Chronic Phosphocreatine Depletion by the Creatine Analogue \( \beta \)-Guanidinopropionate Is Associated With Increased Mortality and Loss of ATP in Rats After Myocardial Infarction

Michael Horn, PhD; Helga Remkes, MSc; Hinrik Strömer, MD; Charlotte Dienesch; Stefan Neubauer, MD

**Background**—The failing myocardium is characterized by reductions of phosphocreatine (PCr) and free creatine content and by decreases of energy reserve via creatine kinase (CK), ie, CK reaction velocity (Flux\(_{\text{CK}}\)). It has remained unclear whether these changes contribute directly to contractile dysfunction. In the present study, myocardial PCr stores in a heart failure model were further depleted by feeding of the PCr analogue \( \beta \)-guanidinopropionate (GP). Functional and metabolic consequences were studied.

**Methods and Results**—Rats were subjected to sham operation or left coronary artery ligation (MI). Surviving rats were assigned to 4 groups and fed with 0% (n = 7, Sham; n = 5, MI) or 1% (n = 7 Sham + GP, n = 8 MI + GP) GP. Two additional groups were fed GP for 2 or 4 weeks before MI. After 8 weeks, hearts were isolated and perfused, and left ventricular pressure-volume curves were obtained. High-energy phosphate metabolism was determined with \(^{31}\)P NMR spectroscopy. After GP feeding or MI, left ventricular pressure-volume curves were depressed by 33% and 32%, respectively, but GP feeding in MI hearts did not further impair mechanical function. Both MI and GP feeding reduced PCr content and Flux\(_{\text{CK}}\), but here, effects were additive. In MI + GP rats, PCr levels and Flux\(_{\text{CK}}\) were reduced by 87% and 94%, respectively. Although ATP levels were maintained in the GP and MI groups, ATP content was reduced by 18% in MI + GP hearts. Furthermore, 24-hour mortality in GP-prefed rats was 100%.

**Conclusions**—Rats with an 87% predepletion of myocardial PCr content cannot survive an acute MI. Chronically infarcted hearts subjected to additional PCr depletion cannot maintain ATP homeostasis. (*Circulation. 2001;104:1844-1849.*)

**Key Words:** magnetic resonance imaging ■ spectroscopy ■ heart failure ■ myocardial infarction ■ creatine kinase

Both experimental and clinical findings indicate that the failing myocardium is characterized by changes in cardiac high-energy phosphate metabolism: Contents of phosphocreatine (PCr) and total creatine (Cr) are depleted by up to 50%, whereas ATP levels remain constant or decrease by up to 30%.\(^5\) The question whether these changes are causally related to the occurrence of contractile dysfunction or whether they are mere epiphenomena has been controversially debated for decades (for review, see Ingwall\(^6\) and Neubauer\(^7\)) and has largely remained unanswered, because previous studies have been correlative.

We hypothesized that if myocardial PCr and Cr levels are specifically depleted by pharmacological intervention before and/or after experimental heart failure (HF) is induced, then the true functional role of high-energy phosphate metabolism in HF should become unmasked. The only pharmacological compound known to specifically reduce PCr and Cr in heart is the creatine analogue \( \beta \)-guanidinopropionate (GP). GP is taken up by the cardiomyocyte\(^8\) via the creatine transporter and is then phosphorylated (P-GP).\(^9\) Its utilization in the CK reaction, however, is 3 orders of magnitude lower than that of PCr,\(^10\) thereby effectively inhibiting that reaction.

Previously, the effects of GP have been studied only in intact hearts.\(^11-14\) We reported that chronic treatment with GP over a period of 8 weeks in intact rats led to a 70% to 80% reduction of PCr and Cr stores. Mechanical function (left ventricular developed pressure, LVDP) of isolated hearts treated in this manner was reduced by 28%. In the present study, we examine how GP treatment affects mechanical function, high-energy phosphate metabolism, and mortality in the rat chronic myocardial infarction (MI) model. Furthermore, because PCr and Cr depletion by GP treatment requires several days, we tested the effect of GP treatment (1) when commenced after coronary ligation and (2) when given as...
pretreatment before coronary ligation. Our results show that rats with an 87% depletion of PCr cannot survive the hemodynamic stress of an acute MI and that rats treated after MI cannot maintain ATP homeostasis.

