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Additive Improvement of Left Ventricular Remodeling and Neurohormonal Activation by Aldosterone Receptor Blockade With Eplerenone and ACE Inhibition in Rats With Myocardial Infarction

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OBJECTIVES We investigated the effects of the aldosterone blocker eplerenone alone and in combination with angiotensin-converting enzyme (ACE) inhibition on ventricular remodeling in rats with left ventricular (LV) dysfunction after extensive myocardial infarction (MI).

BACKGROUND Adding an aldosterone antagonist to ACE inhibition reduces mortality and morbidity in heart failure.

METHODS Starting 10 days after MI, rats were treated with placebo, eplerenone (100 mg/kg/day), the ACE inhibitor trandolapril (0.3 mg/kg/day), or a combination of both for nine weeks.

RESULTS Both monotherapies attenuated the rise in LV end-diastolic pressure (LVEDP) and LV end-diastolic volume (LVEDV) compared with placebo, whereas combined treatment further attenuated LVEDP and LVEDV, significantly improved LV function and reduced plasma norepinephrine levels. The time constant of LV pressure isovolumic decay (τ) was prolonged in placebo MI rats, significantly shortened by eplerenone, and normalized by eplerenone/trandolapril. Increased collagen type I gene expression and collagen content in the noninfarcted LV myocardium from MI placebo rats was attenuated by trandolapril, but almost completely prevented by eplerenone and eplerenone/trandolapril. The addition of eplerenone to ACE inhibition prevented sarcoplasmic-reticulum calcium ATPase downregulation and the increases in LV gene expression of β-MHC and atrial natriuretic factor more effectively than either monotherapy. Furthermore, combination treatment attenuated the increase in myocardial angiotensin II type 1 receptor expression and increased phosphorylated endothelial nitric oxide synthase protein levels.

CONCLUSIONS The aldosterone blocker eplerenone improved LV remodeling in rats with LV dysfunction after extensive MI. Combination therapy with an ACE inhibitor substantially potentiates this effect by a complementary prevention of LV fibrosis, cardiac hypertrophy, and molecular alterations. (J Am Coll Cardiol 2003;42:1666–73) © 2003 by the American College of Cardiology Foundation

Left ventricular (LV) remodeling after myocardial infarction (MI) involves myocyte hypertrophy, chamber dilation, and increased collagen accumulation remote from the infarct site, leading to impaired contractile function and heart failure (1,2).

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Aldosterone production in the heart (3,4) as well as aldosterone plasma levels (5–7) are increased after MI and in congestive heart failure (CHF), correlating with the severity of disease. Moreover, despite complete vascular angiotensin-converting enzyme (ACE) inhibition, plasma aldosterone levels are elevated in CHF patients, suggesting angiotensin II-independent aldosterone production (8). Aldosterone may contribute to the progression of ventricular remodeling by promoting sodium and water retention, sympathoadrenergic activation, endothelial dysfunction, and vascular and myocardial fibrosis (9–13).

Aldosterone receptor blockade (for four weeks, started early [14] or seven days [3] after MI) reduced fibrosis in the viable myocardium of rats with moderate MI. Moreover, long-term monotherapy with eplerenone, a novel aldosterone blocker, prevented progressive LV dysfunction and remodeling in dogs with moderate heart failure (15). The Randomized Aldactone Evaluation Study (RALES) showed that the aldosterone receptor antagonist spironolactone added to ACE inhibition reduces morbidity and mortality among patients with severe heart failure (16). Post hoc substudies of the RALES and subsequent smaller trials in postinfarction patients reported a decrease in serum markers for cardiac collagen synthesis and a reduction of LV dilation (17–19). However, the role of aldosterone in addition to ACE inhibition in the pathophysiology of postinfarction ventricular remodeling is still uncertain.

In the present study, we investigated the effects of

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Abbreviations and Acronyms

ACE	= angiotensin-converting enzyme
ANF	= atrial natriuretic factor
ANOVA	= analysis of variance
AT_1	= angiotensin II type 1
CHF	= congestive heart failure
dP/dt_{max}	= maximal rate of pressure rise
dP/dt_{min}	= maximal rate of pressure decline
eNOS	= endothelial nitric oxide synthase
GAPDH	= glyceraldehyde-3-phosphate-dehydrogenase
LV	= left ventricle/ventricular
LVEDP	= left ventricular end-diastolic pressure
LVEDV	= left ventricular end-diastolic volume
MHC	= myosin heavy chain
MI	= myocardial infarction
MMP	= matrix metalloproteinase
mRNA	= messenger ribonucleic acid
NT-proANP	= N-terminal pro-atrial natriuretic peptide
PCR	= polymerase chain reaction
RALES	= Randomized Aldactone Evaluation Study
RV	= right ventricle/ventricular
SERCA2	= sarcoplasmic-reticulum calcium

long-term (nine weeks) aldosterone receptor blockade with eplerenone alone and in combination with ACE inhibition on hemodynamics, neurohormonal activation, and LV remodeling (dilation, biochemical and molecular alterations) in rats with LV dysfunction after extensive MI.

