British Journal of Pharmacology (2005) 146, 723–731
www.nature.com/bjp

Effects of combined inhibition of the Na^+–H^+ exchanger and angiotensin-converting enzyme in rats with congestive heart failure after myocardial infarction

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1 We investigated the single vs the combined long-term inhibition of Na^+–H^+ exchanger-1 (NHE-1) and ACE in rats with congestive heart failure induced by myocardial infarction (MI).

2 Rats with MI were randomized to receive either placebo, cariporide (3000 p.p.m. *via* chow), ramipril (1 mg kg^{-1} day^{-1} *via* drinking water) or their combination for 18 weeks starting on day 3 after surgery.

3 Cardiac morphology and function was assessed by echocardiography and by means of a 2.0 F conductance catheter to determine left ventricular (LV) pressure volume relationships.

4 MI for 18 weeks resulted in an increase in LV end-diastolic diameter (LVDed) in the placebo-treated group when compared to sham (placebo: 1.1 ± 0.04 cm; sham: 0.86 ± 0.01; $P < 0.05$). Combined inhibition of NHE-1 and ACE, but not the monotherapies, significantly reduced LVDed (1.02 ± 0.02 cm).

5 Preload recruitable stroke work (PRSW), dp/dt_{max} (parameter of systolic function) and end-diastolic pressure volume relationship (EDPVR, diastolic function) were significantly impaired in placebo-treated MI group (PRSW: 39 ± 7 mmHg; dp/dt_{max}: 5185 ± 363 mmHg s^{-1}; EDPVR: 0.042 ± 0.001 mmHg μl^{-1}; all $P < 0.05$). Cariporide treatment significantly improved PRSW (64 ± 7 mmHg), dp/dt_{max} (8077 ± 525 mmHg s^{-1}) and EDPVR (0.026 ± 0.014 mmHg μl^{-1}), and reduced cardiac hypertrophy in rats with MI. Combined inhibition of NHE-1 and ACE had even a more pronounced effect on PRSW (72 ± 5 mmHg) and EDPVR (0.026 ± 0.014 mmHg μl^{-1}), as well as cardiac hypertrophy that, however, did not reach statistical significance compared to cariporide treatment alone.

6 The NHE-1 inhibitor cariporide significantly improved LV remodeling and function in rats with congestive heart failure induced by MI. The effect of cariporide was comparable or tended to be stronger (e.g. systolic function) compared to ramipril. Combined treatment with cariporide and ramipril tended to be more effective on LV remodeling in rats with heart failure than the single treatments. Thus, inhibition of the NHE-1 may be a promising novel therapeutic approach for the treatment of congestive heart failure.
British Journal of Pharmacology (2005) **146,** 723–731. doi:10.1038/sj.bjp.0706381;
published online 5 September 2005

Keywords: Myocardial infarction; heart failure; remodeling; ACE inhibition; Na^+–H^+ exchanger-1; pressure–volume analysis

Abbreviations: ACE, angiotensin-converting enzyme; CHF, chronic heart failure; EDPVR, end-diastolic pressure volume relationship; Ees, end-systolic pressure volume relationship; FS, fractional shortening; LV, left ventricle; MI, myocardial infarction; NHE-1, Na^+–H^+ exchanger-1; PRSW, preload recruitable stroke work; tau (τ), time constant of LV pressure isovolumic decay

Introduction

Congestive heart failure (CHF) is a progressive severe clinical syndrome with a high annual morbidity and mortality (Dhir & Nagueh, 2002). The underlying cause of heart failure today is in approximately two-third of cases a coronary artery disease followed by long-standing hypertension (Bolognese & Cerisano, 1986; McKay *et al*., 1986). Acute myocardial infarction (MI) is associated with left ventricular (LV) remodeling, which is a dynamic process characterized by morphological, functional, biochemical and molecular alterations in the myocardium, finally leading to CHF. This process includes an early maladaptive hypertrophy, finally leading to chamber dilation and systolic and diastolic dysfunction (McKay *et al*., 1986; Mann, 2002).

It is well known that LV remodeling is accompanied by activation of the renin–angiotensin–aldosterone system (RAAS) and blocking RAAS with angiotensin-converting enzyme (ACE) inhibitors has been shown to be beneficial in large, long-term clinical outcome trials (Bolger *et al*., 2002; Sleight, 2002). Those beneficial effects of ACE inhibitors are

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related to peripheral vasodilation, ventricular unloading and reduction of cardiomyocyte hypertrophy and interstitial fibrosis (Pfeffer *et al*., 1985; Schieffer *et al*., 1994; Khalil *et al*., 2001). Although ACE inhibitors improve clinical symptoms and reduced morbidity and mortality, heart failure remains the leading cause of death in patients with coronary heart disease. Therefore, novel therapeutic strategies are needed, which further reduce morbidity and mortality in heart failure in addition to current standard treatment.

There is increasing experimental evidence suggesting that inhibition of the Na^+–H^+ exchanger-1 (NHE-1) is beneficial in animal models of heart failure induced by MI (Kusumoto *et al*., 2001; Chen *et al*., 2004; Rungwerth *et al*., 2004). The NHE represents a family of transporters consisting of six family members that regulate intracellular pH by removing protons in exchange for sodium influx (Karmazyn *et al*., 1999). There is a large body of evidence that pharmacological inhibition of NHE-1, which is the primary isoform in cardiomyocytes, prevents myocardial ischemia and reperfusion injury in experimental as well as humans undergoing percutaneous transluminal coronary angioplasty or coronary artery bypass grafting (Linz *et al*., 1998; Rupprecht *et al*., 2000; Winkelmann, 2004). This has largely been attributed to the prevention of intracellular Ca^{2+} overload during ischemia and reperfusion by NHE-1 inhibitors (Karmazyn *et al*., 2001). Interestingly, the neurohormonal factors angiotensin II (ANGII) and endothelin-1 that are elevated in heart failure activate NHE-1 and cause hypertrophy in isolated cardiomyocytes, both of which are prevented by NHE-1 inhibition (Dostal & Baker, 1998). Moreover, the NHE-1 inhibitors cariporide and EMD-87580 reduce right ventricular (RV) and LV hypertrophy in rat models of CHF induced by MI (Kusumoto *et al*., 2001; Chen *et al*., 2004).

