Effects of ACE inhibition and β-receptor blockade on energy metabolism in rats postmyocardial infarction

Hügel, Stephanie, Michael Horn, Mark De Groot, Helga Remkes, Charlotte Dienesch, Kai Hu, Georg Ertl, and Stefan Neubauer. Effects of ACE inhibition and β-receptor blockade on energy metabolism in rats postmyocardial infarction. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2167–H2175, 1999.—Chronic treatment with β-receptor blockers or angiotensin-converting enzyme (ACE) inhibitors in heart failure can reduce mortality and improve left ventricular function, but the mechanisms involved in their beneficial action remain to be fully defined. Our hypothesis was that these agents prevent the derangement of cardiac energy metabolism. Rats were subjected to myocardial infarction (MI) or sham operation. Thereafter, animals were treated with bisoprolol, captopril, or remained untreated. Two months later, cardiac function was measured in the isolated heart by nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), and enzyme analysis, we define steady-state levels, turnover rates, and free energy change of high-energy phosphates as well as tissue activities ($V_{\text{max}}$) of creatine kinase (CK) isoenzymes.

**MATERIALS AND METHODS**

Animals and experimental MI. Infarcts or sham operations were performed in 12-wk-old Wistar rats kept in a 12:12-h light-dark cycle. Left coronary artery ligation (MI) was induced by a previously described technique (11, 35). A left thoracotomy was performed under ether anesthesia and positive pressure ventilation. The heart was rapidly exteriorized by applying gentle pressure on both sides of the thorax. The left coronary artery was ligated between the pulmonary outflow tract and the left atrium. The heart was then replaced into the thorax, lungs were inflated by increasing positive end-expiratory pressure, and the wound was closed immediately. Sham operation was performed using an identical procedure except that the suture was passed under the light-dark cycle. Left coronary artery ligation (MI) was induced by a previously described technique (11, 35). A left thoracotomy was performed under ether anesthesia and positive pressure ventilation. The heart was rapidly exteriorized by applying gentle pressure on both sides of the thorax. The left coronary artery was ligated between the pulmonary outflow tract and the left atrium. The heart was then replaced into the thorax, lungs were inflated by increasing positive end-expiratory pressure, and the wound was closed immediately. 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%. Surviving rats were kept on commercial rat chow and water ad libitum. The investigation conformed with the “Guiding Principles for Research Involving Animals and Human Beings.”

Bisoprolol and captopril treatment and experimental groups. Rats were randomly assigned to one of six groups: untreated sham (sham, $n = 9$), untreated MI (MI, $n = 8$), bisoprolol-treated sham (sham-Biso, $n = 13$), bisoprolol-treated MI (MI-Biso, $n = 6$), captopril-treated sham (sham-Capto, $n = 11$), and captopril-treated MI (MI-Capto, $n = 6$). After surgery, bisoprolol-treated groups received 60 mg·kg$^{-1}$·day$^{-1}$ bisoprolol with the drinking water. Captopril was added to the drinking water at 2 g/l. These concentrations were chosen because they were shown to exert a small but significant effect of established heart failure drugs such as ACE inhibitors or β-receptor blockers on energy metabolism may fuel the search for therapies targeted more specifically to cellular systems involved in the regulation of energy metabolism.

The purpose of this study was thus to define the geometric, functional, and energetic cardiac effects of chronic treatment with the β-receptor blocker bisoprolol and the ACE inhibitor captopril in a clinically highly relevant model of cardiac dysfunction, occurring post-myocardial infarction (MI) in the rat. Using a combination of nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), and enzyme analysis, we define steady-state levels, turnover rates, and free energy change of high-energy phosphates as well as tissue activities ($V_{\text{max}}$) of creatine kinase (CK) isoenzymes.
hemodynamic effect (10% reduction of blood pressure (bisoprolol) or heart rate (bisoprolol) (3, 24)). Because bisoprolol has a slightly bitter taste, 5% glucose was added to the drinking water of all groups. Drinking water was freshly prepared every other day. Therapy was continued for 8 wk and was stopped 1 day before the isolated heart experiment.