Methods

Animals and Experimental MI
Infarctions or sham operations were carried out in 12-week-old Wistar rats by left coronary artery ligation as previously described.15,16 The mortality rate of infarcted rats for the first 24 hours after the operation was 40% to 50%. The investigation conforms with the Revised Guide for the Care and Use of Laboratory Animals.17

Isolated Heart Preparation
Rats were reanesthetized, and the heart was excised.15 Perfusion of the heart with phosphate-free Krebs-Henseleit buffer was started in the Langendorff mode at 37°C and a coronary perfusion pressure of 100 mm Hg. Coronary flow (CF) was measured by an ultrasonic flow probe (Transonic Systems Inc.). CF per gram viable tissue was calculated assuming a scar weight of 14% of the myocardium.15 For measurement of cardiac performance, a water-filled latex balloon was inserted into the LV.15

LV pressure-volume curves were obtained by stepwise (∆0.05 mL) increases in balloon volume until maximum LVDP was reached or until LV end-diastolic pressure exceeded 50 mm Hg. Thereafter, end-diastolic pressure was set to 10 mm Hg for the duration of the MR measurements. At the end of the protocol, hearts were fixed in formalin for histological determination of infarct size.

Determination of Infarct Size
Infarct size was determined by a previously described technique.15 Sections were stained with picrosirius red, and the lengths of scar and noninfarcted muscle were measured. The average ratios of endocardial and epicardial surfaces defined the infarct. Hearts with an infarct size <25% were excluded.

31P NMR Spectroscopy
The perfused hearts were placed into a 7.05-T NMR system (Bruker) as previously described.15,18 31P NMR spectra at 121.50 MHz were acquired in the pulsed Fourier transform mode.

Single (“one-pulse”) spectra were accumulated over 5-minute periods (averaging 152 free induction decays, pulse angle 45°, interpulse delay of 1.93 seconds). The resonance areas corresponding to ATP, PCr, P-GP, and inorganic phosphate (Pi) were integrated by use of NMR software (Tripos) and were corrected for partial saturation.

Because the protocol (histological determination of LV infarct size) did not allow absolute ATP quantification by high-performance liquid chromatography (HPLC), absolute metabolite concentrations were calculated from NMR data. The average γ-ATP resonance area of all sham-operated hearts during control perfusion was set to 10.8 mmol/L.18 and all other metabolite concentrations were calculated as a fraction thereof. Intracellular pH (pHi) was calculated from the chemical shift between P and PCr.15

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-ATP for 0, 0.3, 0.6, 1.2, 2.4, or 3.6 seconds as previously described.15 Magnetization transfer measurements of the forward CK reaction, PCr → γ-ATP, were analyzed according to the 2-site chemical exchange model of Forsen and Hoffmann,19 providing estimates of the pseudo-first-order rate constant and reaction velocity (FluxCK).20

Experimental Groups and Protocols
In 2 series of experiments, a total of 6 groups of rats were studied: Four groups were fed GP or standard chow after MI or sham operation: Sham-operated untreated (Sham; n=7), infarcted untreated (MI; n=7), sham-operated treated (Sham+GP; n=5), and infarcted treated (MI+GP; n=8) surviving animals. After sham operation or MI, rats were randomized to receive creatine-free chow (fish protein replaced by soybean; Altromin) (untreated) or 1% (treated) GP (Sigma) was added (% refers to the total weight of chow) for a period of 8 weeks. We had previously shown14 a decrease of creatine in the heart to ~42% of control (2 weeks) and to ~30% of control after 8 weeks. After 8 weeks, hearts were isolated and perfused.

After stabilization, pressure-volume curves were obtained as described above. Hearts were allowed to restabilize at an end-diastolic pressure of 10 mm Hg. One 5-minute one-pulse 31P NMR spectrum was then recorded. A saturation transfer measurement of FluxCK followed as described. Finally, another 5-minute one-pulse 31P NMR spectrum was recorded. At the completion of the protocol, the LV was fixed in formalin and used for determination of infarct size.

Two additional groups of rats were fed for 2 or 4 weeks (n=43 and 45, respectively) with GP chow before the infarct operation. Animals then underwent surgery as described above.

Statistical Analysis
Results were compared by an unpaired, Bonferroni-corrected t test. With a maximum of 2 comparisons per group, values of P<0.025 were considered to indicate statistical significance. Data given are mean±SEM. Mortality was tested by a 2×2 contingency table with Fisher’s exact test between the MI and the MI+GP groups as well as the MI+GP versus the prefed MI group.