The aim of the present study was to investigate the effects of long-term inhibition of NHE-1 with cariporide alone and in combination with ACE inhibition with ramipril on LV morphology and function, LV fibrosis and neurohormonal activation in rats with CHF induced by MI.

Methods

Study design

Left coronary artery ligation for the induction of MI or sham operation was performed in male Sprague–Dawley rats (230–280 g; Møllegaards Breeding Center Ltd, Denmark). Rats were anesthetized with thiopental (100 mg kg^{-1}, intraperitoneal (i.p.)), intubated and mechanically ventilated. Subsequently, the thorax was opened and the heart was exteriorized. After 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 38% within the first 24 h. Sham-operated rats underwent the same surgical procedure, except tying the suture around the coronary artery. Echocardiography (2D, M-Mode) was performed after 2–3 days in order to exclude animals with small infarcts. At day 3 after surgery, animals were randomized to one of the following treatment groups: placebo, the NHE-1 inhibitor cariporide (3000 p.p.m. in chow), the ACE inhibitor ramipril (1 mg kg^{-1} day^{-1} *via* drinking water) or a combination of cariporide and ramipril for 18 weeks. The dose of cariporide and ramipril were selected from previous studies in rats in which these compounds demonstrated efficacy in post-MI models (Kusumoto *et al*., 2001; Seeland *et al*., 2002). All animals were treated in accordance to the guidelines of the National Institutes of Health (Publication No. 85-23, revised 1996).

Echocardiography

Serial echocardiography was performed in rats under light anesthesia induced by ketamine hydrochloride (50 mg kg^{-1}) and xylazine (8 mg kg^{-1}) at day 3, and 6 and 18 weeks after surgery with an HDI 3000 ultrasonograph (Philips, Solingen, Germany) using a dynamic 5–8 MHz convex array transducer. M-mode echocardiograms were captured from parasternal, short-axis view. Left ventricular end-diastolic and end-systolic diameter (LVDed and LVDsys) and wall thickness of the anterior wall and the posterior wall were assessed at the midpapillary level. LV fractional shortening (FS) was calculated as FS = (LVDed − LVDsys)/LVDed. Peak velocity of early and late mitral inflow was assessed by pulsed-wave Doppler. All echocardiographic data were analyzed online and recorded on paper at 100 mm s^{-1} and on a commercially available analysis system (SonoWin®-2000, MESO).

Hemodynamic and LV pressure–volume measurements

For the assessment of pressure–volume relationships, rats were anesthetized with thiopental (100 mg kg^{-1}, i.p.), intubated and artificially ventilated. The LV was catheterized retrogradely *via* the right carotid artery using a 2.0 F impedance-micromanometer catheter (Millar Instruments, Houston, TX, U.S.A.). In brief, the method is based on measuring the time-varying electrical conductance signal of two segments of blood in the LV, from which total volume is calculated. Raw conductance volumes were corrected for parallel conductance by the hypertonic saline dilution method. For absolute volume measurements, the catheter was calibrated with known volumes of heparin-treated rat blood. Pressure–volume signals were recorded at steady state and during transient preload reduction achieved by vena cava occlusion. Data were digitized with a sampling rate of 1000 Hz and recorded on a PC using specialized software (HEM, Notocord, Croissy, France). For subsequent analysis of pressure–volume loops, preload recruitable stroke work (PRSW), end-systolic pressure volume relationship (Ees) and end-diastolic pressure volume relationship (EDPVR), PVAN software (Millar Instruments Inc., Houston, TX, U.S.A.) was used.

After hemodynamic measurements were recorded, a blood sample was taken for determination of proatrial natriuretic peptide (pro-ANP) and determination of plasma concentrations of cariporide. Subsequently, the heart was stopped end-diastolic by injecting a saturated potassium chloride solution.

Heart and lung weight, infarct-size and histological analysis

Hearts and lungs were removed and weighed. Subsequently, LV (including septum) and RV were separated and also weighed. In order to confirm an equal distribution of MI sizes among the infarcted groups, infarct-size was determined by planimetric measurement. The infarct area was stated as percentage of the whole LV. All rats with an infarct $<25\%$

were excluded from the study. Subsequently, LV was routinely fixed in 4% unbuffered formalin and then prepared according to standard methods. Serial sections were stained with hematoxylin and eosin (H&E) and elastica van Gieson. Fibrosis in the remote noninfarcted myocardium was quantified using a computerized morphometric system (LeicaQWin, Leica Imaging Systems, Germany) and expressed as percentage of the left myocardium. Myocyte cross-sectional area was measured from sections stained with H&E, and suitable cross-sections were defined as having nearly circular capillary profiles and nuclei. In all, 50 myocytes from either sham-operated or from rats with MI that were treated with placebo, cariporide, ramipril or their combination were analyzed.

Neurohormonal assay

The plasma concentrations of pro-ANP were determined by radioimmunoassay (Immundiagnostik, Bensheim, Germany). The plasma concentrations of cariporide were measured in samples that were collected between 09:00 and 15:00 hours by LC-MS/MS.