Isolated rat heart preparation. Eight weeks after the left coronary artery ligation or sham operation, rats were anesthetized with the injection of 50 mg pentobarbital sodium intraperitoneally. A transverse laparotomy and left and right anterolateral thoracotomy were performed, and the heart was rapidly excised and immersed in ice-cold buffer. The aorta was dissected free and mounted onto a cannula attached to a perfusion apparatus, as previously described (2). Retrograde perfusion of the heart was begun in the Langendorff mode at a constant temperature of 37°C and at a constant coronary perfusion pressure of 100 mmHg. A small vent made out of polyethylene tubing was pierced through the ventricle and connected to a perfusion apparatus, as previously described (2). Retrograde perfusion of the heart was begun in the Langendorff mode at a constant temperature of 37°C and at a constant coronary perfusion pressure of 100 mmHg. A small vent made out of polyethylene tubing was pierced through the ventricle and connected to a perfusion apparatus, as previously described (2). This study was performed at the University Medical Center, Tokyo, Japan. Performance was estimated as the product of heart rate and left ventricular developed pressure (LVDP, mmHg/mmHg/min). Frank-Starling curves were obtained by increasing the volume of the left ventricular balloon by 0.05-mI increments until LVDP reached a maximum.

31P 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 superwide bore (150 mm), a 7.05-Tesla magnet (Bruker, Rheinstetten, Germany) as distributed among the spectra. A complete saturation transfer spectrum was acquired in 32 min. Stability of the magnetization transfer measurements of the forward CK reaction phosphocreatine -> [γ-P]ATP for 0, 0.3, 0.6, 1.2, 2.4, or 3.6 s as previously described (29, 30). Separate studies showed that the narrow-band pulse directly attenuated the PCr magnetization by <5% when the carrier frequency was placed 2.5 ppm downfield from the resonance of PCr. For each of the six saturation transfer spectra, 64 scans were accumulated by repetitively cycling through the six different times of presaturation. Thus any metabolic deterioration occurring during the saturation transfer measurement was equally distributed among the spectra. 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 phosphocreatine -> [γ-P]ATP were analyzed according to the two-site chemical exchange model of Forse and Hoffman (9), providing estimates of the pseudo first-order rate constant (kHz) and the intrinsic longitudinal relaxation time for PCr (T1). Briefly, as the time of saturation at [γ-P]ATP, t, is increased from 0 to 3.6 s, the integrated signal intensity of the PCr resonance peak (Mt) decays from M0 to M∞ (defined as magnetization at zero and infinite saturation times, respectively) with a time constant τ1 as

\[ Mt = M_0 + (M_0 - M_∞)e^{-t/\tau_1} \]

where

\[ 1/\tau_1 = 1/T_1 + k_{off} \]

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

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

\[ \Delta G (kJ/mol) = \Delta G^o + RT \ln ([ADP][P_i][ATP]) \]

where \( \Delta G^o \) (−30.5 kJ/mol) is the value of \( \Delta G \) under standard conditions of molarity, temperature, pH, and [Mg2+] (14); R is the gas constant (8.3 J/mol K); and T is the temperature in Kelvin (K).

31P-NMR magnetization transfer measurements of CK kinetics. For magnetization transfer experiments, each broadband pulse was preceded by a low-power, narrow-band 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 (29, 30). Separate studies showed that the narrow-band pulse directly attenuated the PCr magnetization by <5% when the carrier frequency was placed 2.5 ppm downfield from the resonance of PCr. For each of the six saturation transfer spectra, 64 scans were accumulated by repetitively cycling through the six different times of presaturation. Thus any metabolic deterioration occurring during the saturation transfer measurement was equally distributed among the spectra. 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 phosphocreatine -> [γ-P]ATP were analyzed according to the two-site chemical exchange model of Forse and Hoffman (9), providing estimates of the pseudo first-order rate constant (kHz) and the intrinsic longitudinal relaxation time for PCr (T1). Briefly, as the time of saturation at [γ-P]ATP, t, is increased from 0 to 3.6 s, the integrated signal intensity of the PCr resonance peak (Mt) decays from M0 to M∞ (defined as magnetization at zero and infinite saturation times, respectively) with a time constant τ1 as