METHODS

All procedures conformed to the guiding principles of the American Physiological Society and were approved by the institutional Animal Research Committee.

MI and study protocols. Left coronary artery ligations were performed in adult male Wistar rats (200 to 250 g) (7). Briefly, under ether anesthesia, the thorax was opened, the heart exteriorized, and a ligature was placed around the proximal left coronary artery. The heart was returned to its normal position, and the thorax was closed. Mortality was 46% within the first 24 h. Sham-operated control rats underwent the same surgical procedure except that the suture around the coronary artery was not tied. Starting on the tenth postoperative day, sham-operated animals received placebo treatment, and surviving MI rats were randomly allocated to one of the following four treatment groups: placebo, the aldosterone blocker eplerenone, the ACE inhibitor trandolapril, or a combination of eplerenone and trandolapril for nine weeks. Eplerenone was given in food, and trandolapril was administered by gavage once daily. The dose of eplerenone (100 mg/kg/day) was selected from previous studies in which eplerenone provided marked end-organ protective effects in the heart and kidney of hypertensive rats (20). Trandolapril was used at a dose of 0.3 mg/kg/day, which is the most commonly used dose for this drug in rats with heart failure (7,11).

Hemodynamic and LV volume measurements. Left ventricular systolic pressure, LV end-diastolic pressure (LVEDP), mean arterial pressure, maximal rate of pressure rise and decline (dP/dt_{max} and dP/dt_{min}, respectively), and heart rate were measured 10 weeks after MI, under pentobarbital anesthesia (30 mg/kg body weight, intraperitoneally). Saline-filled catheters (polyethylene-50) were advanced from the right carotid artery into the LV and connected via a three-way stopcock to a Millar micromanometer and Statham transducer. The time constant of LV pressure isovolumic decay (regression of log [pressure] vs. time) was calculated by the Weiss method (21). Correlation coefficients for all studies were $\geq$0.99. The in vivo LV pressure-volume relationship was analyzed using a conductance catheter (SPR-774, Millar Instruments, Houston, Texas). The 1.4 F catheter was advanced from the right carotid artery into the LV through the polyethylene-50, saline-filled catheter after hemodynamic measurements. Pressure-volume signals were acquired by BioBench software (National Instruments, Austin, Texas). Pvan software (Millar Instruments) was used to analyze all pressure-volume loop data recorded at steady-state and during injection of hypertonic saline for the calibration of parallel conductance volume (22). The LV volume was calculated for each rat from conductance volume corrected by the relative parallel conductance volume.

Sample collection, infarct size. The right ventricle (RV) and the LV, including septum, were separated in ice-cold saline and weighed. Infarct size was quantified histologically by planimetry. The LV was cut into three transverse sections: apex, middle ring (~3 mm), and base. From the middle ring, 5-μm sections were cut at 100-μm intervals and stained with picrosirius red. Infarct size (fraction of the infarcted LV) was calculated as the average of all slices and expressed as a percentage of length. Only rats with extensive infarcts (>45%) were included in the study.

Neurohormonal assay. After hemodynamic measurement, a blood sample was collected from the right carotid artery. Plasma norepinephrine was measured with high-performance liquid chromatography, and N-terminal pro-atrial natriuretic peptide (NT-proANP) was measured by radioimmunoassay (Immunodiagnostik, Bensheim, Germany).

Quantification of cardiac gene expression. Total ribonucleic acid was isolated from LV samples (noninfarcted LV myocardium) using TRIzol reagent (Invitrogen, Karlsruhe, Germany). Atrial natriuretic factor (ANF), collagen α1(I), and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) gene expression were determined by competitive polymerase chain reaction (PCR) as described (7). Heterologous internal standards were constructed with a Competitive DNA PCR Kit (TaKaRa, Shiga, Japan). Products of PCR amplification were separated on 2% agarose gel. A given messenger ribonucleic acid (mRNA) level was expressed as a ratio with respect to the level of mRNA for GAPDH. α- and β-myosin heavy chain (MHC) mRNA was amplified by PCR as previously described (7). After

Table 1. Global Parameters of Sham-Operated Rats (Sham) and Rats With LV Dysfunction 10 Weeks After MI: Effects of Aldosterone Receptor Inhibition, ACE Inhibition, or Combined Aldosterone and ACE Inhibition