Statistical analysis

Values are given as mean ± s.e.m. Statistically significance in mean values were tested by two-factor analysis of variance (ANOVA), and differences between groups were assessed by one-factor ANOVA followed by Dunnett's test if appropriate. A value of $P<0.05$ was considered statistically significant.

Materials

Cariporide (HOE 642) was synthesized by Aventis Pharma as described previously (Weichert *et al*., 1997).

Results

Global parameters

MI for 18 weeks resulted in a survival rate of 64% in the placebo-treated MI group and tended to be higher in the MI groups treated with cariporide (69%), ramipril (69%) or their combination (73%). A total of 10 animals (two placebo, three cariporide, three ramipril and two in the combination group) were excluded from further analysis due to an infarct-size <25%. Infarct size of the remaining animals and body weights were similar among all groups (Table 1). LV weight, LV weight/body weight ratio and particularly RV weight and RV weight/body weight ratio as well as lung weight was significantly increased in the placebo-treated MI group compared to sham-operated control rats. Treatment of MI rats with cariporide or ramipril significantly reduced RV and RV weight/body weight as well as lung weight and the combination therapy with cariporide and ramipril prevented both RV and LV hypertrophy compared to MI rats on placebo (Table 1). The effect on cardiac hypertrophy and congestion tended to be stronger by cariporide compared to ramipril treatment. MI for 18 weeks led to a progressive LV dilation in the placebo-treated MI group compared to sham-operated rats as indicated by an increase in LV end-diastolic and end-systolic dimension (LVDed, LVDsys) (Figure 1). Monotherapy with either cariporide or ramipril tended to decrease LV dilation, whereas combined treatment significantly reduced LV dilation.

Hemodynamics and LV remodeling

Cardiac function was assessed *in vivo* by using a miniaturized 2.0 F conductance catheter that simultaneously measures pressure and volume. LV systolic pressure was reduced in

Table 1 Echocardiographic and morphometric parameters in rats 18 weeks after sham surgery or myocardial infarction treated either with placebo, cariporide, ramipril or the combination of cariporide and ramipril

Parameter	*Sham* (n = *14*)	*Myocardial infarction*			
		Placebo (n = *11*)	*Cariporide* (n = *9*)	*Ramipril* (n = *9*)	*Carip/ram* (n = *11*)
Morphometry					
Infarct size (%)	—	31 ± 2	32 ± 2	32 ± 3	34 ± 1
BW (g)	566 ± 10	582 ± 17	560 ± 14	590 ± 10	534 ± 18
LVW (mg)	1.045 ± 26	1.189 ± 36*	1.099 ± 31	1.145 ± 29	988 ± 22†
LVW/BW ratio	1.85 ± 0.04	2.04 ± 0.04*	1.96 ± 0.05	1.94 ± 0.05	1.85 ± 0.05†
RVW (mg)	246 ± 10	386 ± 14*	289 ± 15†	311 ± 23†	234 ± 18†§
RVW/BW ratio	0.47 ± 0.02	0.63 ± 0.03*	0.52 ± 0.02†	0.53 ± 0.03†	0.44 ± 0.02†§‡
LW, g 100 g^{-1} BW	0.42 ± 0.01	0.91 ± 0.11*	0.62 ± 0.08†	0.67 ± 0.09†	0.56 ± 0.07†
Myocyte CSA (μm^2)	276 ± 17	372 ± 21*	298 ± 15†	307 ± 22†	281 ± 11†
Echocardiography					
AWThed (mm)	0.17 ± 0.01	0.09 ± 0.01*	0.08 ± 0.01*	0.09 ± 0.01*	0.1 ± 0.01*
PWThed (mm)	0.17 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
FS (%)	36 ± 2	15 ± 1*	19 ± 2*†	20 ± 1*†	22 ± 1*†
Peak E vel (cm s^{-1})	78 ± 3	86 ± 5	81 ± 4	82 ± 4	76 ± 2
Peak A vel (cm s^{-1})	39 ± 2	23 ± 2*	29 ± 4†*	33 ± 5	38 ± 3†§
E/A ratio	2.0 ± 0.2	3.6 ± 0.6*	2.8 ± 0.4*	2.5 ± 0.4†	2.0 ± 0.2†§‡

Echocardiographic and morphometric data in sham-operated rats (sham) and rats with congestive heart failure 18 weeks after myocardial infarction: effects of placebo, NHE-1 inhibition (cariporide), ACE inhibition (ramipril), or combined NHE-1 and ACE inhibition (Carip/ram). BW = body weight; LVW = left ventricular weight; RVW = right ventricular weight; LW = lung weight; CSA = cross-sectional area; AWThed = anterior wall thickness end-diastolic; PWThed = posterior wall thickness end-diastolic; FS = fractional shortening; peak E vel = early velocity of mitral inflow; peak A vel = active/atrial velocity of mitral inflow. Values are mean ± s.e.m. *$P<0.05$ vs sham, $^{\dagger}P<0.05$ vs placebo, $^{\S}P<0.05$ vs ramipril, $^{\ddagger}P<0.05$ vs cariporide.

the placebo- and ramipril-treated MI groups compared to sham-operated control rats. In contrast, cariporide as well as the combined treatment attenuated the fall in LV systolic pressure (Table 2).

