Preservation of Cardiac Function and Energy Reserve by the Angiotensin-Converting Enzyme Inhibitor Quinapril During Postmyocardial Infarction Remodeling in the Rat

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ABSTRACT

Purpose: *Angiotensin-converting enzyme (ACE) inhibitors show beneficial long-term hemodynamic effects in chronically infarcted hearts. The purpose of this study was to test whether prevention of the deterioration of mechanical function by ACE inhibitors is related to beneficial effects on high-energy phosphate metabolism that is deranged in heart failure.* ***Methods:*** *Twelve-week old rats were randomly assigned to ligation of the left coronary artery [mycardial infarction (MI)] or sham operation (Sham) and to the ACE inhibitor quinapril (+Q) (6 mg/kg/day per gavage) or placebo treatment. Eight weeks later, cardiac function was measured in the isolated heart by a left ventricular balloon (pressure-volume curves), and energy metabolism of residual intact myocardium was analyzed in terms of total and isoenzyme creatine kinase activity (spectrophotometry), steady-state levels [adenosine triptosphate (ATP), phosphocreatine], and turnover rates (creatine kinase reaction velocity) of high-energy phosphates [^{31}P nuclear magnetic resonance (NMR)] and total creatine content [high-performance liquid chromatography (HPLC)].* ***Results:*** *Quinapril prevented post-MI hypertrophy and partially prevented left ventricular contractile dysfunction [maximum left ventricular developed pressure 166 ± 6, 83 ± 16 (p < 0.05 MI vs. Sham), 139 ± 13 mm Hg (p < 0.05 quinapril treated vs. untreated) in Sham, MI and MI+Q hearts]. Residual intact failing myocardium showed a 17% decrease of MM-CK and a 16% decrease of mito-CK activity. Total*

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creatine was reduced by 23%, phosphocreatine by 26% and CK reaction velocity by 30%. Parallel to improved function, treatment with quinapril largely prevented the impairment of energy metabolism occuring post-MI. **Conclusions:** *quinapril treatment results in an improvement of high-energy phosphate metabolism, of energy reserve via the creatine kinase reaction, and of contractile performance post-MI.*

Key Words: *ACE inhibitors; Creatine kinase; Remodeling; Myocardial infarction; Energy metabolism*

INTRODUCTION

The prognosis of patients with heart failure remains poor, the most common cause of heart failure being post-myocardial infarction (MI) remodeling. Angiotensin-converting enzyme (ACE) inhibitors are widely used to treat patients with post-MI left ventricular dysfunction (1,2). Clinical trials have shown that preventive treatment of heart failure patients with ACE inhibitors is cardioprotective and reduces morbidity and mortality (1). Hemodynamic improvements include decreased afterload, increased coronary perfusion, and reduced left ventricular (LV) dilatation (2,3).

These clinical observations were predicted by animal studies in rats with experimental MI, where the ACE inhibitor captopril attenuated progressive ventricular dilatation (4) and prevented LV and right ventricular (RV) hypertrophy occurring in residual intact myocardium post-MI (5–8). The precise mechanisms by which ACE inhibition influences the remodeling process has not yet been elucidated (7), but it has been attributed predominantly to their hemodynamic effects. However, the biochemical and molecular mechanisms whereby ACE inhibitors exert their beneficial action post-MI are just beginning to unfold. In a pilot study (9), we showed earlier that the ACE inhibitor quinapril preserved the phosphocreatine-to-ATP ratio post-MI.

In the present work, we studied the effects of quinapril on LV dysfunction and dilatation post-MI by obtaining pressure–volume curves and fully analyzing the metabolism of high-energy phosphates using ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectroscopy, MR saturation transfer, high-pressure liquid chromatography, and enzyme analysis.

METHODS

Animals, Myocardial Infarction, and Determination of Infarct Size

Infarcts or sham operations were performed in 12-week-old Wistar rats. Left coronary artery ligation was induced by a previously described technique (9–11). The left coronary artery was ligated after left thoracotomy under ether anesthesia. Sham operation was performed using an identical procedure except that the suture was passed under the coronary artery without ligation. Mortality rate of infarcted rats for the first 24 h after the operation was 40–50%. All procedures conformed to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1985).

