Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure

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Submitted 28 November 2004; accepted in final form 9 May 2005

Karram, Tony, Anan Abbasi, Shlomo Keidar, Eliahu Golomb, Irit Hochberg, Joseph Winaver, Aaron Hoffman, and Zaid Abassi. Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure. Am J Physiol Heart Circ Physiol 289: H1351–H1358, 2005. First published May 13, 2005; doi:10.1152/ajpheart.01186.2004.—Angiotensin-converting enzyme (ACE)-2 is a newly described enzyme with antagonistic effects to those of the classical ACE (ACE-1). Both ANG II and aldosterone play an important role in the pathophysiology of congestive heart failure (CHF) and in the adverse cardiac remodeling during its development. In this study, we examined the effects of experimental CHF induced by an aorticaval fistula (ACF) and of its treatment with ANG II and aldosterone inhibitors on the relative levels of ACE-1 and ACE-2. We also compared the effects of spironolactone, an aldosterone antagonist, and eprosartan, an ANG II receptor antagonist, on heart hypertrophy and fibrosis in rats with ACF. Spironolactone (15 mg·kg⁻¹·day⁻¹ ip, via minipump) or eprosartan (5 mg·kg⁻¹·day⁻¹, via minipump) was administered into rats with ACF for 14 and 28 days. Specific antibodies were used to determine the protein levels of myocardial ACE-1 and ACE-2. ACF increased the cardiac levels of ACE-1 and decreased those of ACE-2. Heart-to-body weight ratio significantly increased from 0.30 ± 0.004% in sham-operated controls to 0.50 ± 0.018% and 0.56 ± 0.044% (P < 0.001) in rats with ACF, 2 and 4 wk after surgery, respectively, in association with increased plasma levels of aldosterone. The area occupied by collagen increased from 2.33 ± 0.27% to 6.85 ± 0.65% and 8.03 ± 0.93% (P < 0.01), 2 and 4 wk after ACF, respectively. Both spironolactone and eprosartan decreased cardiac mass and collagen content and reversed the shift in ACE isoforms. ACF alters the ratio between ACE isoforms in a manner that increases local ANG II and aldosterone levels. Early treatment with both ANG II and aldosterone antagonists is effective in reducing this effect. Thus ACE isoform shift may represent an important component of the development of cardiac remodeling in response to hemodynamic overload, and its correction may contribute to the beneficial therapeutic effects of renin-angiotensin-aldosterone system inhibitors.

aldosterone antagonist; angiotensin antagonist

THE INVOLVEMENT OF VASOCONSTRICTOR neurohormonal systems in the pathogenesis of congestive heart failure (CHF) has been increasingly recognized (12, 14, 27). Numerous studies in patients and in experimental models of CHF have established the important role of the renin-angiotensin-aldosterone system (RAAS) in the progression of cardiovascular and renal dysfunction in CHF. It is now accepted that excessive neurohumoral activation may adversely affect cardiac function and the hemodynamic condition by enhancement of systemic vasoconstriction and promotion of salt and water retention by the kidney. In addition, prolonged activation of the RAAS may have direct deleterious actions on the myocardium, independent of their systemic hemodynamic effects (27). Specifically, ANG II has been shown to stimulate myocyte hypertrophy and to enhance fibrosis and apoptosis, leading ultimately to progressive remodeling and further deterioration in cardiac performance (23).

The concept that CHF is also a neurohormonal disorder has led to the use of angiotensin-converting enzyme (ACE) inhibitors and ANG II receptor antagonists aldosterone antagonists, as well as β-blockers, that are now central to the treatment of CHF (22, 23, 27). Yet, ANG II comprises only one of the two major components of the RAAS (14). The role of the other component, namely, aldosterone, in cardiac remodeling has emerged in the last few years. It is widely accepted that structural remodeling of the interstitial collagen matrix is regulated by both ANG II and aldosterone (3, 5, 37, 38, 40). These effects have been attributed to both the elevated circulatory and local cardiac levels of these two active components of the RAAS, activated in both experimental and clinical severe CHF (33). Recently, Mizuno et al. (25) showed that cardiac aldosterone production is increased in patients with CHF, especially when caused by systolic dysfunction. Convincing evidence for the local production of aldosterone was provided by the finding that CYP11B2 mRNA (aldosterone synthase) is expressed in cultured neonatal rat cardiac myocytes (21). The adverse contribution of aldosterone to the functional and structural alterations of the failing heart was elegantly demonstrated by Suzuki et al. (36). These authors showed that eplerenone, a specific aldosterone antagonist, prevented progressive left ventricular (LV) systolic and diastolic dysfunction in association with reducing interstitial fibrosis, cardiomyocyte hypertrophy, and LV chamber sphericity in dogs with CHF. Similarly, Delyani et al. (9) reported that eplerenone attenuated the development of ventricular remodeling.