MI significantly impaired systolic function as indicated by a significant reduction in dP/dt_{max}, cardiac output index (COI), ejection fraction (EF), stroke work (SW), FS and particularly in the load-independent parameter PRSW and Ees (Tables 1 and 2; Figure 2). In addition, there was a rightward shift of the LV P–V loops in the MI/control group as well as a markedly decreased slope of the end-systolic pressure volume relationship (ESPVR) in the MI/control group compared to sham-operated animals (Figure 3). Treatment of MI rats with ramipril significantly increased FS, as assessed by echocardiography, EF and SW and tended to enhance dP/dt_{max}, COI and PRSW, whereas cariporide significantly improved FS, EF, SW, dP/dt_{max}, COI as well as Ees and PRSW. The combined treatment of MI rats with cariporide and ramipril tended to further improve systolic function as indicated by a further increase in FS, COI, PRSW and Ees (Tables 1 and 2; Figures 2 and 3). In all treatment groups, the rightward shift of the LV P–V loops was partially reversed and the slope of ESPVR was less depressed compared to MI/control (Figure 3).

In the placebo-treated MI group, dP/dt_{min} was significantly reduced, and LVEDP, the time constant of LV pressure isovolumic decay (tau, τ) and the load independent EDPVR were significantly increased when compared to sham-operated control rats, indicating impaired diastolic function (Table 2; Figures 2 and 4). In addition, LV end-diastolic volume (EDV) and the E/A ratio were significantly increased in

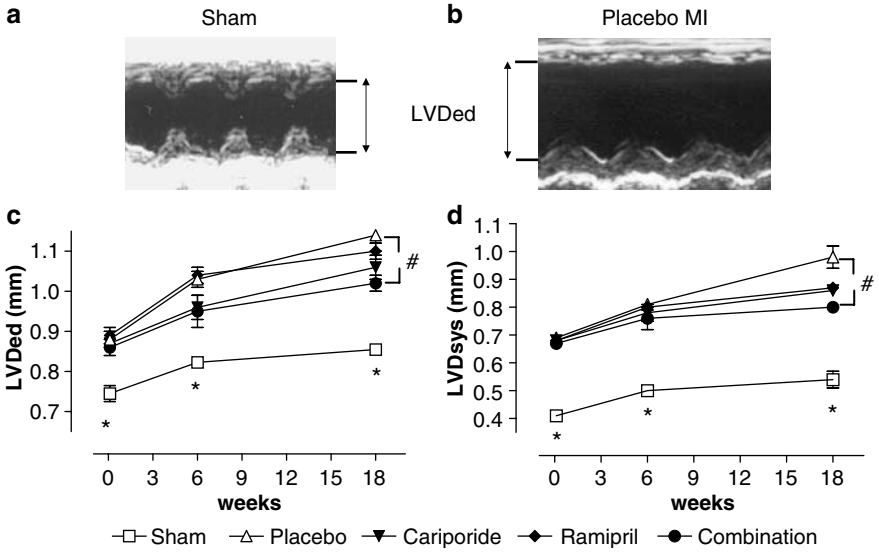

Figure 1 Representative M-mode echocardiograms obtained from sham-operated (a) and placebo-treated rats with myocardial infarction (b) at 18 weeks after surgery. The progress of cardiac enlargement shown as LV end-diastolic diameter (LVDed) (c) and LV end-systolic diameter (LVDsys) (d) of sham- ($n=14$), MI/placebo- ($n=11$), MI/ramipril- ($n=9$), MI/cariporide- ($n=9$) and MI/ramipril/cariporide- ($n=11$) treated rats at day 3, and 6 and 18 weeks after surgery. Values are expressed as mean ± s.e.m. $^*P<0.05$ vs sham. $\#P<0.05$ vs MI/placebo.

Table 2 Hemodynamic parameters in rats 18 weeks after sham surgery or myocardial infarction treated either with placebo, cariporide, ramipril or the combination of cariporide and ramipril

Parameter	*Sham operation* (n = *14*)	*Myocardial infarction*			
		Placebo (n = *11*)	*Cariporide* (n = *9*)	*Ramipril* (n = *9*)	*Carip/ram* (n = *11*)
Heart rate (bpm)	366±8	355±5	377±12	344±7	374±13
LVSP (mmHg)	156±5	113±8*	143±7†	123±9*	140±4†
LVEDP (mmHg)	5±1	18±2*	12±2†	9±1$^{\dagger q}$	11±1†
LVESV (μl)	154±16	308±10*	226±19†	200±24†	213±16†
MAP (mmHg)	140±8	103±9*	126±7†	106±9*	121±6†
dp/dt_{max} (mmHg s^{-1})	8618±435	5185±363*	8077±525†§	6332±527†	7395±446†
EF (%)	56±2	28±3*	41±3†	46±4†	40±2†
SW (mmHg μl^{-1})	18,156±1099	6925±1033*	15,231±1565†	14,028±1864†	12,577±1070†
Ees (mmHg μl^{-1})	0.64±0.05	0.35±0.06*	0.40±0.06§	0.30±0.05	0.47±0.08†§
dp/dt_{min} (mmHg s^{-1})	−7686±263	−4199±357*	−4734±398	−5030±195†	−4745±331
EDPVR (mmHg μl^{-1})	0.0144±0.004	0.042±0.001*	0.026±0.014*†	0.0098±0.004†‡	0.012±0.003†‡

Hemodynamic parameter in sham-operated rats (sham) and rats with congestive heart failure 18 weeks after myocardial infarction: effects of placebo, NHE-1 inhibition (cariporide), ACE inhibition (ramipril), or combined NHE-1 and ACE inhibition (Carip/ram). LVESP = left ventricular systolic pressure; LVEDP = LV end-diastolic pressure; LVESV = LV end-systolic volume; MAP = mean arterial pressure; EF = ejection fraction; dp/dt_{max} = maximal rate of pressure development; dp/dt_{min}; maximal rate of decay of pressure; SW = stroke work; Ees = end-systolic pressure volume relationship; EDPVR = end-diastolic pressure volume relationship. Values are mean ± s.e.m. $^*P<0.05$ vs sham, $^{\dagger}P<0.05$ vs placebo, $^{\S}P<0.05$ vs ramipril, $^{\ddagger}P<0.05$ vs cariporide.