After experiments were finished, left ventricles were embedded in paraffin, and 20-μm sections were cut serially from apex to base of the heart. Sections were stained for collagen using Picrosirius Red staining. Slices were digitized by using the NIH Image 1.59/ppc scanner software (National Institutes of Health, Bethesda, USA) and lengths of scar and noninfarcted muscle for both endocardial and epicardial surfaces were determined for each section. Final infarct size was determined as the average of endo- and epicardial surfaces and is given as a percentage. All hearts with an infarct size $<30\%$ were excluded from the analysis (untreated $n = 12$, and quinapril $n = 11$).

Quinapril Treatment and Experimental Groups

Before surgery rats were randomly assigned to one of the four groups: untreated sham ($n = 11$), untreated MI ($n = 7$), quinapril-treated sham ($n = 9$) or quinapril-treated MI ($n = 7$). Rats received 6 mg/kg/day of the ACE inhibitor quinapril orally (p.o.), initiated after recovery from surgery. Quinapril was administered by gavage daily at the same time of day. Quinapril treatment was discontinued 24 h before the hearts were isolated. A dosage of 6 mg/kg/day was chosen because in previous experiments this dose decreased mean aortic blood pressure in vivo by 10% (data not shown) when therapy was initiated early (30 min after occlusion).

Isolated Rat Heart Preparation

Eight weeks after left coronary artery ligation or sham operation, rats were anaesthetized by injecting 50 mg pentobarbital sodium intraperitoneally. Hearts were iso-

lated in a previously described technique (9,12). Retrograde perfusion of the heart was begun in the Langendorff mode at a constant temperature of 37°C and a constant coronary perfusion pressure of 100 mm Hg. For control perfusion, phosphate-free Krebs-Henseleit buffer was used as previously described (13). Coronary flow was continuously measured by an ultrasonic flow probe (Transonic Systems Inc. Ithaca, NY) built into the perfusate inflow tubing.

Cardiac Performance Measurements

All cardiac performance measurements were made using standard procedures. A water-filled latex balloon was inserted into the left ventricle through an incision in the left atrial appendage via the mitral valve, and secured by a ligature. The balloon was connected to a Statham P23Db pressure transducer (Gould Instruments, Glen Burnie, MD) via a small-bore stainless-steel tube for continuous measurement of pressure and heart rate (HR) on a four-channel recorder (Graphtec Corp., Tokyo, Japan) (9,12,13). Frank-Starling curves were obtained by increasing the volume of the intraventricular balloon by 0.05 mL increments to raise the end-diastolic pressure and to allow left ventricular developed pressure to reached a maximum (peak developed pressure).

^{31}P-NMR Spectroscopy

The perfused hearts were placed into a 20-mm NMR sample tube and inserted into a probe seated in the bore of a superconducting super-wide-bore (150 mm) 7.05-Tesla magnet (Bruker, Rheinstetten, FRG), as previously described (14). Hearts were bathed in their own perfusate. An Aspect 3000 computer (Bruker, Rheinstetten, FRG) was used in the pulsed Fourier transform mode to generate ^{31}P-NMR spectra at 121.50 MHz. A 14-channel Shim unit served to homogenize the magnetic field. Single (''one pulse'') spectra were accumulated over 5-min periods, averaging data from 152 free-induction decays obtained using a pulse time of 37.6 μs, a pulse angle of 45°, and an interpulse delay of 1.93 s. The resonance areas corresponding to ATP, phosphocreatine, and inorganic phosphate (P_i), which are proportional to the number of phosphorus atoms of the respective compound, were measured by integration using the NMR1 software (TRIPOS, Munich, Germany) and were corrected for partial saturation. In each heart, the area of the [γ-P]ATP resonance of the first spectrum obtained under control conditions was arbitrarily set to 100% and used as the

reference value for all resonances in the sequence of ^{31}P NMR spectra obtained for the protocol. Absolute ATP concentrations were previously determined for sham hearts as 10.8 ± 0.8 mmol/L, for residual intact LV tissue of MI hearts as 10.6 ± 0.8 mmol/L by HPLC (14). Since the protocol (histologic determination of LV infarct size, cutoff of the right ventricle for creatine analysis within 20 s) did not allow absolute ATP quantification by HPLC, ATP concentrations for sham and MI hearts were assumed to be the same as for the previous study (14). In addition, it was assumed that quinapril does not affect normal ATP content, which is most likely the case: ATP cannot increase above normal levels, since mitochondrial ATP production is subject to close feedback inhibition by ATP. ATP could not be determined by HPLC in this study, since left ventricles were fixed in formalin for infarct size measurements and right ventricles were cut off and frozen, allowing determination of total creatine (Cr) and creatine (CK) kinase activity. ATP levels were not determined in right ventricles because we cut off the right ventricle from the beating heart and froze it after several seconds because freeze-clamping the beating heart was impossible when we wanted to determine infarct size. In our experience, ATP levels cannot reliably be determined when tissue is not directly freeze-clamped. Intracellular pH (pH_i) was measured by comparing the chemical shift between inorganic phosphate and phosphocreatine with values obtained from a standard curve (15). The free cytosolic ADP concentration was calculated by using [ATP], [PCr], and [H$^+$] measured in the intact beating heart by ^{31}P-NMR spectroscopy and total Cr measured chemically in RV homogenates, by assuming that CK is in equilibrium and by using a CK equilibrium constant of 1.66×10^9 M^{-1} (16,17):