\[ Mt = M_0 + (M_0 - M_∞)e^{-t/\tau_1} \]

where

\[ 1/\tau_1 = 1/T_1 + k_{off} \]
Nonlinear regression of PCr magnetization at different saturation times of 1T–P iATP was used to determine M0, M∞, and τ1. T1 and κΓM were then calculated by solving the equations

\[ k_{\text{for}} = \frac{(M_0 - M_\infty)}{(M_0 - M_\infty)} \]

and

\[ T_1 = \frac{(M_0 - M_\infty)}{M_0} \tau_1 \]

Multiplying the rate constant by substrate concentration yielded reaction velocity (2).

Biochemical measurements. Because biochemical measurements cannot be made in Formalin-pretreated hearts (necessary for histological 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 CK isoenzyme activities in chronically infarcted hearts are similar for left and right ventricles (23).

HPLC measurements. A piece of tissue (−10 mg) was separated with a Mininot 40/E drill (Praxxon, Niersbah, Germany) under liquid nitrogen and was analyzed for total creatine and total adenine nucleotide (TAN) content as previously described (22, 32). Briefly, the powder was homogenized in 0.42 N perchloric acid at 0°C, and an aliquot of the homogenate was removed for protein determination. The homogenate was neutralized and centrifuged for 5 min. The supernatant was used for measuring total creatine and TAN by HPLC as previously described. Noncollagen protein was measured by the method of Lowry et al. (26). Metabolite concentrations were expressed in millimoles per liter.

Enzyme analysis. From each sample, 5–10 mg of tissue were homogenized in 0.1 M K2HPO4 buffer, pH 7.4, containing 1 mM EGTA, 1 mM β-mercaptoethanol, and 0.1% Triton X-100 at 4°C (final tissue concentration 5 mg/ml). Before the addition of Triton, aliquots for measurements of protein and creatine content were taken. All samples were kept on ice. CK (39), citrate synthase (38), and lactate dehydrogenase (1) 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) as a separation unit and the REP CK Isoforms Kit (Helena Diagnostika) for agarose gel and incubation solution were used. The agarose gel contains a Tris-barbital buffer with sodium azide as a preservative. The Electrophoresis Data Center (EDC, Helena Diagnostika) automatically quantified the separated isoenzyme bands.

Experimental protocols. All hearts were given 10–15 min for stabilization where left ventricular end-diastolic pressure was set to 10 mmHg by adjusting the balloon volume in the left ventricle. After baseline left ventricular pressures (mmHg), heart rate (beats/min), and coronary flow (ml/min) were recorded, and the balloon was emptied. A left ventricular pressure-volume curve was performed by stepwise inflation of the balloon by 0.05 ml until maximum LVDP was obtained or until the end-diastolic pressure exceeded 50 mmHg. 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 stabilization period (end-diastolic pressure set to 10 mmHg), a 5-min one-pulse spectrum was recorded. Thereafter, a set of six 31P NMR magnetization transfer spectra was recorded in 32 min. After a final one-pulse 31P NMR spectrum was obtained, 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.

Determination of infarct size. The left ventricle was embedded in paraffin, and 20-µm sections were cut serially from the apex to the base of the heart. Sections were stained for collagen using Picosirius red stain. A sustained increase in collagen content, measured as the Sirius red-positive area on each section, determines the infarct area. Slices were digitized by using the NIH Image 1.59/ppc scanner software (National Institute of Health, Bethesda, MD), and lengths of scar and noninfarcted muscle for both endocardial and epicardial surfaces were determined by cursor measurements for every section using the above software. The ratio of the lengths of scar and surface circumferences defined the infarct size for endo- and epicardial surfaces, respectively. Final infarct size was determined as the average of endo- and epicardial surfaces and is given in percentage. Average infarct size including all hearts was 29 ± 3% in the untreated, 27 ± 3% in the bisoprolol-treated, and 27 ± 4% in the captopril-treated group. To test whether treatment with bisoprolol or captopril affects the remodeling process post-MI, all hearts with an infarct size <30% were excluded from the analysis (untreated n = 12, bisoprolol n = 11, and captopril n = 12) to ensure comparability of the infarcted groups and to only include hearts where impairment of left ventricular mechanical function occurs.