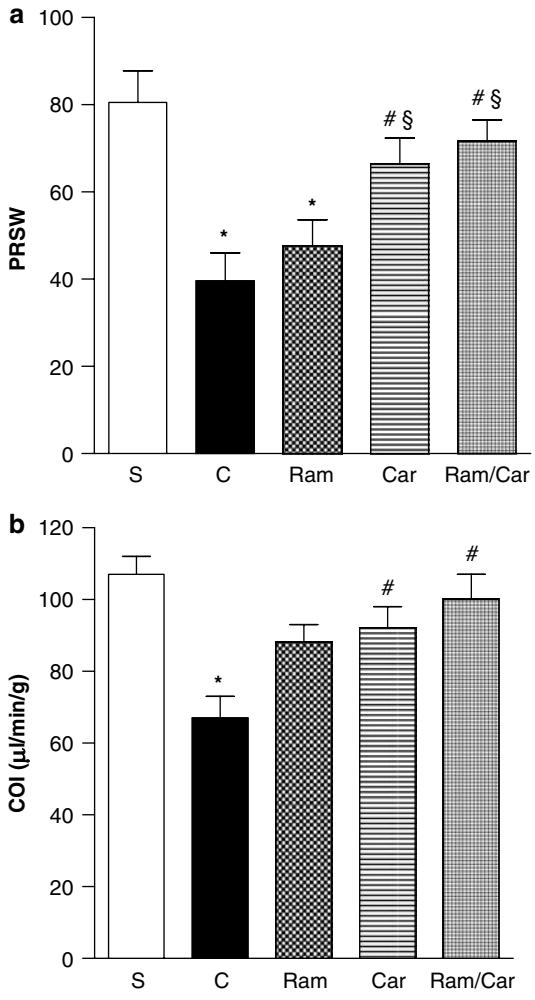

Figure 2 (a) Preload recruitable stroke work (PRSW) and (b) cardiac output index (COI) in sham-operated (S) and rats with myocardial infarction that were treated with placebo (C), ramipril (Ram), cariporide (Car) or the combination of ramipril and cariporide (Ram/Car) for 18 weeks. Values are mean $\pm$ s.e.m. $N=9$–14 per group. $^{*}P<0.05$ vs sham, $^{\#}P<0.05$ vs C, $^{\S}P<0.05$ vs ramipril.

placebo-treated MI rats when compared to sham rats (Table 1; Figures 3 and 4). Treatment of MI rats with ramipril significantly reduced the increase in E/A ratio, LVEDP and EDPVR and increased dP/dt_{min}, whereas cariporide only significantly reduced LVEDP and EDPVR. Combination therapy with ramipril and cariporide did not only improve E/A ratio, LVEDP, EDPVR, dP/dt_{min} but also reduced the increase in EDV and τ (Table 1; Figures 3 and 4). Heart rate was similar among all experimental groups (Tables 1 and 2).

Neurohormonal activation, cardiac myocyte hypertrophy and fibrosis

MI for 18 weeks was characterized by a marked increase in cardiac myocyte cross-sectional area and plasma pro-ANP that was significantly reduced by monotherapy with ramipril or cariporide and normalized by combined ACE and NHE-1 inhibition (Table 1; Figure 5). In addition, CHF was also accompanied by substantial LV fibrosis in the remote noninfarcted myocardium when compared to sham-operated hearts (Figure 6). Treatment of rats with either ramipril or cariporide alone reduced the degree of LV fibrosis that was further reduced by the combined treatment with ramipril and cariporide (Figure 6).

Supplementation of chow with cariporide (3000 p.p.m.) resulted in an efficient plasma concentration of $1.89\pm0.3\,\mu g\,ml^{-1}$ that has been shown to completely block NHE-1 activity *in vivo* (Rungwerth *et al*., 2004).

Discussion

CHF is a severe cardiovascular disease with increasing incidence and prevalence. Despite recent advances in heart failure therapy, mortality remains high (Tendera & Ochala, 2001). Therefore, new therapeutic approaches are needed to decrease morbidity and mortality in heart failure patients. Importantly, novel therapeutic modalities have to be effective in addition to current therapy. In the present study, we investigated the long-term effect of the NHE-1 inhibitor cariporide alone and, particularly from the clinical point of view, in combination with the ACE inhibitor ramipril in rats with CHF induced by MI. We observed that cariporide attenuated LV dilation and improved LV function without decreasing blood pressure. In addition, cariporide inhibited cardiac hypertrophy and the activation of the neurohormonal system as well as the fibrosis in the remote noninfarcted myocardium of the LV. The effect of cariporide on LV morphology was comparable or tended to be stronger to the effect observed with the ACE inhibitor ramipril. Cariporide improved the following hemodynamic parameters: PRSW, Ees, LVSP, dP/dt_{max}, MAP, EF, CO, LVEDP and EDPVR in rats with CHF. Most importantly, combined inhibition of NHE-1 and ACE in rats with CHF had even a greater beneficial effect on LV remodeling and function than the single approaches alone.