	Sham (n = 16)	Placebo MI (n = 24)	Eplerenone MI (n = 12)	Trandolapril MI (n = 16)	Eplerenone + Trandolapril MI (n = 21)
Infarct size (%)	—	53.3 ± 0.9	55.2 ± 1.4	55.2 ± 1.3	54.3 ± 1.4
BW (g)	451 ± 9	448 ± 10	440 ± 20	433 ± 11	426 ± 12
LV (g)	0.83 ± 0.02	0.81 ± 0.03	0.77 ± 0.04	0.76 ± 0.02	0.69 ± 0.03*†
LV/BW (mg/g)	1.84 ± 0.04	1.81 ± 0.05	1.78 ± 0.1	1.74 ± 0.05	1.64 ± 0.06*‡
RV (g)	0.23 ± 0.01	0.49 ± 0.02*	0.40 ± 0.03*†	0.39 ± 0.02*†	0.30 ± 0.02*§‖
RV/BW (mg/g)	0.50 ± 0.02	1.11 ± 0.02*	0.94 ± 0.08*‡	0.92 ± 0.04*†	0.74 ± 0.05*§‖
MAP (mm Hg)	124 ± 3	95 ± 3*	106 ± 3*	96 ± 7*	103 ± 3*
LVSP (mm Hg)	136 ± 3	112 ± 3*	112 ± 4*	109 ± 3*	110 ± 2*
LVEDP (mm Hg)	4.3 ± 0.5	21.7 ± 2*	14.8 ± 3*‡	15.9 ± 3*‡	11.6 ± 2*§
dP/dt_{max} (mm Hg/s)	11,845 ± 335	7,688 ± 237*	8479 ± 477*	8,453 ± 288*	8,901 ± 267*†
dP/dt_{min} (mm Hg/s)	8,855 ± 424	4,841 ± 165*	5477 ± 305*	5,278 ± 254*	5,521 ± 166*‡
Heart rate (beats/min)	400 ± 9	353 ± 9*	358 ± 7*	363 ± 11*	376 ± 12
NT-proANP (nmol/l)	0.71 ± 0.14	3.19 ± 0.48*	2.47 ± 0.26*	2.31 ± 0.24*‡	1.43 ± 0.23§‖
Norepinephrine (pg/ml)	213 ± 25	469 ± 71*	305 ± 57	312 ± 60	217 ± 19†

Values are mean ± SEM. *$p < 0.05$ versus Sham. †$p < 0.01$, ‡$p < 0.05$, §$p < 0.001$ versus placebo MI. ‖$p < 0.05$ versus eplerenone MI and trandolapril MI.
ACE = angiotensin-converting enzyme; BW = body weight; dP/dt_{max} = maximal rate of pressure rise; dP/dt_{min} = maximal rate of pressure decline; LV = left ventricular; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; MI = myocardial infarction; NT-proANP = N-terminal pro–atrial natriuretic peptide.

digestion with the restriction enzyme *Tru*9I, fragments of the PCR amplification product were separated on 8% polyacrylamide gel.

Myocardial hydroxyproline. For hydroxyproline determination, LV samples (noninfarcted LV myocardium) were freeze-dried, weighed, and hydrolyzed in 6N HCl at 110°C for 24 h. Hydroxyproline concentration was measured spectrophotometrically, and collagen content was expressed in μg/mg dry tissue weight assuming that collagen contains an average of 13.4% in hydroxyproline (7).

Western blot analysis. The LV samples (noninfarcted LV myocardium) were homogenized in ice-cold RIPA buffer (150 mmol/l NaCl, 50 mmol/l Tris-Cl, 5 mmol/l ethylenediaminetetraacetic acid, 1% v/v Nonidet P-40, 0.5% w/v deoxycholate, 10 mmol/l NaF, 10 mmol/l sodium pyrophosphate, 100 mmol/l phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, and 2 μg/ml leopeptin). Proteins were determined by Bradford assay. Myocardial extracts (30 μg protein per lane) were mixed with sample loading buffer and under reducing conditions separated on 10% sodium dodecyl sulfate-polyacrylamide gel. Proteins were electrotransferred overnight at 4°C onto polyvinylidene difluoride membrane (Immun-Blot, Bio-Rad, München, Germany). The bands were detected using chemiluminescence assay (ECL+Plus, Amersham, Freiburg, Germany). Primary antibodies used recognize the following: matrix metalloproteinase 13 (MMP-13) (MAB-13426, Chemicon International, Hofheim, Germany); angiotensin II type 1 (AT_1) receptor (sc-579, Santa Cruz Biotechnology, Heidelberg, Germany); sarcoplasmic-reticulum calcium adenosine triphosphatase (SERCA2 ATPase; MA3-919, Affinity BioReagents, Alexis Biochemicals, Grunberg, Germany); endothelial nitric oxide synthase (eNOS) (N-30020, Transduction Laboratories, BD Biosciences, Heidelberg, Germany); and phosphorylated eNOS at Ser^{1177} (9571, Cell Signaling Technology, Frankfurt, Germany).