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eleng in reactive but not reparative fibrosis after myocardial infarction in rats. These findings are in agreement with the results observed in clinical trials. Randomized Aldactone Evaluation Study, therapy with spironolactone, reduced overall mortality in patients with advanced heart failure by 30% compared with placebo (30). Recently, the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival (EPHESUS) study showed that addition of eplerenone to optimal medical therapy reduces morbidity and mortality among patients with acute myocardial infarction complicated by LV dysfunction and heart failure (29).

It has recently been discovered that the local levels of ANG II and aldosterone depend not only on renin activity but also on the ratio between two isoforms of ACE. The classic ACE converts ANG I into ANG II and is the target of ACE inhibitors. Its inhibition exerts well-established beneficial effects in various cardiovascular disorders, including CHF. In contrast, ACE-2, an enzyme recently identified, was shown to possess a different biochemical activity (8). It converts ANG I into ANG 1–9 and Ang II into ANG 1–7, therefore antagonizing ACE activity. In addition, the resulting ANG 1–7 possesses opposite effects to those of ANG II, including vasodilatation, antihypertrophy, and diuresis (10). The role of aldosterone in the pathophysiology of heart failure was shown to involve amplification of tissue ACE expression (17), and spironolactone treatment reduced ACE activity (13). Similarly, ANG II receptor blockers (ARBs) have also been an effective treatment for CHF. The relative contribution of ANG II and aldosterone to myocardial hypertrophy and fibrosis in CHF has not been comparatively investigated.

Therefore, the present study was designed to examine the changes in ACE and ACE-2 during the development of CHF and the effects of early treatment with either eprosartan (an ARB) or spironolactone (an aldosterone antagonist) on these changes, as well as on cardiac hypertrophy and fibrosis. We used the animal model of CHF induced by an aortocaval fistula (ACF), a well-established and thoroughly characterized model of volume overload in rats.

In Vivo Experiments
Effects of treatment with either spironolactone or eprosartan on rats with ACF. This protocol was designed to evaluate the effects of long-term administration (14 and 28 days) of either spironolactone or eprosartan through osmotic minipumps: Alzet model 2ML2 for 14 days of treatment, Alzet model 2ML4 for 28 days of treatment (Alza, Palo Alto, CA) on the changes in the cardiac ACE and ACE-2 immunoreactivity, as well as on the development of cardiac hypertrophy and fibrosis in rats with ACF (n = 6–30). Sham-operated rats (n = 6–23) or untreated ACF animals (n = 6–20) served as controls. Spironolactone (Sigma) was dissolved in polyethylene glycol 400 (35 mg/ml) and was adjusted to a final concentration sufficient to deliver 15 mg·kg⁻¹·day⁻¹ for either 14 or 28 days, according to the manufacturer’s specifications. Eprosartan (Merek) was dissolved in NaHCO₃ (20 mg/ml) and was adjusted to a final concentration sufficient to deliver 5 mg·kg⁻¹·day⁻¹ for either 14 or 28 days. The osmotic minipumps containing either spironolactone, eprosartan, or vehicles were implanted into the peritoneal cavity during the creation of the ACF. After the operation the animals were transferred into metabolic cages, and daily measurements of urinary sodium and potassium excretion were performed for either 14 or 28 days.

After completion of the spironolactone or eprosartan treatment, animals were euthanized and their blood was collected in precooled tubes, their chests opened, and the hearts were removed immediately, placed on absorbent paper to remove excess blood, weighed, and halved. Half of the cardiac tissue was frozen for immunoblot analysis, and half was embedded in paraffin for histological analysis.

In Vitro Experiments
Morphological analysis. Fixed blocks of the myocardial tissue were embedded in paraffin, and 4- to 5-μm sections were cut from the blocks of the different experimental groups. The sections were placed in Harris hematoxylin for 5 min and washed three times with distilled water. Sections were stained with Trichrome solution for 10 min and washed three times with 0.2% acetic acid as described by Masson (24). The sections were dehydrated in two changes of 95% ethanol, and cleared in four changes of xylol. Collagen volume was determined by measuring the area of stained tissue within a given field and expressed as the proportion of the total area under observation by using Image-Pro Plus (version 4.5, resolution 760 × 590 pixels, MediaCybernetics).