$$[ADP] = ([ATP][Cr]_{free})/([PCr][H^+]1.66 \times 10^9) \quad (1)$$

The free energy change of ATP hydrolysis (ΔG) was calculated as

$$\Delta G(\text{KJ/mol}) = \Delta G° + RT\ln([ADP][P_i]/[ATP]) \quad (2)$$

where $\Delta G°$ (-30.5 KJ/mol) is the value of ΔG under standard conditions of molarity, temperature, pH, and [Mg^{2+}] (18), R is the gas constant (8.3 J/molK), and T is the temperature in Kelvin (K).

^{31}P-NMR Magnetization Transfer Measurements of Creatine Kinase Kinetics

For magnetization transfer experiments, each broadband pulse was preceded by a low-power, narrowband

pulse at the resonance frequency of [γ-P]ATP for 0, 0.3, 0.6, 1.2, 2.4, or 3.6 s as previously described (14,19). For each of the 6 saturation transfer spectra, 64 scans were accumulated by repetitively cycling through the six different times of presaturation. A complete saturation transfer experiment was acquired in 32 min. Stability of the preparation was assessed by comparing one-pulse spectra obtained before and after each magnetization transfer experiment. Magnetization transfer measurements of the forward CK reaction

$$\text{Phosphocreatine} \rightarrow [\gamma\text{-P}]\text{ATP} \tag{3}$$

were analyzed according to the two-site chemical exchange model of Forsen and Hoffman (20), providing estimates of the pseudo-first-order rate constant (k_{for}) and the intrinsic longitudinal relaxation time for PCr (T_1). Multiplying the rate constant by substrate concentration yielded reaction velocity (21).

Biochemical Measurements

Since biochemical measurements cannot be made in formalin-pretreated hearts (necessary for histologic determination of infarct size), right ventricles were separated after the NMR-experiment and frozen in liquid nitrogen. We have previously shown that changes of total creatine and creatine kinase isoenzyme activities in chronically infarcted hearts are mostly similar for left and right ventricles (22).

High-Pressure Liquid Chromatography Measurements

A piece of tissue (~10 mg) was separated with a Minimot 40/|E drill (Proxxon GmbH, Niersbach, Germany) under liquid nitrogen and was analyzed for total creatine content as previously described (23,24). Free Cr was then calculated for each heart as total Cr minus PCr. Noncollagen protein was measured by the method of Lowry et al. (25). Metabolite concentrations were expressed as mmol/L, assuming that 50% of wet weight represents intracellular H_2O (26).

Enzyme Analysis

From each sample, 5–10 mg of tissue were homogenized as previously described (22). Before the addition of Triton, aliquods for measurements of protein and Cr content were taken. Creatine kinase (27), citrate synthase

(28), and lactate dehydrogenase (29) enzyme activities were measured using an Ultraspec III spectrophotometer (Pharmacia Biosystems, Freiburg, FRG). To measure the CK isoenzyme distribution, the Rapid Electrophoresis System (REP, Helena Diagnostika GmbH) as separation unit and the REP CK Isoforms Kit (Helena Diagnostika GmbH) for agarose gel and incubation solution were used. The Electrophoresis Data Center (EDC, Helena Diagnostika GmbH) automatically quantified the separated isoenzyme bands.

Experimental Protocols

All hearts were given 10–15 min for stabilization where LV end-diastolic pressure was set to 10 mm Hg by adjusting the balloon volume in the left ventricle. After ''baseline'' left ventricular pressures (mm Hg), heart rate (min^{-1}), and coronary flow (ml/min) were recorded, the balloon was emptied. A LV pressure–volume curve was performed. Recordings of all parameters were made at each step when a new steady state was reached, which occurred within 2 min. After another 15 min of stabilization (end-diastolic pressure set to 10 mm Hg), a 5-min one-pulse spectrum was recorded. Thereafter, a set of six ^{31}P-NMR magnetization transfer spectra was recorded in 32 min. After obtaining a final one-pulse ^{31}P-NMR-spectrum, the right ventricle was separated and rapidly frozen in liquid nitrogen for HPLC and enzyme measurements and the left ventricle was fixed in formalin for determination of infarct size.