Statistical analysis. The main effects of the drug were tested by two-factor analysis of variance (ANOVA) for repeated measures, and differences between the groups were assessed by one-factor ANOVA with post hoc comparisons by the Fisher protected least significant difference test. Statistical analysis was performed using the SuperANOVA statistic program (Abacus Concepts, California). Statistical significance was assumed at $p < 0.05$. Relationships between two variables were tested by linear regression analysis.

RESULTS

Global parameters. Infarct size and body weight were similar among the experimental groups (Table 1). The LV weight and LV weight/body weight were reduced in the eplerenone/trandolapril-treated MI group compared with placebo. The RV weight and RV weight/body weight were markedly higher in placebo-treated MI rats compared with sham-operated control rats and were significantly reduced by both monotherapies. Combination therapy led to a significant further decrease in RV compared with monotherapy with either eplerenone or trandolapril (Table 1).

Hemodynamics and LV remodeling. Mean arterial pressure and LV systolic pressure were reduced in all MI groups irrespective of treatment (Table 1). The MI rats on placebo developed elevated LVEDP and a markedly lower dP/dt_{max} and dP/dt_{min}. Monotherapy with eplerenone or trandolapril attenuated LVEDP and tended to enhance dP/dt_{max} and dP/dt_{min}, whereas combined treatment further prevented the rise in LVEDP and significantly improved dP/dt (Table 1). The time constant of LV pressure isovolumic decay (τ) was prolonged in placebo MI rats, attenuated by trandolapril, significantly shortened by eplerenone, and normalized by eplerenone/trandolapril (Fig. 1A). Myocardial infarction resulted in a rightward shift of the LV pressure-volume loops to high volumes (Fig. 1B). Eplerenone and trandola-