Western blot analysis. Western blot analysis was performed on heart tissue stored at −70°C. One hundred micrograms of total ventricular homogenate were resolved in a polyacrylamide gel (4–20%, Bio-Rad). Proteins were transferred onto nitrocellulose membranes (Optitran; Schleicher & Schuell, Keene, NH) using a semidy transfer apparatus (Bio-Rad). After the blocking in 3% nonfat milk was completed, membranes were incubated with specific antibodies (anti-ACE and ACE-2, Santa Cruz) for 2 h and with secondary antibody for 1 h at 24°C. After each incubation, three 10-min Tris-buffered saline-Tween washes were performed. Blots were developed with an enhanced chemiluminescence detection kit (Amersham, Amersham, UK). The corresponding bands were quantified by densitometric scan (Bioprolif Imaging, Wilber Lourmat, France).

Determination of ACE Activity
Cardiac homogenates were analyzed for their ACE activity using a commercial kit (Buhlmann; Zurich, Switzerland). The kinetics of ACE-mediated cleavage of the synthetic substrate furyl-acryloyl-phenylalanylglycyl-glycine to furyl-acryloyl-phenylalanine and glycine are measured by reduced absorbance at 340 nm (31). The absorbance kinetic was measured in a UV microplate reader (PowerWave, Biotech) and standardized to a known calibrator activity.
ACE-2 Activity Assay

ACE-2 activity determination is a modification of the method described by Huang et al. (19). Briefly, ACE-2 cleaves the leucine at the COOH-terminal of the decapeptide ANG I (Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8-His9-Leu10), and the assay is based on measurement of free leucine released. With the addition of β-NAD and leucine dehydrogenase (Sigma), NADH is formed and the latter is coupled to diaphorase-mediated conversion of resazurine to resorufin, which is fluorescent (excitation 565 nm, emission 585 nm). Fluorescence kinetic is measured for 1 h at room temperature in the Fluostar Galaxy plate reader (BMG Labtechnologies, Germany).

The assay was adapted to measure ACE-2 in myocardial homogenate. Leucine dehydrogenase and diaphorase concentrations were determined. Leucine dehydrogenase and diaphorase-mediated conversion of resazurine to resorufin, which is fluorescent (excitation 565 nm, emission 585 nm). Thus, increase in fluorescence is dependent on the presence of leucine dehydrogenase and an external supply of NAD, indicating the specific measurement of free leucine released. ANG II alone was unable to induce an increase of fluorescence. Preincubation of homogenate with a specific antibody directed against the ectopic domain of ACE-2 (R&D systems, Minneapolis, MN) completely abolished the ANG I-induced increase of fluorescence, indicating the association of the ANG I degradation activity with ACE-2. Activity results are expressed as femtomole leucine formation per minute and normalized to milligram tissue protein.

Chemical Analysis

Sodium and potassium concentrations in plasma and urine were determined by flame photometry (model IL 943, Instrumentation).

Statistical Analysis

Data are presented as means ± SE. One-way ANOVA was used when multiple comparisons were made followed by Dunnett’s test. For comparison of the graphs representing control and experimental groups, two-way ANOVA was used. A value of P < 0.05 was considered statistically significant.

RESULTS

Effects of Spironolactone or Eprosartan on Rats With ACF

Figure 1 depicts that plasma levels of aldosterone significantly increased after 2 and 4 wk from the placement of ACF, compared with sham-operated controls, in line with our previous observation in rats with ACF for 1 wk (1, 7, 41). Aldosterone levels were further elevated after spironolactone treatment for 2 and 4 wk. In agreement with its inhibitory effects on ANG II-induced aldosterone production, eprosartan significantly attenuated the elevation of aldosterone levels in rats with ACF. Administration of the vehicles did not affect plasma levels of aldosterone (data not shown). These findings demonstrate that chronic administration of either spironolactone or eprosartan through osmotic minipumps appears to be an effective and reliable approach for the experimental blockade of aldosterone or ANG II, respectively.