Results

Body Weight, Heart Weight, and Survival
The 2 infarcted groups had similar infarct sizes (35.6±1.3% versus 34.7±2.1%). Table 1 shows a trend toward increased heart weight after MI (by 19% in MI versus Sham, P=0.036; by 41% in MI+GP versus Sham+GP, P=0.0281). At the same time, body weight was reduced in MI+GP rats. The heart weight/body weight ratio showed a marked increase in the MI+GP rats. Food intake was similar in all groups (32.4±0.5 g/d).

In large infarcts (>30% of the LV circumference), mortality is typically ~40% within the first 24 hours after surgery (P=0.87, χ²=0.026, MI versus MI+GP). After this initial time, all animals fed GP or standard rat chow after MI or Sham operation survived without further mortality over the time of the study (2 months).

If rats were fed with GP before the operation, none of the animals survived: In both groups, 2 weeks (n=43) and 4 weeks (n=45) of prefeding, animals with MI died either during surgery or within 24 hours (P<0.0001, χ²=27.69, MI+GP versus prefed MI). In a subgroup of these animals (n=12), hearts were excised postmortem. Neither extraordinary infarct sizes nor gross overt morphological changes were seen. There were no deaths among animals fed with GP before sham operation (n=5).

Cardiac Performance
Table 1 shows unchanged heart rate and CF or CF per gram viable tissue in Sham and MI rats with or without GP feeding. Figure 1 shows LV pressure-volume curves demonstrating reduced cardiac performance in MI as well as in GP hearts. In
TABLE 1. Characteristics of Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>Sham+GP</th>
<th>MI+GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>MI size, %</td>
<td>0</td>
<td>35.6±1.3</td>
<td>0</td>
<td>34.7±2.1</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.81±0.06</td>
<td>2.14±0.10</td>
<td>1.80±0.08</td>
<td>2.54±0.22</td>
</tr>
<tr>
<td>BW, g</td>
<td>510±32</td>
<td>555±17</td>
<td>518±18</td>
<td>473±16*</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>3.62±0.25</td>
<td>3.85±0.13</td>
<td>3.49±0.14</td>
<td>5.37±0.43†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>259±8</td>
<td>234±16</td>
<td>267±5</td>
<td>255±11</td>
</tr>
<tr>
<td>CF, mL/min</td>
<td>29.8±1.8</td>
<td>23.4±5.7</td>
<td>25.7±1.77</td>
<td>26.3±4.7</td>
</tr>
<tr>
<td>CF/viable tissue, mL · min⁻¹ · g⁻¹</td>
<td>17.3±2.0</td>
<td>12.6±1.1</td>
<td>13.9±1.3</td>
<td>14.0±2.6</td>
</tr>
</tbody>
</table>

HW indicates heart weight; BW, body weight; and HR, heart rate.
*P<0.025 treated vs untreated.
†P<0.025 Sham vs MI.

MI and in GP hearts, LVDP_{max} at a given end-diastolic pressure was reduced compared with Sham. Furthermore, GP-treated hearts showed similarly reduced LV function, although the cardiodepressant effects of MI and GP were not additive.

NMR Spectroscopy

Figure 2 shows typical spectra of the 4 groups investigated. The integral area of the PCr resonance is lower in MI hearts than in Sham hearts. With GP treatment, an additional depletion in an animal model of HF due to ventricular remodeling after MI in the rat. We15 and others16,21 have fully characterized this model, in which, over a period of 2 months after coronary ligation, LV dysfunction and dilatation occur21 and CF is unchanged.15 Furthermore, in residual intact myocardium, PCr levels are reduced by up to 30%15 and Flux_{CK} up to 50%,15 but ATP and P_i levels remain constant.

This HF model is well suited to study the effects of pharmacological intervention: eg, ACE inhibitors18,22 and β-receptor blockers22 have been found to preserve cardiac structure, function, and energetics, whereas the Ca^{2+} channel blocker anipamil23 exerted detrimental effects in this model.