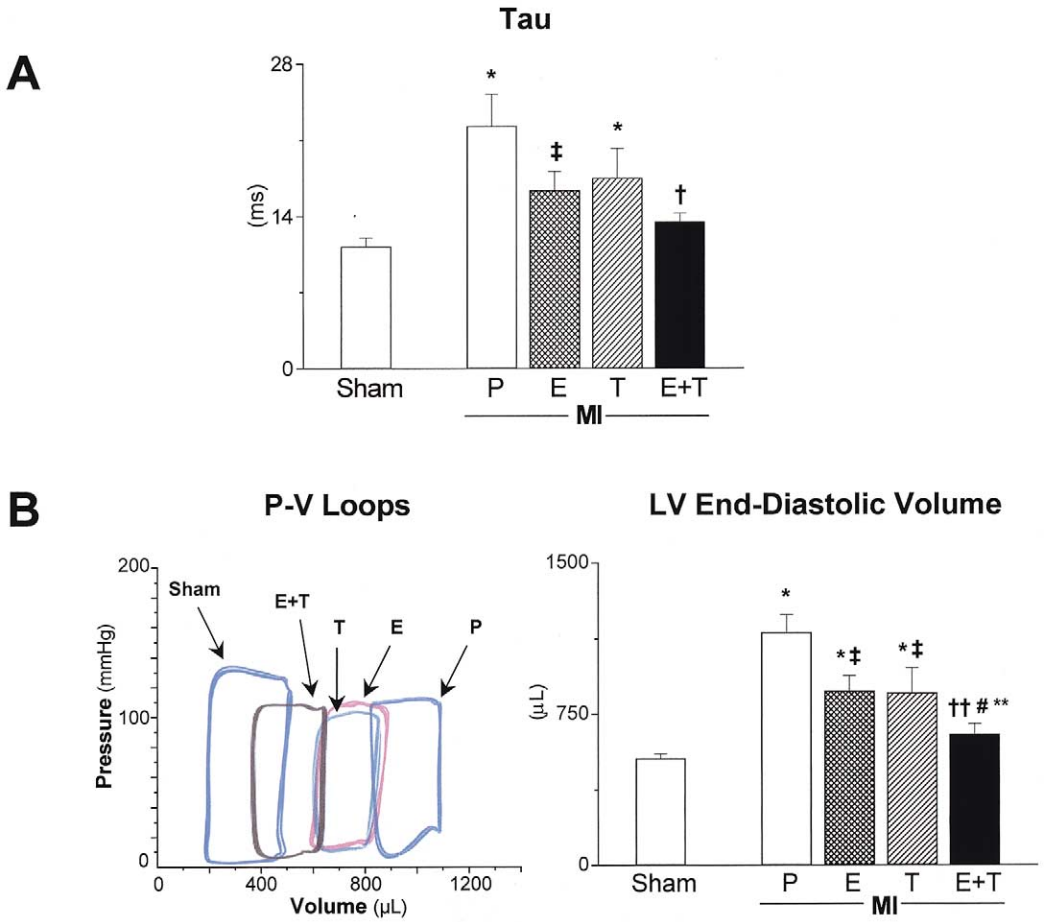

Figure 1. (A) The time constant of left ventricular pressure decay (Tau), and **(B)** left ventricular (LV) pressure–volume (P-V) loops and left ventricular end-diastolic volume measured in vivo with conductance catheter in sham-operated rats (Sham) and in rats with extensive myocardial infarction (P MI): Effects of aldosterone receptor inhibition (E), ACE inhibition (T), or combined aldosterone and ACE inhibition (E+T). Mean ± SEM (n = 6 to 12). *$p < 0.05$ versus Sham; ‡$p < 0.05$, †$p < 0.001$, ††$p = 0.0001$ versus P MI; #$p < 0.05$ versus E MI; **$p = 0.059$ versus T MI.

pril prevented the rightward shift of LV volume and significantly attenuated the LV end-diastolic volume (LVEDV) compared with placebo. However, combination therapy led to a substantial further leftward shift of the LV pressure-volume curve and a decrease in LVEDV compared with monotherapies. The LV ejection fraction was lower in placebo MI rats compared with control rats (30 ± 2% vs. 69 ± 4%, $p < 0.001$) and was significantly improved only in the eplerenone/trandolapril-treated MI group (42 ± 3%, $p < 0.01$).

NT-proANP and norepinephrine. Myocardial infarction was characterized by a marked increase in circulating NT-proANP and norepinephrine levels (Table 1). The NT-proANP levels tended to be reduced by eplerenone ($p = 0.11$) and were significantly decreased by trandolapril. However, combination therapy significantly further attenuated plasma NT-proANP concentrations compared with monotherapies. The increase in plasma norepinephrine levels was attenuated by eplerenone ($p = 0.06$) and trandolapril ($p = 0.06$) monotherapy and significantly reduced by eplerenone/trandolapril treatment.

LV fetal gene expression. The ratio of LV β-MHC to α-MHC mRNA and ANF gene expression, molecular markers of hypertrophy, were substantially increased in placebo MI rats and attenuated by trandolapril and, to a lesser degree, by eplerenone monotherapy. Eplerenone/trandolapril treatment led to an additional attenuation of cardiac β-MHC and ANF mRNAs (Fig. 2).

LV fibrosis. The LV collagen concentrations were increased in placebo MI rats, attenuated by trandolapril, and nearly normalized by eplerenone and eplerenone/trandolapril (Fig. 3). The rise in collagen type I gene expression in the noninfarcted LV myocardium from MI placebo rats was attenuated by trandolapril but almost completely prevented by eplerenone and eplerenone/trandolapril. Collagen type I mRNA levels significantly correlated with dP/dt_{max} ($r = 0.52$, $p < 0.01$), dP/dt_{min} ($r = 0.52$, $p < 0.01$), and τ ($r = 0.82$, $p < 0.01$). Left ventricular MMP-13 (59 kDa) protein expression was increased in placebo MI rats compared with control rats. This increase was attenuated by eplerenone and trandolapril and further inhibited by eplerenone/trandolapril.