CHF in the rats induced by MI is accompanied by LV dilation and myocardial dysfunction (Pfeffer *et al*., 1979; Kusumoto *et al*., 2001). Several studies in rats with heart failure induced by MI have demonstrated that monotherapy with either ACE or NHE-1 inhibitors provided beneficial effects on LV remodeling and function (Schieffer *et al*., 1994; Litwin *et al*., 1996; Kusumoto *et al*., 2001; Seeland *et al*., 2002; Chen *et al*., 2004; Rungwerth *et al*., 2004). Noteworthy, the long-term effect of the ACE inhibitors captopril and trandolapril on LV dilation and function in rats with extensive MI was only minor (Litwin *et al*., 1996; Fraccarollo *et al*., 2003). This is in line with our results showing that the ACE inhibitor ramipril also has only minor effects on LV dilation and systolic function but significantly improved diastolic function. In contrast, NHE-1 inhibition with cariporide significantly improved systolic function in rats with CHF, whereas it also had only a minor effect on LV dilation and LV relaxation (e.g. dp/dt_{min}). We demonstrate for the first time that combined inhibition of ACE by ramipril and NHE-1 by cariporide significantly improved LV systolic and diastolic function and attenuated LV dilation in rats with CHF induced by chronic MI. In addition, cariporide and to a lesser extent ramipril reduced LV and, particularly, RV hypertrophy in these animals, whereas combination therapy prevented LV and RV hypertrophy. However, combined treatment tended to be more

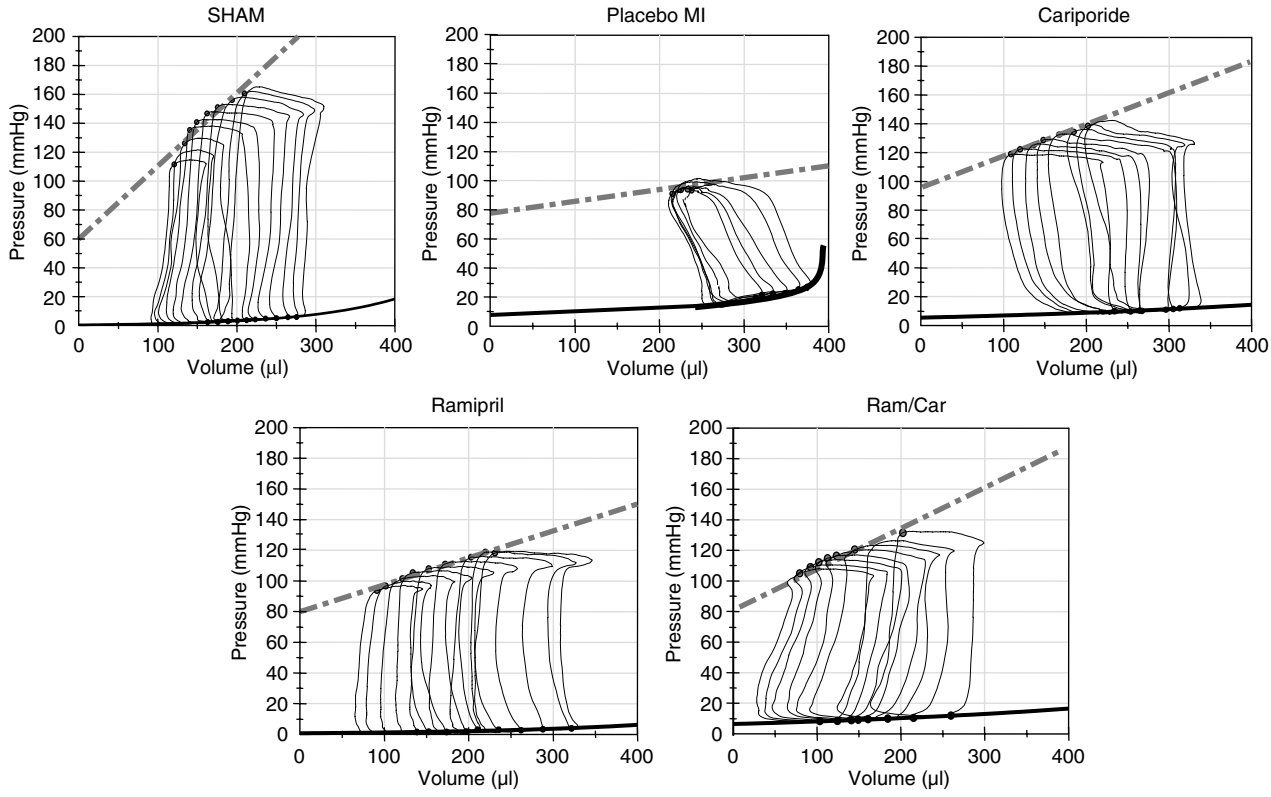

Figure 3 Representative pressure–volume loops during transient reduction of cardiac preload in sham-operated (sham) and rats with myocardial infarction that were treated with placebo (placebo MI), ramipril, cariporide or the combination of ramipril and cariporide (Ram/Car) for 18 weeks.

effective than either with cariporide or ramipril alone but did not reach statistical significance.