Statistical Analysis

All data are presented as mean ±S.E.M. Results of quinapril-treated and untreated sham and MI hearts were compared by multicomparison analysis of variance (ANOVA) (30). Calculations were performed by a commercially available program, Stat View SE+Graphics (Brainpower Inc., Calabasas, CA, USA). A *p*-value of less than 0.05 was considered significant.

RESULTS

Heart Weight, Body Weight, and Infarct Size

Table 1 shows infarct size, body weight, heart weight, LV weight, RV weight, and their ratios for the four studied groups of rats. Infarct size was comparable for the two infarcted groups. Body weight was significantly re-

Table 1

Characteristics of Study Groups

	Sham	MI	Sham + quin	MI + quin
n	11	7	9	7
MI size [%]	—	41 ± 2	—	38 ± 3
Body weight [g]	449 ± 11	506 ± 17	482 ± 17	$438 \pm 9^{\dagger}$
Heart weight [g]	1.52 ± 0.06	$2.69 \pm 0.18*$	1.59 ± 0.07	$1.93 \pm 0.09^{\dagger}$
Heart weight/body weight	3.37 ± 0.11	$5.12 \pm 0.25*$	3.30 ± 0.10	$4.39 \pm 0.15*^{\dagger}$
LV weight [g]	1.07 ± 0.05	$2.08 \pm 0.15*$	1.16 ± 0.05	$1.39 \pm 0.07^{\dagger}$
LV weight/body weight	2.38 ± 0.08	$4.11 \pm 0.22*$	2.41 ± 0.07	$3.17 \pm 0.12*^{\dagger}$
RV weight [mg]	309 ± 15	392 ± 48	273 ± 14	309 ± 18
RV weight/body weight	687 ± 30	777 ± 95	568 ± 30	703 ± 38

LV = left ventricular; RV = right ventricular.
* $p = 0.05$ sham vs. MI.
$\dagger$ $p < 0.05$ quinapril-treated vs. untreated.

duced in quinapril-treated infarcted rats. Heart weight and LV weight were substantially increased in the infarcted untreated group. Quinapril treatment prevented these increases. Similar effects were observed for their ratios. RV weight in absolute and relative terms was not affected by either MI or treatment.

Cardiac Performance

Table 2 shows HR, coronary flow, and left ventricular developed pressure (LVDP) for the four experimental groups, all recorded at an end-diastolic pressure set to 10 mm Hg. LV developed pressure was significantly reduced in untreated, infarcted hearts (62 ± 11 vs. 130 ± 4 mm Hg in sham). Although treatment did not affect left ventricular–developed pressure in sham-operated hearts, quinapril treatment prevented the decrease in LV developed pressure in infarcted hearts (110 ± 8 in quinapril-treated vs. 62 ± 11 mm Hg in untreated infarcted hearts).

Figure 1 shows LV developed pressure to LV volume relations for the four groups. The curve for the infarcted untreated group was shifted to the right with a significantly reduced maximal developed pressure compared to the sham-operated untreated group (Table 2). The shift of the curves was partially prevented by quinapril treatment, the reduction of the maximum developed LV pressure in infarcted hearts was prevented by quinapril. On average, heart rate was 273 ± 4 beats/min and was not significantly different among groups. Coronary flow was 22.7 ± 0.6 mL/min on average and was not significantly different among groups.

High-Energy Phosphate Metabolism

Representative ^{31}P-NMR spectra obtained from sham-operated and infarcted hearts, untreated and quinapril-treated, are shown in Figure 2. Mean values for high- and low-energy phosphates and pH_i are shown in Table 3.