Although the changes in cardiac energy metabolism described above have been observed for several decades, it has remained unclear whether alterations of high-energy phosphates do in fact play a causal role in HF. Therefore, we studied the effects of additional substantial depletion of PCr content by GP feeding in the rat HF model. Functional and metabolic effects of chronic GP feeding in normal hearts have been described previously.10,11,14,24,25 Over a period of 8 weeks, chronic GP feeding leads to up to 79% depletion of PCr and up to 70% depletion of Cr stores, and Flux_{CK} is reduced even further to 15% of normal as a result of reduction of both its substrate PCr and its rate constant. In the isolated perfused heart, a 28% decrease of LV pressure was reported, whereas the adverse hemodynamic effects observed in the intact rat in vivo are more subtle.14 These functional and metabolic effects of GP feeding in normal rats were fully reproduced.

(Sub)acute Effects of GP Prefeeding After MI

The most remarkable finding of the present study is that when hearts were prefed with GP for 2 (n=43) or 4 (n=45) weeks, leading to a 65% and 74% reduction of PCr content, respectively,14 the 24-hour mortality of prefed rats undergoing coronary ligation was 100% (P<0.0001, χ²=27.69, MI+GP versus prefed MI), whereas in untreated rats, mortality for this procedure is 40% to 50%.15,16,21 This finding clearly shows that rat hearts with a 65% depletion of PCr and a 75% reduction of Flux_{CK} are unable to cope with the stress of an acute MI, or, in other words, that an intact PCr-CK system is needed to survive an acute coronary ligation. The reasons for the 100% mortality of GP-prefed animals may be that in PCr-depleted hearts, the residual intact myocardium is inca-
able of meeting the acute hemodynamic stress placed on it by the acute loss of contractility of the infarcted zone, or alternatively, may be related to induction of lethal ventricular arrhythmias, or both. In this respect, a limitation of our study is that because ECG monitoring was unavailable, we cannot discriminate between these possibilities. Furthermore, we cannot rule out that other, nonspecific, effects of GP have contributed to increased mortality. Conversely, our study provides solid evidence for the functional relevance of the PCr-CK system in acutely induced HF.

Chronic Effects of GP Feeding After MI

Because none of the rats fed with GP before coronary ligation survived for >24 hours after the procedure, we were unable to test the chronic functional and energetic effects of severe PCr depletion present from the initiation of HF. Instead, we could only test for the chronic functional consequences if GP feeding was started after rats recovered from coronary ligation. In this situation, at the time of acute surgery, PCr levels are not yet decreased initially but instead are continuously reduced over several weeks after MI. Pressure-volume curves obtained in perfused hearts 8 weeks after Sham/MI operation and placebo/GP feeding\textsuperscript{14,15} showed LVDP to be reduced by 28% by GP feeding and by 29% in post-MI hearts, but the cardiodepressant effects of GP and MI were not additive, and thus, LVDP was not further reduced in the MI+GP group. This demonstrates that for the baseline perfusion conditions, PCr depletion by GP did reduce mechanical performance in normal hearts but did not further impair performance from the reduced level of chronically infarcted hearts. The reasons for this are currently unclear, but together with our findings of 100% mortality in the acute MI study arm, this may suggest that the chronically failing heart can, over a period of weeks, adapt to a further depletion of PCr content, thereby maintaining LV contractile function at a minimal level necessary for survival (a further reduction of LVDP by GP in MI rats would have resulted in cardiogenic shock), whereas the acutely infarcted heart cannot adapt to a 65% PCr depletion within a 24-hour period. Clearly, such potential chronic adaptive mechanisms need to be further studied.

We also showed that heart weights tended to be higher in infarcted hearts in GP-fed animals, suggesting exacerbation of the post-MI hypertrophic response (not significant). Mekhfi et al\textsuperscript{11} showed previously that in normal rats, GP feeding started at a young age (3 weeks) over prolonged periods (10 to 12 weeks) also induces cardiac hypertrophy.