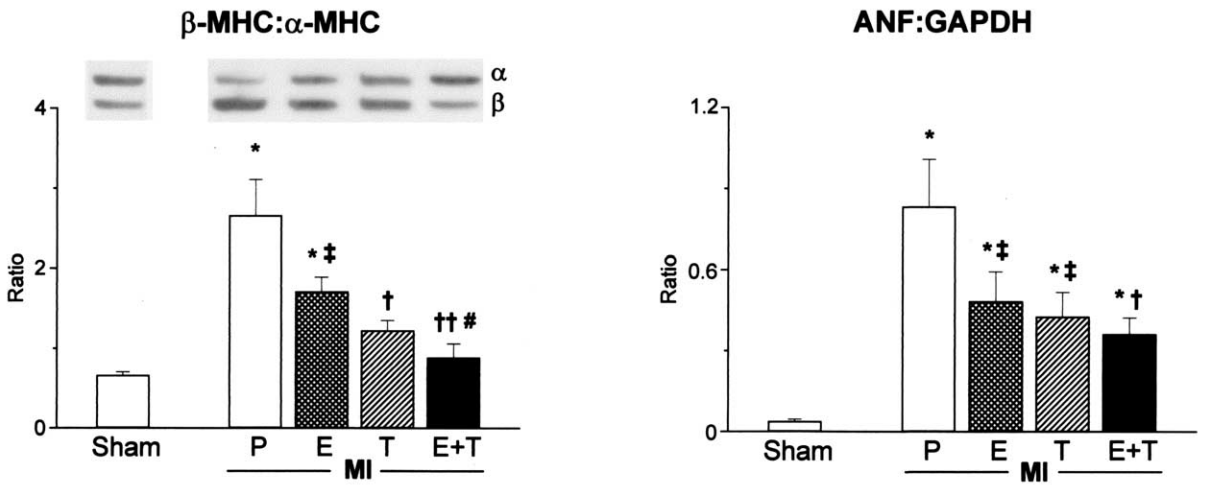

Figure 2. Ratios of messenger ribonucleic acid expression β-myosin heavy chain (MHC) to α-MHC and of atrial natriuretic factor (ANF) to glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) in the left ventricle of sham-operated rats (Sham) and in the noninfarcted left ventricular myocardium of rats with extensive myocardial infarction (P MI): Effects of aldosterone receptor inhibition (E), ACE inhibition (T), or combined aldosterone and ACE inhibition (E+T). Mean ± SEM (n = 5 to 7). *p < 0.05 versus Sham; ‡p < 0.05, †p < 0.005, ††p = 0.0001 versus P MI; #p < 0.05 versus E MI.

AT_1 receptor, SERCA2 ATPase, eNOS protein. The increase in AT_1 receptor protein expression in the noninfarcted LV from placebo MI rats tended to be attenuated by eplerenone and trandolapril monotherapy; however, it was significantly prevented only by combination therapy (Fig. 4). The SERCA2 ATPase downregulation in placebo MI rats was attenuated by trandolapril and completely prevented by eplerenone/trandolapril. The SERCA2 ATPase protein expression significantly correlated with dP/dt_{max} ($r = 0.45$, $p < 0.01$), dP/dt_{min} ($r = 0.45$, $p < 0.01$), and τ ($r = 0.60$, $p < 0.01$). There was no difference in total eNOS protein expression among the groups studied (data not shown). In contrast, treatment with eplerenone/trandolapril resulted in a significant eNOS phosphorylation at Ser^{1176} in MI rats (Fig. 4).

DISCUSSION

Our study shows improvement of LV remodeling by long-term aldosterone receptor antagonism in rats with severe LV dysfunction after extensive MI. We suppose that the favorable effects of eplerenone were likely mediated in part by the reduction of LV fibrosis in the remote noninfarcted myocardium, a major determinant of ventricular remodeling in ischemic cardiomyopathy (2). Alterations in the collagen matrix impair myocyte relengthening, leading to relaxation abnormalities, progressive diastolic dysfunction, and heart failure (2,13). In patients with hypertensive heart disease, regression of myocardial fibrosis was associated with improvement of LV relaxation (23). In dogs with moderate heart failure, Suzuki et al. recently reported that eplerenone reduced interstitial fibrosis and improved ventricular func-

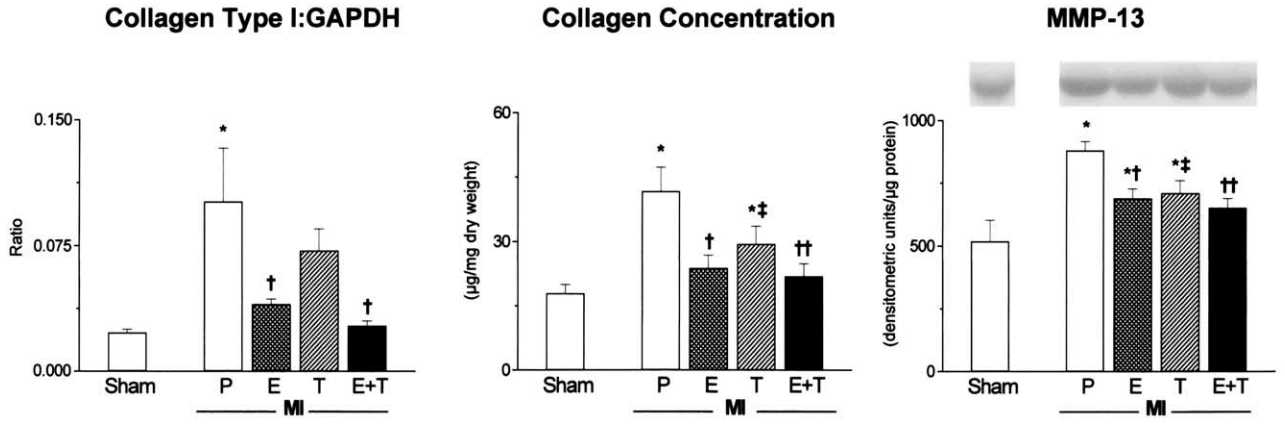

Figure 3. Type I collagen messenger ribonucleic acid levels, collagen concentration, and matrix metalloproteinase (MMP)-13 protein expression in the left ventricle of sham-operated rats (Sham) and in the noninfarcted left ventricular myocardium of rats with extensive myocardial infarction (P MI): Effects of aldosterone receptor inhibition (E), ACE inhibition (T), or combined aldosterone and ACE inhibition (E+T). Mean ± SEM (n = 5 to 11). *p < 0.05 versus Sham; ‡p < 0.05, †p < 0.01, ††p < 0.001 versus P MI. GAPDH = glyceraldehyde-3-phosphate-dehydrogenase.