Statistical analysis. All data are presented as means ± SE. With six experimental groups, 15 statistical comparisons are conceivable. Testing for this high number of comparisons with multifactorial ANOVA would overcome significance levels. We therefore limited the statistical analysis by seven “biologically meaningful” comparisons: sham vs. MI, sham-Bis vs. MI-Bis, sham-Capto vs. MI-Capto, sham vs. sham-Bis, sham vs. sham-Capto, MI vs. MI-Bis, and MI vs. MI-Capto. Comparisons of variables between two groups were made by using an unpaired Student’s t-test. Bonferroni’s correction for multiple comparisons was applied to yield a significance level of 0.05:7 = 0.007. Calculations were performed by a commercially available program, StatView SE–Graphics (Brainpower, Calabasas, CA).

RESULTS

Heart weight, body weight, and infarct size. Table 1 shows infarct size, body weight, heart weight, heart-to-body weight ratios, and left and right ventricular weight of the six groups studied. In the three infarcted groups, infarct size was 40% of the left ventricular circumference and was not significantly different. There was a tendency for body weight reduction in captopril-treated, sham-operated rats and in the bisoprolol-treated MI group. With bisoprolol the increase in heart weight and left ventricular weight, which occurs in infarcted hearts, was no longer significant. Heart weight was substantially reduced in both sham-operated and infarcted captopril-treated rats. Infarction led to an increase in right ventricular weights, which was prevented by captopril (P < 0.007) but not significantly by bisoprolol.

Cardiac performance and pressure-volume relations. Table 2 shows heart rate, coronary flow, and LVDP of the six experimental groups, all recorded at an end-diastolic pressure of 10 mmHg. On average, heart rate was 273 ± 3 beats/min and was not significantly different among groups. In contrast, LVDP was signifi-
LV wt, left ventricular weight; RV wt, right ventricular weight.

Resonance in untreated chronically infarcted hearts, but there was a significant reduction of the PCr were comparable in sham and chronically infarcted hearts. Pi resonances showed in Fig. 2. Mean values for high- and low-energy phosphates and pHi are given in Table 3. Pi resonances in untreated, infarcted hearts did not reach significance, an indication that the shift between captopril-treated, sham-operated and untreated groups were also shifted rightward and downward and not significantly affected by treatment. However, the shift between captopril-treated, sham-operated and infarcted hearts did not reach significance, an indication that structural dilation was less than in untreated or bisoprolol-treated infarcted hearts.

High-energy phosphate metabolism. Representative $^{31}$P NMR spectra from the various groups of hearts are shown in Fig. 2. Mean values for high- and low-energy phosphates and pHi are given in Table 3. P, resonances were comparable in sham and chronically infarcted hearts, but there was a significant reduction of the PCr resonance in untreated chronically infarcted hearts (10.6 ± 0.6 vs. 13.5 ± 0.7 mM in untreated sham-operated hearts, P < 0.007). With bisoprolol treatment, PCr remained almost unchanged and also captopril could limit the extent of the PCr reduction. There was no effect of treatment on $^{31}$P metabolites in the sham-operated groups. The total creatine pool tended to be reduced by chronic infarction (19.7 ± 1.2 vs. 23.0 ± 1.0 mM in sham-operated rats). Treatment with bisoprolol prevented this reduction, whereas captopril did not. Free creatine was, on average, 9.4 ± 0.5 mM and tended to reduce in infarcted hearts but not in treated hearts. Intracellular pH was 7.15 ± 0.00, and the values were comparable among sham and infarcted, treated and untreated, hearts. Free cytosolic ADP concentrations were, on average, 89 ± 5 µM, and ΔG values were −59.5 ± 0.4 kJ/mol. TANs measured in the right ventricle were 32.1 ± 1.0 nmol/mg protein on average. There were no significant differences among groups (Table 3).