Our finding that cariporide has beneficial effects on LV remodeling and function in rats with CHF is in line with previous findings demonstrating that cariporide reduces hypertrophy of cardiomyocytes in spontaneously hypertensive rats (SHR) (Camilion de Hurtado *et al*., 2002), rats and rabbits with heart failure induced by MI (Kusumoto *et al*., 2001; Rungwerth *et al*., 2004) as well as in β1-adrenergic receptor transgenic mice (Engelhardt *et al*., 2002). Importantly, the effect of NHE-1 inhibition by cariporide on cardiac remodeling is, in contrast to ACE inhibition, blood pressure- and, hence, afterload-independent (Kusumoto *et al*., 2001; Rungwerth *et al*., 2004). This is further supported by our finding that cariporide significantly improved PRSW, a modification of the Frank–Starling mechanism that is more or less not influenced by ventricular geometry, preload-independent and afterload-insensitive over the physiological range, in rats with CHF as assessed by means of a conductance catheter. The precise mechanism explaining the involvement of NHE-1 in cardiac hypertrophy or heart failure is still unclear. An increased NHE-1 expression and activity has been demonstrated in myocardial samples from rats with ischemia-induced heart failure (Loennechen *et al*., 2002), in SHR (Camilion de Hurtado *et al*., 2002), in β1-adrenergic receptor transgenic mice with heart failure (Engelhardt *et al*., 2002) and, most importantly, in patients with heart failure (Yokoyama *et al*., 2000). In addition, treatment of isolated rat cardiac myocytes with hypertrophic agonists such as α-adrenoceptor agonists (Yokoyama *et al*., 1998), endothelin-1 (Kramer *et al*., 1991), ANGII (Matsui *et al*., 1995) or thrombin (Yasutake *et al*., 1996) results in an increase in NHE-1 activity. Interestingly, ANGII-induced cardiomyocyte hypertrophy is prevented by NHE-1 inhibition (Dostal & Baker, 1998). Therefore, it is conceivable to assume that in our study both treatments (cariporide and ramipril) caused beneficial effects due to reduction in NHE-1 activity. This would also explain why combined inhibition of NHE-1 and ACE only tended to be more effective compared to either cariporide or ramipril treatment alone.

It has been proposed that the NHE-1-dependent sodium influx is a major contributor to the hypertrophic response by hypertrophic stimuli and involves sodium-induced activation of PKC (Hayasaki-Kajiwara *et al*., 1999). In addition, other cell signaling mechanisms such as Raf-1 and MAP kinase may also be involved in the NHE-1-mediated induction of cardiomyocyte hypertrophy (Yamazaki *et al*., 1998). Beside sodium, calcium handling is also altered in heart failure as evident by increased diastolic and decreased systolic cytoplasmatic calcium and sarcoplasmatic reticulum (SR) calcium content that is related to increased expression and activity of the sodium–calcium exchanger. In a rabbit model of volume and pressure overload-induced heart failure, cariporide has been shown not only to prevent heart failure and LV hypertrophy but also to partly normalize intracellular sodium, end-diastolic intracellular calcium, and impaired calcium handling of the SR and reduced the incidence of calcium after-transient. In addition, the negative force–frequency relation of the failing hearts and/or myocytes was almost entirely reversed to a positive relationship as observed in

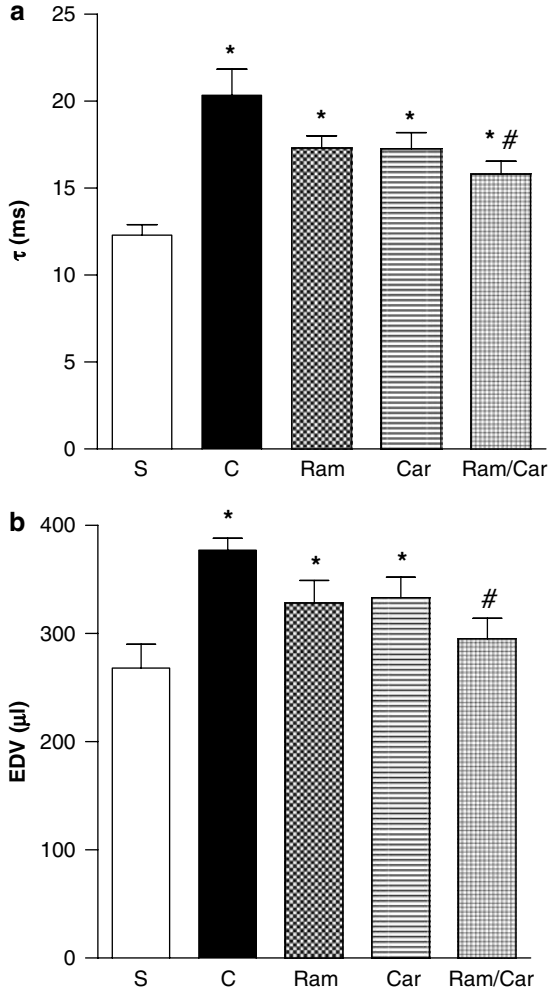

Figure 4 (a) The time constant of LV pressure isovolumic decay (τ) and (b) end-diastolic volume (EDV) in sham-operated (S) and rats with myocardial infarction that were treated with placebo (C), ramipril (Ram), cariporide (Car) or the combination of ramipril and cariporide (Ram/Car) for 18 weeks. Values are mean$\pm$s.e.m. $N=9$–14 per group. $^{*}P<0.05$ vs sham, $^{\#}P<0.05$ vs C.

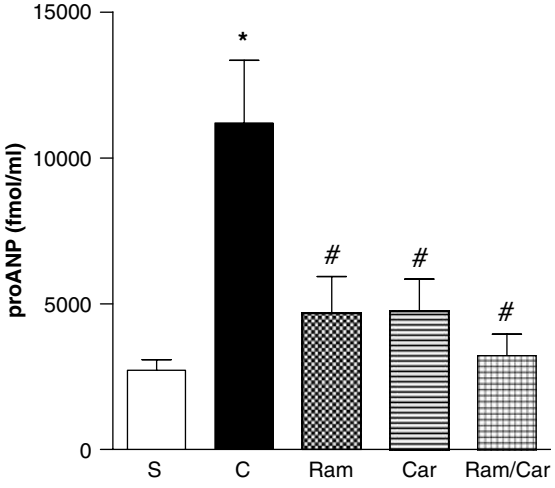

Figure 5 Plasma concentrations of proatrial natriuretic peptide (pro-ANP) in sham-operated (S) and rats with myocardial infarction that were treated with placebo (C), ramipril (Ram), cariporide (Car) or the combination of ramipril and cariporide (Ram/Car) for 18 weeks. Values are mean$\pm$s.e.m. $N=9$–14 per group. $^{*}P<0.05$ vs sham, $^{\#}P<0.05$ vs C.