Rats with ACF for 2 and 4 wk display a moderate elevation in plasma concentrations of K+ (5.54 ± 0.47 and 5.67 ± 0.39 μeq/l), compared with sham-operated (4.38 ± 0.17 μeq/l, P < 0.05) and normal Na+ levels [143.2 ± 1.11 and 142.9 ± 0.79 μeq/l, compared with sham-operated controls, 141.1 ± 1.79 μeq/l, P = not significant (NS)]. Spironolactone slightly, but significantly, decreased plasma Na+ levels to 138.4 ± 0.97 μeq/l (P = 0.007) and increased circulating K+ to 6.69 ± 0.43 μeq/l (P < 0.11) after 4 wk of treatment. These effects of spironolactone are well known in patients and further support our conclusion regarding the validity of the drug administration via osmotic minipumps.

Figure 2A shows that rats with ACF have lower urinary potassium excretion compared with sham-operated rats, and spironolactone treatment has a further potassium sparing effect, contributing to the hyperkalemic effect of this agent. The decrease in urinary potassium excretion by spironolactone was significant after the fifth day of treatment (P < 0.05 by 2-way ANOVA). This dose of the aldosterone antagonist did not significantly affect urine sodium excretion (data not shown). ANG II blockade by eprosartan in rats with ACF for 2 or 4 wk did not affect Na+ and K+ plasma levels but significantly (P < 0.05, 2-way ANOVA) improved natriuresis in ACF rats (Fig. 2B).

The effect of spironolactone on cardiac hypertrophy in rats with ACF is depicted in Fig. 3. Rats with ACF display a significant increase in HW/BW after 2 (0.502 ± 0.018%, P < 0.001) and 4 wk (0.56 ± 0.044%, P < 0.001) from the creation of the fistula compared with sham-operated controls (0.3 ± 0.004%). Administration of spironolactone for 2 and 4 wk partially but significantly attenuated the development of cardiac hypertrophy (HW/BW decreased to 0.409 ± 0.012%, P < 0.05, and 0.485 ± 0.0035%, P = NS, respectively). Similar to spironolactone, administration of eprosartan for 2 or 4 wk reduced cardiac mass in rats with ACF to 0.453 ± 0.022 (P < 0.01) and 0.454 ± 0.035, respectively (P < 0.05) (Fig. 3).

Figure 4 quantitatively summarizes the effects of either spironolactone or eprosartan on the interstitial fibrosis in cardiac ventricular tissue (combined right and left). The myocardial collagen volume fraction in rats with ACF for 2 (6.85 ± 0.65%) and 4 wk (8.03 ± 0.93%) was significantly (P < 0.01) higher compared with sham-operated rats (2.33 ± 0.27%), indicating that fibrosis is characteristic of the ACF model. Administration of spironolactone for 2 and 4 wk significantly reduced myocardial collagen volume fraction in rats with ACF (5.05 ± 0.41%, P < 0.5, and 3.10 ± 0.39%, P < 0.01 respectively). Administration of eprosartan for 2 and 4 wk produced an even more profound reduction in myocardial collagen volume fraction in rats with ACF (2.87 ± 0.24% and 1.86 ± 0.26%, respectively, P < 0.01) (Fig. 4).
Changes in ACE and ACE2 Immunoreactivity in Untreated and Treated Rats With ACF

Figure 5 shows an increase in the level of ACE and a decrease in the level of ACE-2, 2 and 4 wk after the placement of ACF. Treatment with both spironolactone and eprosartan restored the immunoreactive levels of ACE and ACE-2 to comparable levels of the sham-operated controls. The increase in ACE-2 in response to eprosartan was greater than that in response to spironolactone. Densitometric analysis of ACE-2 in the various experimental groups is presented in Fig. 6.

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Effect of the Spironolactone and Eprosartan on Cardiac ACE and ACE-2 Activities in Rats With CHF

To determine whether the effects of spironolactone or eprosartan on ACE and ACE-2 immunoreactive levels are associated with similar trend in activities, the ACE and ACE-2 activities were determined in the different experimental groups.

ACE activity in ventricular homogenate derived from rats with CHF for 2 wk increased by 15% ($P < 0.05$, NS). Treatment of CHF animals with spironolactone and eprosartan reduced ACE activity by 41% ($P = 0.086$) and 8% ($P = 0.766$) compared with untreated CHF (Fig. 7).

In contrast to ACE, ACE-2 activity decreased by 14% ($P = 0.63$) in the myocardium of rats with CHF for 2 wk. Both spironolactone and eprosartan increased ACE-2 activity by 42% ($P = 0.067$) and 100.5% ($P = 0.042$), respectively, in rats with ACF compared with untreated animals (Fig. 7).