CK reaction velocity. Figure 3 depicts stacked plots of $^{31}$P NMR spectra obtained from saturation transfer experiments for sham-operated untreated, infarcted untreated, infarcted bisoprolol-treated, and infarcted captopril-treated hearts. The spectra show that the extent of saturation transfer from the PCr to $[^g$-P]ATP resonance is reduced in infarcted hearts and that this reduction is partially prevented by treatment either with bisoprolol or captopril.

Mean data from saturation transfer experiments are shown in Table 3 and in Fig. 4. On average, the $T_1$ of PCr was 3.49 ± 0.14 s and was not different among groups (Table 3). There was a trend for a decrease of the CK rate constant in all infarcted groups. CK reaction velocity was reduced in all infarcted hearts but significantly less so in hearts treated with either bisoprolol or captopril (Fig. 4). However, the reduction post-MI did not reach significance in the bisoprolol-treated group. Thus energy reserve via CK remained at higher levels when infarcted hearts were treated with either the β-blocker bisoprolol or the ACE inhibitor captopril.

Table 1. Infarct size, body weight, heart weight, left and right ventricular weight, and their ratios for all experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>Sham + Biso</th>
<th>MI + Biso</th>
<th>Sham + Capto</th>
<th>MI + Capto</th>
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<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>MI size, %</td>
<td>42±2</td>
<td>42±2</td>
<td>42±2</td>
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<tr>
<td>BW, g</td>
<td>477±13</td>
<td>475±21</td>
<td>475±8</td>
<td>475±8</td>
<td>475±8</td>
<td>475±8</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.49±0.06</td>
<td>2.11±0.09</td>
<td>1.58±0.09</td>
<td>1.82±0.10</td>
<td>1.27±0.04†</td>
<td>1.51±0.06†</td>
</tr>
<tr>
<td>HW/BW</td>
<td>3.13±0.08</td>
<td>4.74±0.29†</td>
<td>3.51±0.19</td>
<td>4.53±0.19†</td>
<td>3.00±0.15</td>
<td>3.70±0.12*</td>
</tr>
<tr>
<td>LV wt, g</td>
<td>1.09±0.06</td>
<td>1.44±0.07*</td>
<td>1.11±0.07</td>
<td>1.32±0.08</td>
<td>0.88±0.03†</td>
<td>1.13±0.06*</td>
</tr>
<tr>
<td>RV wt, g</td>
<td>0.26±0.02</td>
<td>0.38±0.03*</td>
<td>0.28±0.02</td>
<td>0.28±0.02</td>
<td>0.25±0.02</td>
<td>0.23±0.01†</td>
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</table>

Values are means ± SE; n, number of rats. MI, myocardial infarction; Biso, bisoprolol; Capto, captopril; BW, body weight; HW, heart weight; LV wt, left ventricular weight; RV wt, right ventricular weight. *P < 0.007 sham vs. MI; †P < 0.007 Biso- or Capto-treated vs. untreated.

Table 2. Cardiac mechanical function and coronary flow for all experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>Sham + Biso</th>
<th>MI + Biso</th>
<th>Sham + Capto</th>
<th>MI + Capto</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>282±13</td>
<td>256±8</td>
<td>268±4</td>
<td>285±9</td>
<td>276±6</td>
<td>281±6</td>
</tr>
<tr>
<td>CF, ml/min</td>
<td>23.3±0.9</td>
<td>21.3±1.6</td>
<td>25.4±1.9</td>
<td>21.5±1.7</td>
<td>22.1±1.66</td>
<td>23.3±1.4</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>135±0.9</td>
<td>85±11*</td>
<td>142±7</td>
<td>138±13†</td>
<td>145±16</td>
<td>113±19</td>
</tr>
<tr>
<td>LVDPmax, mmHg</td>
<td>185±11</td>
<td>133±9*</td>
<td>239±12†</td>
<td>223±14†</td>
<td>214±11</td>
<td>160±10</td>
</tr>
</tbody>
</table>

