulation of SR Ca\(^{2+}\)-ATPase gene expression may include load independent factors.

Acknowledgements

The expert technical assistance of Mr. E. Eichlisberger, Mr. P. Forgiarini, Mr. A. Theuer, and Mr. W. Theilkäs is gratefully acknowledged. This study was supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG Dr. 148/6-1 and 5-1).

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