TABLE 2. Quantitative Measurements of High-Energy Phosphate Metabolism

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Sham</th>
<th>MI</th>
<th>Sham + GP</th>
<th>MI + GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP, mmol/L</td>
<td>10.8±0.6*</td>
<td>10.6±0.7*</td>
<td>10.6±1.0</td>
<td>8.9±0.6†‡</td>
</tr>
<tr>
<td>PCr, mmol/L</td>
<td>13.4±0.9</td>
<td>11.7±1.0†</td>
<td>3.1±0.3‡</td>
<td>1.8±0.3‡</td>
</tr>
<tr>
<td>P-GP, mmol/L</td>
<td>...</td>
<td>...</td>
<td>5.8±0.3</td>
<td>8.0±0.6†</td>
</tr>
<tr>
<td>P, mmol/L</td>
<td>2.5±0.3</td>
<td>2.7±0.2</td>
<td>2.5±0.4</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.15±0.01</td>
<td>7.16±0.01</td>
<td>7.14±0.01</td>
<td>7.15±0.01</td>
</tr>
<tr>
<td>K, s\textsuperscript{-1}</td>
<td>0.86±0.09</td>
<td>0.74±0.21</td>
<td>0.50±0.09†</td>
<td>0.26±0.16†</td>
</tr>
<tr>
<td>Flux, mmol/L/s</td>
<td>11.5±1.2</td>
<td>8.0±1.3†</td>
<td>1.7±0.4‡</td>
<td>0.7±0.4‡</td>
</tr>
</tbody>
</table>

Concentration of \textsuperscript{31}P metabolites (ATP, PCr, P). K indicates CK reaction rate constant.
*Taken from HPLC measurements.\textsuperscript{15}
†P<0.025 sham vs MI.
‡P<0.025 treated vs untreated.
The precise molecular mechanisms involved in such a growth response remain to be further elucidated, but it is conceivable that the heart may respond to the energetically unfavorable situation induced by chronic GP feeding by increasing its wall thickness, thereby reducing wall tension and energy demands. Furthermore, GP effects were unrelated to CF levels (Table 1).

Using 31P NMR spectroscopy, we showed that GP feeding reduced PCr levels down to 23%, the CK rate constant to 58%, and FluxCK to 15% of normal levels. In GP-fed MI hearts, these values were even lower, at 13%, 30%, and 6% of control. Remarkably, a 94% reduction of FluxCK was unable to induce additional myocardium dysfunction in MI hearts, demonstrating that the chronically failing myocardium can adapt over the long term to such a marked decrease in ATP availability via CK. In contrast to cardiac function, however, the effects of GP feeding and post-MI remodeling were in fact additive in terms of changes of high-energy phosphate metabolism. Interestingly, the extent of GP accumulation was significantly greater in chronically failing hearts (P-GP 8.0±0.6 mmol/L) than in sham-operated hearts (P-GP 5.8±0.3 mmol/L, P<0.025). It is currently unclear why the failing myocardium accumulates higher levels of GP than the normal heart. It is generally assumed that GP is taken up by cardiomyocytes solely by the action of the specific creatine transporter, a membrane-bound protein,26,29 and expression of this transporter is in fact downregulated by 25% in this HF model.26 Conversely, Seppet et al27 showed that in rat hearts made hypertrophic by chronic T3 treatment, creatine uptake was increased. Clearly, creatine and creatine analogue transport kinetics in HF will need to be studied in greater detail to answer these open questions.

In all groups, P and pH levels were unaltered and thus are unlikely to play a causal role in the functional and metabolic effects observed in this study.

The other remarkable finding was that the chronically infarcted, GP-treated group was unable to maintain normal ATP concentrations, ie, 2 months after MI and GP feeding, an 18% decrease of myocardial ATP was observed. This is in contrast to both the untreated MI groups and sham-operated groups, in which ATP remained constant.14,15 This shows that under the combined stress of post-MI remodeling and PCr depletion by GP, leading to a 94% reduction of energy reserve via CK, myocardial ATP decreases. Thus, ATP homeostasis is disrupted in failing heart by additional GP feeding, again demonstrating the relevance of a PCr-CK system in HF. This is in line with studies showing that ATP content remains normal in mild and moderately severe HF15,28–30 but decreases progressively with severe stages of cardiac failure.3,12 This also suggests that the GP-fed MI group may not have been in a metabolic steady state, and it would be interesting to study longer periods of time after MI to examine whether late mortality (after several months) is again increased by GP feeding in chronically infarcted hearts.

Because ATP levels in residual intact myocardium could not be measured by HPLC (as LV histology is required for infarct size stratification), a limitation of the study is that ATP levels had to be quantified on the basis of 31P NMR peak area comparisons among groups, which is less accurate.

In summary, our study points to the relevance of the PCr-CK system in the rat coronary ligation HF model: Rats with depleted myocardial PCr content cannot survive an acute MI, whereas PCr depletion induced later during HF development causes loss of ATP homeostasis.

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References


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