Values are means ± SE; HR, heart rate; CF, coronary flow; LVDP, left ventricular developed pressure measured at an end-diastolic pressure of 10 mmHg; LVDPmax, maximum left ventricular developed pressure. *P < 0.007 sham vs. MI; †P < 0.007 Biso- or Capto-treated vs. untreated.
Fig. 1. A: pressure-volume curves for left ventricular developed pressure (LVDP) of all experimental groups. B: pressure-volume curves for left ventricular end-diastolic pressure (EDP) of all experimental groups. MI, myocardial infarction; Capto, captopril; Biso, bisoprolol. * P < 0.007 sham vs. MI, † P < 0.007 Biso- or Capto-treated vs. untreated.

Fig. 2. Representative $^{31}$P nuclear magnetic spectroscopy (NMR) spectra of all experimental groups. PCr, phosphocreatine.
Biochemical analysis. Table 4 summarizes the results of enzyme analysis of right ventricular homogenates. Total CK activity showed a trend for reduction after MI from 8.1 ± 0.5 to 6.0 ± 0.7 IU/mg protein in untreated but not in hearts treated with bisoprolol or captopril. CK isoenzyme distribution showed a trend for the changes characteristic of failing myocardium: a decrease of the absolute MM-CK and mitochondrial CK activities and an increase of the relative activities of fetal β-containing isoenzymes. Treatment with bisoprolol or captopril completely prevented all changes in total CK and isoenzyme activities after MI both in absolute and relative terms. Activities of the mitochondrial enzyme citrate synthase and of the glycolytic enzyme lactate dehydrogenase did not change in any experimental group.

DISCUSSION

Definition of model. The present study defines cardiac function and energy metabolism under various forms of treatment in a clinically highly relevant model of heart failure, occurring post-MI. For untreated groups, changes of cardiac geometry and function were as previously reported (23, 30). End-diastolic and systolic pressure-volume relations were shifted to the right in infarcted hearts, indicating structural dilatation (40). At the same time, maximum LVDP was substantially reduced, attesting to contractile dysfunction. This model is well suited to study beneficial or
adverse effects of various forms of pharmacological treatment (10, 17).

Similarly, changes in cardiac energy metabolism in untreated infarcted hearts were as previously reported: steady-state TAN levels are unaltered, whereas PCR is reduced by 22% and total creatine content by 14% (20, 21, 30). On the basis of CK equilibrium assumptions, we calculated unchanged free ADP and ΔG levels at least for the baseline performance conditions studied here. CK reaction velocity, a measure of ATP transfer from mitochondrion to myofibrils (21), was reduced by 41%. Also, changes in total CK and CK isoenzyme distribution, measured in the right ventricle, were as previously described (23, 30), indicating that the chronically failing heart is characterized by reduced MM-CK and mitochondrial CK activity. The alterations of energy metabolism found here are characteristic of many models of cardiac hypertrophy and failure, i.e., they occur independent of the etiology of heart failure (5, 20, 21).

Effects of β-receptor blockade and ACE inhibition on cardiac geometry and function. In untreated infarcted hearts, substantial left and right ventricular hypertrophy occurred, as indicated by increased left and right ventricular weights occurring despite the loss of ~40% of left ventricular tissue due to infarction. The increase in heart weight was reduced by both captopril and bisoprolol treatment, most effectively in the right ventricle, indicating that both forms of treatment blunted the hypertrophic response post-MI. However, even under captopril treatment, there was still significant hypertrophy after MI. This is in agreement with previous findings on β-blockers (13, 24) and ACE inhibitors (17, 46). Aside from a small increase in maximum LVDP by bisoprolol, both forms of treatment did not affect function and geometry in sham-operated hearts, as assessed by pressure-volume curves. In contrast, in infarcted groups, ACE inhibition with captopril partially prevented the rightward and downward shift of the systolic and diastolic pressure-volume curves, a well-characterized effect (12, 33, 46). Data are controversial on the functional effects of chronic β-receptor blockade in the post-MI rat model. Several studies suggested that β-blockers promote left ventricular dilation (13, 18). In contrast, our present work showed unchanged end-diastolic pressure to end-diastolic volume relations with bisoprolol treatment. In addition, bisoprolol improved LVDP (end-diastolic pressure = 10 mmHg) and maximum LVDP in infarcted hearts studied under the same loading conditions as sham-operated hearts. The present study clearly demonstrates that chronic bisoprolol treatment partially prevented the decrease of LVDP at various loading conditions but did not change end-diastolic pressure-volume relations. For the dosages used, the extent of the beneficial effect was similar to that exerted by captopril.