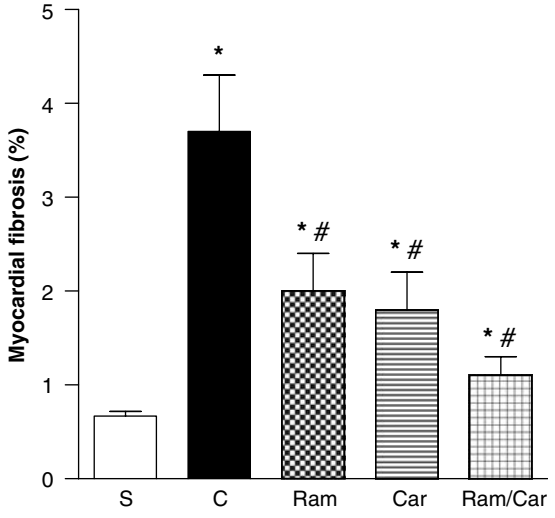

Figure 6 Fibrosis in the remote noninfarcted myocardium in sham-operated (S) and rats with myocardial infarction that were treated with placebo (C), ramipril (Ram), cariporide (Car) or the combination of ramipril and cariporide (Ram/Car) for 18 weeks. Values are mean$\pm$s.e.m. $N=9$–14 per group. $^{*}P<0.05$ vs sham, $^{\#}P<0.05$ vs C.

healthy myocardium (Baartscheer *et al*., 2003a, b; 2005). These data suggest that the beneficial effects of cariporide in animal models of heart failure (incl. the present study) may be mediated at least in part by inhibition of elevated intracellular sodium due to increased activity of the NHE-1. Noteworthy, the beneficial effects of cariporide in heart failure are most likely mediated by different mechanisms (as discussed above) than its beneficial effects on myocardial ischemia–reperfusion injury that are well documented in animals as well as now in humans (Linz *et al*., 1998; Rupprecht *et al*., 2000; Winkelmann, 2004). Indeed, in the present model of heart failure induced by sustained coronary occlusion without reperfusion, infarct-size was not affected by cariporide but LV remodeling was improved.

LV remodeling after MI is characterized by interstitial and perivascular deposition of fibrillar collagen leading to cardiac muscle stiffness and LV dysfunction and, hence, to progressive cardiac dysfunction and heart failure (Caulfield & Borg, 1979). Fibrosis in the remote noninfarcted myocardium is a major determinant of ventricular remodeling in ischemic cardiomyopathy (Beltrami *et al*., 1994). In line with previous reports, we confirmed that both ACE inhibition by ramipril or NHE-1 inhibition by cariporide reduces reactive fibrosis of the LV after MI (Seeland *et al*., 2002; Rungwerth *et al*., 2004). Importantly, the combined inhibition of ACE and NHE-1 reduced LV fibrosis even more pronounced than the single treatments which, at least in part, may contribute to a more marked improvement of LV remodeling and function. The signaling pathways leading to myocardial fibrosis are very complex and not yet fully understood. Several growth factors including TGFβ and components of the RAAS are potent activators of collagen synthesis in (myo)fibroblasts (Weber, 1997; Fraccarollo *et al*., 2003). Indeed, inhibition of all components of the RAAS by ACE inhibitors (Seeland *et al*.,

2002), ANGII AT_1 receptor blockers (Xia *et al*., 1999) or aldosterone antagonists (Fraccarollo *et al*., 2003) reduce myocardial fibrosis in animal models of heart failure. There is evidence that the beneficial effects of ACE inhibitors on extracellular matrix remodeling is due to the inhibition of collagen synthesis as well as by influencing matrix metalloproteinases (MMP), particularly MMP-2 (Seeland *et al*., 2002). In contrast, the mechanism by which cariporide inhibits interstitial fibrosis has not yet been elucidated. It could be speculated that cariporide reduced interstitial fibrosis by inhibiting cardiomyocyte death and, hence, replacement fibrosis. In addition, cariporide may also have direct effects on cardiac fibroblast. Indeed, the expression of NHE-1 is activated by mitogenic stimuli such as insulin, thrombin and epidermal growth factor in cardiac fibroblasts (Besson *et al*., 1998). The pronounced inhibition on interstitial fibrosis in rats with CHF by combined inhibition of ACE and NHE-1 suggest a synergistic effect by cariporide and ramipril and, hence, independent effects on myocardial fibrosis.

In conclusion, the Na^+/H^+ exchange inhibitior cariporide improved LV remodeling and particularly systolic LV function in rats with CHF induced by MI. Combination of cariporide with the ACE inhibitor ramipril tended to further improve LV remodeling and enhanced both systolic and diastolic LV function. The beneficial effect of NHE-1 inhibition in addition to ACE inhibition may have important clinical implications for patients with heart failure, particularly, as the effect of NHE-1 inhibition is, in contrast to current treatment options with ACE inhibitors, β receptor blockers, vasodilators, aldosterone antagonists and angiotensin AT1 receptor blockers, blood pressure independent. Thus, inhibition of the NHE-1 may be a promising novel therapeutic approach for the treatment of CHF.

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(Received June 13, 2005
Revised July 26, 2005
Accepted August 4, 2005
Published online 5 September 2005)