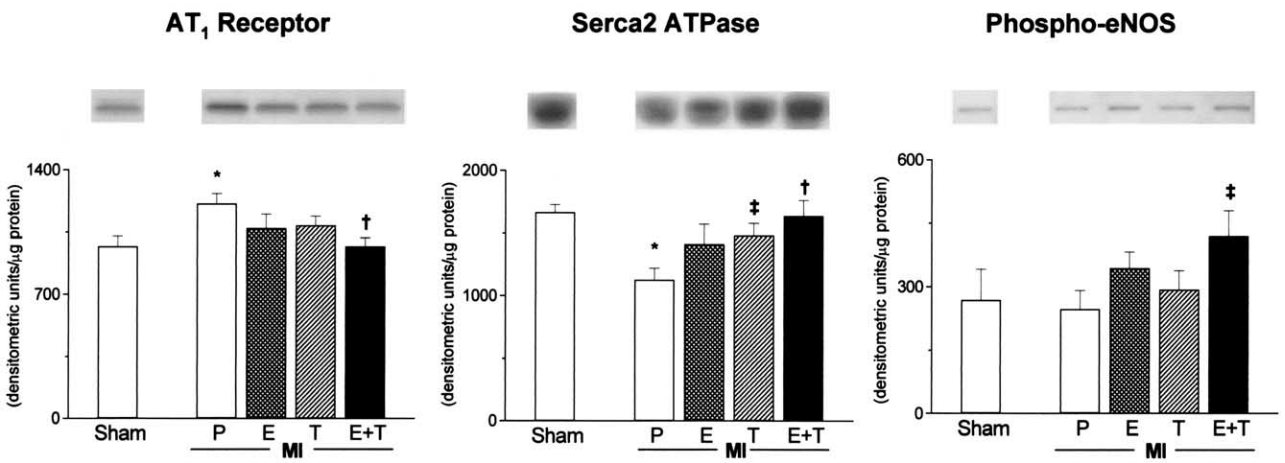

Figure 4. Protein expression of angiotensin II type 1 (AT_1) receptor, sarcoplasmic-reticulum calcium (SERCA2) ATPase, and phosphorylated endothelial nitric oxide synthase (eNOS) in the left ventricle of sham-operated rats (Sham) and in the noninfarcted left ventricular myocardium of rats with extensive myocardial infarction (P MI): Effects of aldosterone receptor inhibition (E), ACE inhibition (T), or combined aldosterone and ACE inhibition (E+T). Mean ± SEM (n = 7 to 11). *$p < 0.05$ versus Sham; ‡$p < 0.05$, †$p < 0.005$ versus P MI.

tion (15). Thus, the beneficial effects on isovolumetric relaxation by eplerenone might partly be due to the prevention of collagen deposition in the remote LV myocardium. Limitation of the aldosterone-related excessive collagen synthesis may be one of the various extrarenal mechanisms contributing to the clinical benefit of spironolactone in the RALES trial (17). In fact, serum markers for cardiac collagen synthesis were associated with poor outcome in CHF patients and were decreased by spironolactone therapy. Moreover, the reduction of MMP-13 could account for the improvement of LV remodeling. Indeed, upregulation of MMPs results in increased deposition of poorly structured fibrotic tissue in the myocardium and contributes to the development of progressive ventricular dilation and failure (24). Locally produced or circulating aldosterone stimulates cardiac fibrosis either directly via mineralocorticoid receptors or indirectly by interfering with AT_1 receptors (13,25). In addition, involvement of other factors has been implicated in aldosterone-induced myocardial fibrosis such as endothelin (26), bradykinin (27), and calcium (28). Reduction of LV AT_1 receptor expression by eplerenone may contribute to decreased cardiac fibrosis, similar to observations in aldosterone-salt-treated rats, where spironolactone attenuated the upregulation of cardiac AT_1 receptor (25).

The beneficial effects of long-term eplerenone treatment on LV remodeling were probably also related to the prevention of pathologic hypertrophy, as shown by the reduction of fetal genes such as β-MHC and ANF. Recent evidence that targeted overexpression of human mineralocorticoid receptor resulted in dilated cardiomyopathy and increased cardiac ANF expression supports our hypothesis (29). Experimental animal data suggest a role for aldosterone in mediating cardiac hypertrophy. Aldosterone receptor blockade attenuated cardiac hypertrophy in dogs with CHF (15) and in stroke-prone spontaneously hypertensive rats (30). In rats harboring the human renin and angiotensinogen genes, aldosterone antagonism, like AT_1 receptor inhibition, reduced cardiac hypertrophy and fibrosis, suggesting that angiotensin II-induced end-organ damage might arise via aldosterone-related mechanisms (31). However, the mechanisms underlying aldosterone-induced cardiac hypertrophy are not completely understood. Increased AT_1 receptor expression as well as calcineurin activity caused by aldosterone may play a role (32).