In the isolated isovolumic heart preparations used in this study, global coronary flow was not significantly different among the six groups of hearts. Therefore, the beneficial effects of chronic β-blocker or ACE inhibitor treatment on function and energy metabolism may be due at most in part to improved global perfusion and microcirculation after MI. Because isolated hearts were not perfused with bisoprolol or captopril, these changes reflect chronic alterations of myocardial perfusion rather than acute coronary vascular effects of these drugs.

Effects of β-receptor blockade and ACE inhibition on cardiac energy metabolism. In clinical studies of heart failure, both β-receptor blockers (7, 44) and ACE inhibitors (34, 37) have been shown to chronically reduce mortality and improve left ventricular function. The exact mechanisms of these beneficial effects remain to be determined. Energy metabolism is compromised in heart failure (19), and these compounds may, at least in part, act by maintaining normal energy metabolism. Previous studies on this subject are limited: Sanbe et al. (36) showed in a post-MI rat model that improvement of cardiac index and high-energy phosphate levels by various ACE inhibitors after MI was associated with an increased mitochondrial oxidative function. A more recent study by Nascimento et al. (27) using Syrian myopathic hamsters showed maintenance of CK reaction velocity by treatment with enalapril. Waagstein et al. (45) showed that myocardial lactate production turns to lactate extraction in dilated cardiomyopathy patients treated chronically with metoprolol. Previous work by Laser et al. (24) showed that the changes in CK and lactate dehydrogenase isoenzyme composition could be prevented by bisoprolol treatment in the post-MI rat model. Also, in six patients with dilated cardiomyopa-