DISCUSSION

Cardiac remodeling occurs in response to a wide variety of prolonged hyperfunction stimuli. It consists of biochemical and structural-morphological changes. Furthermore, it serves as an adaptive mechanism in the short term but is a major pathophysiological component of compensation and the development of severe cardiac dysfunction in the long run with the evolvement of heart failure, major arrhythmias, and ischemia (15, 34, 36, 37, 39). Previously, we have shown that rats with ACF, an experimental model of cardiac hypertrophy, display neurohormonal, renal, and cardiac characteristics that closely mimic those observed in patients with clinical CHF. These include increased activity of the RAAS, sympathetic nervous system, and arginine vasopressin and a marked decrease in renal function in association with sodium and water retention (2). In addition, rats with ACF develop proportionate cardiac hypertrophy to the severity of the cardiac dysfunction (28). The present study demonstrates that early treatment with either spironolactone or eprosartan for either 2 or 4 wk partially and comparably reduced the increase in cardiac mass in rats with ACF. Both drugs remarkably diminished the interstitial cardiac fibrosis, though eprosartan was more effective than spironolactone in this respect.

This study demonstrated, to the best of our knowledge for the first time, that cardiac hyperfunction due to volume overload affects the local angiotensin levels by modulating the ratio between ACE and ACE-2: Rats with heart failure not only express high levels of cardiac ACE but also exhibit reduced levels of cardiac ACE-2. Thus higher levels of ANG II are formed by ACE, given the reduced competition of ACE-2 as demonstrated by activity determination tests. Furthermore, ANG II is not metabolized into ANG 1–7, which further augments ANG II levels. Moreover, both spironolactone and eprosartan were found to be effective in reducing the levels of ANG II, thereby attenuating the deleterious effects of ANG II on the myocardium.

Fig. 5. Representative Western blot analysis of angiotensin-converting enzyme (ACE) and ACE-2 in cardiac tissue of control rats and rats with CHF before and after treatment with spironolactone or eprosartan. Bands at ~150 kDa represent ACE, and at ~100 kDa, ACE-2 immunoreactive proteins.

Fig. 6. Densitometric analysis of bands at ~100 kDa corresponding to ACE-2 immunoreactive proteins in cardiac tissue of control rats and rats with CHF before and after treatment with spironolactone or eprosartan. *$P < 0.05$ vs. sham-operated rats; #$P < 0.05$ vs. untreated appropriate rats with ACF. Values are means ± SE.
eprorsartan treatment in rats with ACF restores the ratio between cardiac ACE and ACE-2 immunoreactivity by down-regulating cellular ACE and upregulating ACE-2 in association of similar alterations in the activity of these enzymes. These effects were associated with a significant lowering in the cardiac hypertrophy and fibrosis.

Studies performed on myocytes and fibroblast cultures taken from rat hearts showed that ANG II, and to a lesser extent aldosterone, induced collagen synthesis in a dose-dependent manner and decreased the activity of matrix metalloproteinases, key enzymes in interstitial collagen degradation (6, 32, 43). Likewise, in vivo studies have demonstrated that aldosterone and ANG II can stimulate the accumulation of collagen within the cardiac interstitium and therefore lead to LV diastolic dysfunction and ultimately systolic dysfunction. Brilla et al. (4) demonstrated that spironolactone prevents the development of interstitial fibrosis in hypertensive and normotensive rats given intravenous aldosterone. Similarly, the prognosis of CHF patients correlates with plasma procollagen, a collagen synthesis marker. Procollagen also served as a marker for the responsiveness to spironolactone therapy; patients with high levels of plasma procollagen showed decreased morbidity and mortality in response to the aldosterone antagonist. These results suggest that reducing the excessive extracellular matrix turnover may be one of the various extrarenal mechanisms contributing to the beneficial effect of spironolactone in patients with CHF (42). Our finding that spironolactone modulates the ratio between ACE and ACE-2 offers an additional mechanistic explanation for the beneficial effects of aldosterone inhibition. This finding is in agreement with reports that aldosterone upregulates tissue ACE expression in cardiomyocytes, indicating that this hormone increases local production of ANG II (17). In the current study, spironolactone therapy lowered cardiac ACE immunoreactivity in rats with ACF, which could lead to the blockade of the ANG II-induced aldosterone production and eventually to the reduction in hypertrophy and fibrosis of the heart in our experimental model of heart failure. The significance of ACE-2 in physiological and pathophysiological processes in the heart is starting to be studied, and there are contradictory reports on the potential consequences of high expression of this protein. On one hand, Crackower et al. (8) found that ACE-2 null mice exhibited a severe impairment in myocardial contractility in association with increased ANG II levels. Moreover, genetic ablation of ACE in the ACE-2 null mice rescued the cardiac phenotype (26). In contrast to these findings, ACE-2 transgenic mice have a high incidence of sudden death that correlates with transgenic expression levels (11). However, the relative roles of ACE and ACE-2 should be studied at physiological levels in conditions mimicking common human pathological disorders, such as hypertension, CHF, and cardiac ischemia.