Table 2

Cardiac Performance and Coronary Flow for All Experimental Groups

	Sham	MI	Sham + quin	MI + quin
HR [min^{-1}]	283 ± 9	267 ± 4	269 ± 5	261 ± 6
CF [mL/min]	21.3 ± 0.9	25.1 ± 1.7	23.1 ± 1.65	22.9 ± 1.0
LVDP [mm Hg]	130 ± 4	$62 \pm 11*$	119 ± 5	$110 \pm 8^{\dagger}$
LVDP$_{max}$ [mm Hg]	166 ± 6	$83 \pm 16*$	155 ± 8	$139 \pm 13^{\dagger}$

HR = heart rate; CF = coronary flow; LVDP = left ventricular developed pressure; LVDP$_{max}$ = maximum LVDP.
* $p < 0.05$ sham vs. MI.
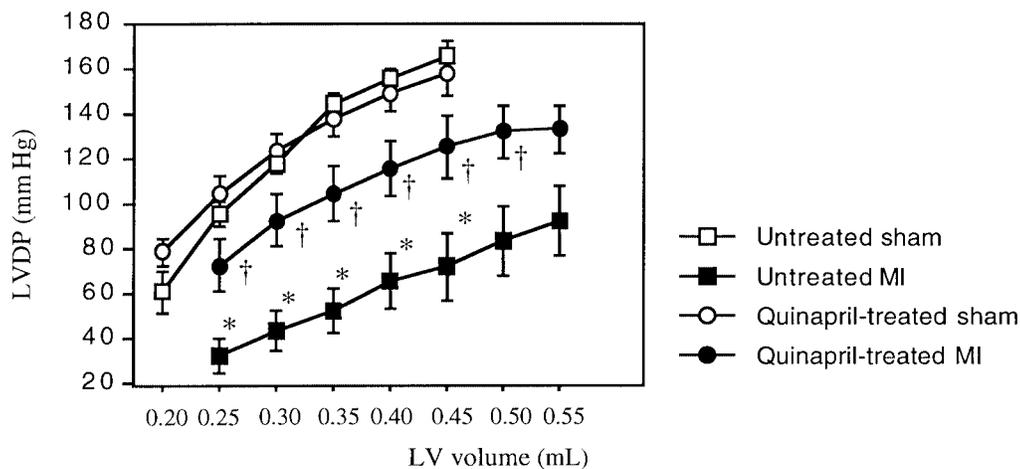$\dagger$ $p < 0.05$ quinapril-treated vs. untreated.

Figure 1. LV developed pressure to volume relations for the four groups studied. There is a rightward and downward shift in the untreated infarcted hearts, which was partially prevented by quinapril treatment.

Inorganic phosphate resonances were not significantly different among groups. The reduction of PCr concentration in infarcted hearts was prevented by quinapril treatment. The calculated mean values for free cytosolic ADP concentrations were $106 \pm 7\ \mu M$ and for the free energy change of ATP hydrolysis were -57.8 ± 0.2 J/mol, respectively. There were no differences among groups (Table 3). As also shown in Tab. 3, intracellular pH was 7.16 ± 0.01 and values were comparable for sham and

infarcted, treated and untreated, hearts. The total Cr pool was significantly reduced in untreated infarcted hearts. Treatment with quinapril prevented this reduction.

Creatine Kinase Reaction Velocity

Data from saturation transfer experiments are also shown in Table 3. On average, the T_1 of PCr was 3.16 ± 0.17 s and was similar for all groups. Both the rate and
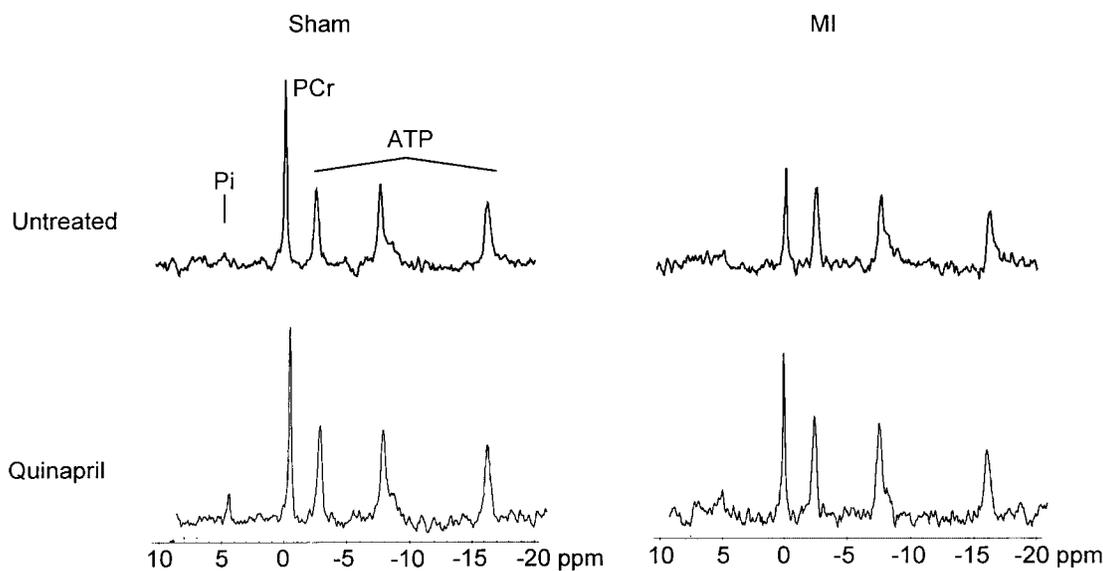
Figure 2. Representative ^{31}P-NMR spectra for the four groups studied. A reduction of PCr in the untreated infarcted heart and the preservation of PCr in the quinapril-treated infarcted heart is visible.