### Table 4. Enzyme activities in right ventricle for all experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>Sham + Biso</th>
<th>MI + Biso</th>
<th>Sham + Capto</th>
<th>MI + Capto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CK activity</td>
<td>8.1 ± 0.5</td>
<td>6.0 ± 0.7</td>
<td>7.8 ± 0.4</td>
<td>7.4 ± 0.3</td>
<td>7.3 ± 0.4</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>MM-CK activity</td>
<td>4.09 ± 0.27</td>
<td>3.03 ± 0.28</td>
<td>3.45 ± 0.25</td>
<td>4.40 ± 0.33†</td>
<td>4.06 ± 0.20</td>
<td>3.69 ± 0.17</td>
</tr>
<tr>
<td>Mito-CK activity</td>
<td>2.33 ± 0.22</td>
<td>1.47 ± 0.18</td>
<td>2.46 ± 0.21</td>
<td>2.25 ± 0.17</td>
<td>2.45 ± 0.20</td>
<td>2.23 ± 0.15</td>
</tr>
<tr>
<td>MB-CK activity</td>
<td>1.18 ± 0.08</td>
<td>1.35 ± 0.16</td>
<td>0.96 ± 0.06</td>
<td>0.97 ± 0.07</td>
<td>0.96 ± 0.05</td>
<td>0.96 ± 0.10</td>
</tr>
<tr>
<td>BB-CK activity</td>
<td>0.55 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>%MM</td>
<td>53 ± 1</td>
<td>54 ± 2</td>
<td>55 ± 1</td>
<td>56 ± 1</td>
<td>54 ± 1</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>%Mito-CK</td>
<td>31 ± 1</td>
<td>24 ± 2*</td>
<td>30 ± 1</td>
<td>29 ± 1</td>
<td>29 ± 1</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>%MB-CK</td>
<td>14 ± 1</td>
<td>19 ± 2*</td>
<td>12 ± 1</td>
<td>13 ± 1†</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>%BB-CK</td>
<td>1.4 ± 0.1</td>
<td>2.1 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>LDH</td>
<td>1.05 ± 0.06</td>
<td>0.90 ± 0.05</td>
<td>0.95 ± 0.05</td>
<td>1.03 ± 0.05</td>
<td>0.91 ± 0.06</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>0.03 ± 0.06</td>
<td>0.75 ± 0.06</td>
<td>0.70 ± 0.04</td>
<td>0.81 ± 0.03</td>
<td>0.76 ± 0.05</td>
<td>0.81 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. 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); lactate dehydrogenase (LDH, IU/mg protein) and citrate synthase (IU/mg protein). *P < 0.007 sham vs. MI; †P < 0.007 bisoprolol- or captopril-treated vs. untreated.
thy, we showed that the myocardial PCr-to-ATP ratio, measured noninvasively with $^{31}$P NMR spectroscopy, increased during chronic drug therapy, including (in 4 of 6 cases) metoprolol (31). In the present work, we systematically analyze the effects of β-receptor blockers and ACE inhibitors on the various components of cardiac energy metabolism in the post-MI rat model. Unequivocally, we demonstrate that the beneficial functional effects of both bisoprolol and captopril treatment are accompanied by beneficial effects on cardiac energy metabolism: increased PCr content and CK reaction velocity; increased MM and mitochondrial CK activities; and prevention of the fetal reprogramming with relative increase of the β-containing CK isoenzymes. For the dosages used, both compounds were similarly effective, one exception being that bisoprolol also prevented the loss of total creatine whereas captopril did not. The reason for this discrepancy warrants further study. It is likely that β-receptor blockers and ACE inhibitors exert their beneficial effects mainly by chronically reducing the energetic needs of the heart, via reduction of heart rate and pre- and afterload, respectively. In addition, it is possible that these agents interfere more directly with energy metabolism, one potential site of action being the sarcolemmal creatine transporter (15). This remains to be further evaluated.

Are the observed beneficial effects on cardiac energy metabolism causally related to the improvement of left ventricular function and geometry, or are they merely epiphenomena of the treatment with these compounds? Our data do not provide a final answer but allow us to speculate: In principle, energy metabolism could limit the extent, by captopril. Thus maintenance of ATP transfer, i.e., energy reserve via CK, may be involved in the protective effect of β-receptor blockers and ACE inhibitors. Finally, ΔG values remained unchanged for the baseline performance conditions studied here. However, Tian et al. (40) and Tian and Ingwall (41) have demonstrated that hearts with a compromised CK system show reduced contractile reserve. It is thus conceivable that the effects of reduced CK flux and the inability to maintain high ΔG combine to limit the contractile reserve of the failing heart during inotropic stimulation, and β-receptor blockers and ACE inhibitors may be able to maintain energy metabolism under these conditions. This remains to be studied. Therefore, our results do not prove a causal relation between the observed beneficial functional and energetic effects, but our findings are consistent with the view that preservation of energy metabolism explains, at least in part, the favorable effects of β-receptor blockers and ACE inhibitors in chronic heart failure.

Study limitations. Enzyme and HPLC analyses were performed on intact residual right ventricular tissue, because the left ventricle was Formalin pretreated for histological determination of infarct size. However, Laser et al. (23) previously showed that changes in energy metabolism are very similar for the left and right ventricle. The present study involves 53 successful long-term experiments, yet only a single dose for each compound, one that showed a mild hemodynamic effect, was tested. Therefore, it is open whether the agents tested here exert dose-dependent effects on energy metabolism. Our study, however, shows that alterations in energy metabolism can be largely prevented when, post-MI, rats are chronically treated with β-receptor blockers or ACE inhibitors.

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