Our results are somewhat contradictory to those reported by Zisman et al. (44) who demonstrated that failing human hearts display high cardiac levels of ANG 1–7, an evidence of upregulation of ACE-2. Likewise, Goulter et al. (16) showed that cardiac ACE-2 is upregulated in patients with both idiopathic and ischemic cardiopathy. However, it should be emphasized that these patients received different conventional pharmacological therapies, including ACE inhibitors, aldosterone, and Ang II antagonists, which, according to our study, may significantly contribute to their finding.

To summarize, the yin-yang regulation of ACE and ACE-2 observed in this study could be a central key for the understanding of mechanisms affecting ANG II production and its pathophysiological consequences. ACE-2 may be an important therapeutic target, and drugs that specifically influence its activity may have considerable clinical value. Therefore, determination of ACE-2 levels and activity in the cardiac tissue could represent an important marker for the assessment of the pathophysiological status and of the effect of therapeutic agents.

The reduction in cardiac fibrosis associated with spironolactone or eprorsartan treatment in the ACF model exceeded the extent reported by similar studies in other models. For instance, Suzuki et al. (35, 36) showed that in dogs with moderate CHF, long-term aldosterone receptor blockade with eplerenone prevented the progressive LV systolic and diastolic dysfunction, to a lesser extent than our results: compared with controls, eplerenone treatment was associated with a 37% reduction of volume fraction of replacement fibrosis and a 34% reduction of volume fraction of reactive interstitial fibrosis. Eplerenone did not alter body weight, electrolytes, blood urea nitrogen, or creatinine, which suggests that regulation of fluid volume did not play a primary role in cardioprotective actions.

Treatment with aldosterone antagonists has become widespread in different cardiac disorders. Hayashi et al. (18) have recently recommended that an aldosterone blocker should be maintained indefinitely in patients with severe chronic CHF.

Fig. 7. ACE and ACE-2 activities in cardiac tissues of control rats and rats with CHF before and after treatment with spironolactone or eprosartan. A: ACE activity expressed in units. B: activity of ACE-2 (ANG I-induced ACE-2-mediated release of leucine) expressed in femtomole per minute, and bars represent means ± SE; n = 5–6 rats; *P < 0.05 vs. untreated rats with CHF.
due to systolic LV dysfunction, as should be an ACE inhibitor and a β-adrenergic blocker. Moreover, mineralocorticoid receptor antagonist combined with ACE inhibitor can prevent postinfarct LV remodeling better than the ACE inhibitor alone in association with the suppression of a marker of collagen synthesis (29, 42). Moreover, Farquharson and Struthers (13) demonstrated that spironolactone improves endothelial dysfunction in patients’ CHF class II and III by increasing nitric oxide bioactivity and inhibiting ANG I and ANG II conversion, suggesting novel mechanisms for this agent’s beneficial effect on cardiovascular mortality.

Using a different model than ours, Ishiyama et al. (20) found recently that both losartan and olmesartan completely reversed the reduction in cardiac ANG II type-1 receptors mRNA observed after coronary artery ligation while augmenting ACE-2 mRNA by approximately threefold. The latter correlated significantly with ANG II, ANG 1–7, and ANG I levels. This group suggested that the effect of ANG II blockade on cardiac ACE-2 may be due to direct blockade of angiotensin receptor type 1 receptors or a modulatory effect of increased ANG 1–7.

In conclusion, treatment with both spironolactone antagonist and ARB was associated with the reduction in cardiac hypertrophy and fibrosis in rats with experimental heart failure. We propose a novel mechanism for the beneficial effects of these agents in CHF, which involves downregulation of cardiac ACE and upregulation of ACE-2.

GRANT

This project was funded by The Bruce Rappaport Institute.

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