Table 3

High-Energy Phosphate Metabolism for All Experimental Groups

	Sham	MI	Sham + quin	MI + quin
ATP (mM)	10.8 ± 0.5[‡]	10.6 ± 1.2[‡]	set to 10.8	set to 10.6
PCr (mM)	14.4 ± 0.6	10.7 ± 0.2*	14.9 ± 0.4	13.5 ± 0.4[†]
Free creatine (mM)	15.0 ± 1.0	11.2 ± 1.0	16.8 ± 1.9	15.4 ± 1.4
Total creatine (mM)	28.4 ± 1.0	22.0 ± 1.0*	31.2 ± 1.5	28.7 ± 1.6[†]
P_i (mM)	2.7 ± 0.4	3.1 ± 0.3	2.5 ± 0.2	3.0 ± 0.2
ADP (μM)	101 ± 9	111 ± 16	102 ± 14	111 ± 16
pH_i	7.15 ± 0.02	7.17 ± 0.01	7.16 ± 0.01	7.17 ± 0.01
T_1 (s)	3.31 ± 0.29	2.97 ± 0.23	3.63 ± 0.35	2.93 ± 0.21
k_{for} (s^{-1})	0.96 ± 0.04	0.75 ± 0.05*	0.95 ± 0.04	0.93 ± 0.06
CK flux (mM/s)	12.0 ± 0.7	8.6 ± 0.5*	12.2 ± 0.8	12.1 ± 0.4[†]
ΔG (kJ/mol)	−58.6 ± 0.4	−57.4 ± 0.5	−58.2 ± 0.4	−57.4 ± 0.5

PCr = phosphocreatine; P_i = inorganic phosphate; pH_i = intracellular pH; T_1 = longitudinal relaxation time of PCr and P_i = rate constant (k_{for}).

[*] $p < 0.05$ sham vs. MI.

[†] $p < 0.05$ quinapril-treated vs. untreated.

[‡] Taken from previous study (20).

the extent of saturation transfer from PCr to [γ-P]ATP were decreased in untreated infarcted hearts. The pseudo–first-order unidirectional rate constant for untreated infarcted hearts decreased significantly from 0.96 ± 0.04/s to 0.75 ± 0.05/s. Quinapril prevented this decrease. As shown in Figure 3, Cr reaction velocity was reduced in untreated infarcted hearts by 30%. This decrease was completely prevented by quinapril treatment.
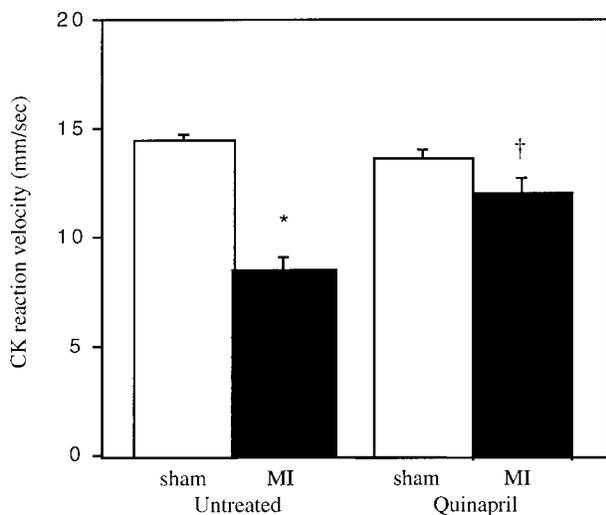

Figure 3. Mean values for creatine kinase reaction velocity (CK flux) for the four groups studied, demonstrating a substantial reduction of CK reaction velocity in the untreated group post-MI. Quinapril treatment prevented this reduction. * = $p < 0.05$ sham vs. MI; † = $p < 0.05$ quinapril-treated vs. untreated.