Additive effects of combination therapy with ACE inhibitor and eplerenone. Several studies have shown that monotherapy with ACE inhibitors after MI improved LV loading conditions, remodeling, and neurohormonal activation, as confirmed in the present study (33). However, ACE inhibition combined with aldosterone antagonism provided additional beneficial effects on LV hemodynamics and remodeling. This may be related to the observation of complementary effects on LV fibrosis and hypertrophy of the respective monotherapies: Although eplerenone was superior to ACE inhibition in reducing LV fibrosis, ACE inhibition had a greater effect on fetal gene expression and SERCA2 ATPase protein levels. Therefore, aldosterone receptor and ACE inhibition might be a particularly favorable combination regarding their action profile.

Combination therapy improved LV contractile function and both the load-dependent (dP/dt_{min}) and load-independent (τ) indices of LV relaxation (34,35). Because SERCA2 ATPase downregulation in hypertrophied and/or failing myocardium is linked to systolic and diastolic dysfunction (36), the improved cardiac function by combined therapy might partly be due to prevention of SERCA2 ATPase downregulation. In rats with heart failure, SERCA2 ATPase gene transfer improved not only contractile function but also survival, cardiac energetics, and remodeling (37), suggesting that impaired SERCA2 ATPase function represents a key mechanism linking deleterious

effects of the upregulated renin-angiotensin-aldosterone system post MI to contractile dysfunction, hypertrophy, and heart failure. Moreover, the favorable functional effects of beta-blockers in CHF patients are related to SERCA ATPase upregulation (38).

Combination therapy particularly increased myocardial phosphorylated eNOS protein levels. Phosphorylation of eNOS activates the enzyme leading to nitric oxide production (39). The pivotal role of eNOS for LV remodeling after MI was recently demonstrated in eNOS knockout mice that exhibited excessive LV dysfunction, hypertrophy, and dilation (40). Furthermore, in these mice, the beneficial effects of ACE inhibitor and AT_1 antagonist therapy on LV remodeling were substantially attenuated (41), suggesting that an increase in cardiac eNOS activity contributes to the cardioprotective effects of various established pharmacologic interventions. The addition of spironolactone to ACE inhibitor therapy in rats with heart failure after MI particularly increased the bioavailability of endothelium-derived nitric oxide associated with reduced superoxide anion formation (11). The impact of oxidative stress on LV remodeling after MI has been convincingly demonstrated in recent studies (42). As eplerenone reduces superoxide formation (43), a beneficial shift in the balance of nitric oxide and superoxide may have improved cardiac performance and remodeling.

The reduction in plasma norepinephrine may have contributed to more benefit of combination therapy by preventing the adverse cardiovascular effects of excessive sympathetic stimulation. Because in post-MI rats the ANF system (44) is activated in relation to the increase in intracardiac pressures, in the treated groups hemodynamic improvement and prevention of cardiac failure likely accounted for the reduction of atrial natriuretic peptide. Results from the RALES neurohormonal substudy (45) and of Tsutamoto et al. (18) also showed decreased circulating levels of natriuretic peptides in patients with severe CHF after spironolactone treatment.

Clinical implications. Although smaller trials in postinfarction patients and post hoc substudies of the RALES trial reported a reduction in LV dilation and a decrease in myocardial collagen synthesis after spironolactone therapy (17–19), our results provide important new insights into the potential mechanisms underlying cardioprotection by aldosterone antagonism combined with ACE inhibition.

Study limitations. Similar to previous observations in placebo rats with extensive MI, arterial pressure was lowered (42) without an additional hypotensive effect of the pharmacologic intervention. As we did not assess changes in systemic vascular resistance, it remains unclear from this study whether the more favorable effects of combined treatment were achieved in part by afterload reduction. Furthermore, we did not evaluate the LV end-systolic pressure-volume relationships at different loading conditions. Thus, future experiments should elucidate the effects of eplerenone and eplerenone/trandolapril on the inotropic state under acutely changing conditions independent of end-diastolic volume and the systolic pressure. Finally, the employed post hoc Fisher protected least significant difference test is limited to exert a real control for multiple comparisons.

In summary, aldosterone receptor blockade with eplerenone improved LV remodeling in rats with LV dysfunction after extensive MI. Combination therapy with an ACE inhibitor substantially potentiated this effect. Complementary prevention of neurohormonal activation, cardiac fibrosis, and cardiac hypertrophy appear to contribute to these beneficial effects.

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