Biochemical Analysis

Table 4 summarizes the results of enzymatic analysis of right ventricles frozen after the experiment. Total CK activity was 8.4 ± 0.3 IU/mg protein and was not different among groups. CK isoenzyme distribution showed an increase of the fetal BB and MB isoenzymes, whereas the mitochondrial CK isoenzyme showed a trend for a decrease. Treatment with quinapril prevented the increase of the fetal CK isoenzymes after myocardial infarction. There was no difference in the reduction of MM isoenzyme in sham-operated and infarcted, untreated and treated hearts (Tab. 4). Lactate dehydrogenase activity, a glycolytic enzyme, was 1.1 ± 0.0 mIU/mg protein and citrate synthase activity, a marker for mitochondrial mass, was 0.9 ± 0.0 IU/mg protein, and these enzyme activations were not significantly different between groups.

DISCUSSION

Definition of the Model

In this study, we employ the clinically most relevant model of chronic heart failure, the rat heart postmyocardial infarction. In untreated infarcted hearts, changes in cardiac structure and function were as previously described (14,22,31): Impaired contractile performance was accompanied by structural dilatation, indicated by a rightward and downward shift of the pressure–volume

Table 4

Enzyme Activities in Right Ventricle of All Experimental Groups

	Sham	MI	Sham + quin	MI + quin
Total CK activity	8.8 ± 0.3	8.1 ± 0.7	8.2 ± 0.7	8.4 ± 0.9
MM activity	4.7 ± 0.3	3.9 ± 0.3	4.6 ± 0.4	4.9 ± 0.5
Mito-CK activity	2.5 ± 0.2	2.1 ± 0.3	2.3 ± 0.7	2.4 ± 0.3
MB-CK activity	1.4 ± 0.1	2.0 ± 0.2	1.2 ± 0.1	1.3 ± 0.1[†]
BB-CK activity	0.16 ± 0.03	0.38 ± 0.08*	0.09 ± 0.02	0.11 ± 0.06[†]
% MM	54 ± 1	47 ± 2	56 ± 2	58 ± 1[†]
% mito-CK	28 ± 1	26 ± 2	28 ± 2	26 ± 1
% MB-CK	16 ± 3	22 ± 2*	15 ± 1	15 ± 1[†]
% BB-CK	1.8 ± 3.0	4.2 ± 0.8*	1.1 ± 0.2	1.2 ± 0.7[†]
LDH	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 1.0	1.2 ± 0.1
Citrate sythase	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1

Total creatine kinase (Total CK activity; IU/mg protein); MM-, mito-, MB- and BB-CK isoenzymes (absolute activity in IU/mg protein and percentage of total creatine kinase activity); LDH (lactate dehydrogenase; IU/mg protein) and citate synthase (IU/mg protein).

* $p < 0.05$ sham vs. MI.

[†] $p < 0.05$ quinapril-treated vs. untreated.

relation with a reduced maximum LV developed pressure.

Untreated infarcted hearts also showed impairment of energy metabolism characteristic of failing myocardium: Reduction of total and phosphorylated Cr levels (14,32–34), while steady state ATP levels were unaltered (14). Based on CK equilibrium assumptions, this also indicates unchanged free ADP and ΔG levels. However, metabolic data presented in this study show ''baseline'' performance conditions only. The rate of phosphoryl transfer (V_{for}) measured as CK reaction velocity was reduced by 30%. A reduction of energy reserve via the creatine kinase reaction as indicated by decreased PCr and CK reaction velocity may contribute to the development of contractile failure (35). The alterations of CK isoenzyme distribution were as previously described (14,22) and showed increased MB-CK and BB-CK activity. The heart failure model used here is well suited to study the chronic effects of both protective (6,9,36) and deleterious (37) interventions.

Effects of Quinapril on Cardiac Geometry and Function Postmyocardial Infarction

In clinical heart failure studies (1,6), ACE inhibitors have been shown to chronically decrease mortality, reduce LV hypertrophy, and improve LV function. However, the exact mechanisms underlying these beneficial effects remain to be determined. For example, Nascimben et al. (38) showed that supplying the ACE inhibitor

enalapril to severely failing cardiomyopathic hamsters improved survival and hemodynamic performance. The present study shows that quinapril treatment prevented the decrease of LV developed pressure occurring post-MI, whereas quinapril had no effect on LV developed pressure in hearts of sham-operated animals. As assessed by Frank-Starling curves, ACE inhibition with quinapril counteracted dilatation and preserved LV function in the MI model, an effect that has also been observed in vivo (6). Part of the effect may be explained by alterations in the collagen content (39).

In the isolated isovolumic heart preparations used in this study, coronary flow was not significantly changed in hearts from quinapril-treated animals. Our data indicate that global coronary perfusion was not significantly altered by chronic MI or ACE inhibition. Therefore, the beneficial effects of chronic ACE inhibitor treatment on function and energy metabolism are unlikely to be related to effects on improved global perfusion and microcirculation after MI. However, the isolated buffer-perfused rat heart model is not an appropriate model to study flow changes, since coronary flow is ten times higher than it is in vivo under conditions of blood perfusion.

Effects of Quinapril Treatment on Cardiac Energy Metabolism

Energy metabolism is compromised in heart failure (35), and one hypothesis is that ACE inhibitors like quinapril may, at least in part, exert their beneficial effects

on cardiac function by maintaining normal energy metabolism. In the present study, we demonstrate that the functional effects of quinapril are accompanied by beneficial effects on cardiac energy metabolism: PCr content, CK reaction velocity, and total Cr content were all maintained at normal levels, and the fetal reprogramming of CK isoenzymes was prevented. Maintaining energy reserve via CK at normal levels, ACE inhibitors may, among multifaceted other biochemical mechanisms of the compounds, exert their beneficial effects.

Only a few investigators have previously studied the effects of ACE inhibition on cardiac energy metabolism. Sanbe et al. (40) showed that energy metabolism and mitochondrial oxidative function was improved by treatment with various ACE inhibitors postmyocardial infarction. Using a different model of heart failure, Nascimben et al. (38) showed that, in Syrian cardiomyopathic hamsters, decreased flux through the CK reaction leads to decreased capacity for ATP synthesis and may contribute to reduced contractile performance. Here, enalapril treatment resulted in increased phosphoryl transfer through the CK reaction in failing myocardium, and this increase was coupled to improved cardiac performance. It is particularly interesting that, in Nascimben's study (38), the increase of CK reaction velocity was mainly due to an increase of the first-order rate constant K, while in our study both K and substrate concentration (PCr) were increased. This suggests that ACE inhibitors do not simply maintain PCr and total Cr levels but, in addition, have effects on the kinetic properties of the enzyme itself.

The mechanisms whereby ACE inhibitors maintain phosphocreatine and total Cr at normal levels remain to be determined. However, since total Cr levels seem to be mainly determined by the activity of the creatine transporter (41), effects on this protein appear to be the most likely mechanism involved. The present study showed unchanged ADP and ΔG values, at least for the baseline performance conditions studied here. However, reduced CK flux and inability to maintain high ΔG may combine to limit the contractile reserve of the failing heart during inotropic stimulation (42), and ACE inhibitors may be able to maintain energy metabolism under these conditions. This remains to be studied. Long-term ACE inhibition leads to increased AT1 and AT2 receptor gene expression and, furthermore, limits infarct size, prevents cardiac hypertrophy and, therefore, improves heart function post-MI (43,44). However, ACE inhibitors may also have effects on other factors such as bradykinin. For example, Blais et al. (45) showed that the ACE inhibitor enalapril significantly prevented the rapid degradation of bradykinin. ACE-inhibitor induced effects on cardiac function and metabolism can be abolished by chronic bradykinin receptor blockade (46). On the other hand, Yamaguchi et al. (47) reported an attenuation of the reduction in ryanodine receptor density in the viable left ventricle of the infarcted rat with CHF and an attenuation of the sarcoplasmic reticulum dysfunction which is in part attributed to prevention of downregulating of the Ca^{2+} release channel.

Study Limitations

Because our study design required that the animals were killed at 2 months, we could not quantitatively assess differences in survival rates due to treatment. Enzyme and HPLC analyses were performed in intact residual RV tissue, because the left ventricle was formalin-pretreated for histologic determination of infarct size. However, we previously showed (22) that changes in energy metabolism are mostly similar for the left and right ventricles. Another limitation of the present study is that only a single dose of quinapril, one that showed a mild hemodynamic effect, was tested. Thus, a dose-dependence of quinapril's effects on energy metabolism also remains to be established.

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