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Beneficial effects of MET-88, a γ-butyrobetaine hydroxylase inhibitor in rats with heart failure following myocardial infarction

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Abstract

Myocardial ischemia can cause myocardial infarction and as a consequence, heart failure. 3-(2,2,2-trimethylhydrazinium) propionate (MET-88) inhibits γ-butyrobetaine hydroxylase and has cardioprotective effects on the ischemic heart. We now examined the effects of MET-88 in rats with congestive heart failure following myocardial infarction. Congestive heart failure was produced by left coronary artery ligation in rats. MET-88 at 100 mg/kg/day was orally administered from the 2nd day after surgery. We performed a survival study for 181 days, and measured ventricular remodeling, cardiac function, and myocardial high-energy phosphate levels after treatment for 20 days. MET-88 prolonged survival with a median 50% survival of 103 days compared to 79 days for the heart-failure control rats. The expansion of the left ventricular cavity (ventricular remodeling) in heart-failure rats was prevented by treatment with MET-88, and the effect of MET-88 was similar to that of captopril at 20 mg/kg. MET-88 attenuated the rise in right atrial pressure in heart-failure rats and augmented cardiac functional adaptability against an increased load. Also, MET-88 improved the myocardial energy state in heart-failure rats. The present results indicate that MET-88 improves the pathosis in rats with heart failure induced by myocardial infarction.

Keywords: γ-Butyrobetaine hydroxylase inhibitor; Heart failure; Survival rate; Ventricular remodeling; Cardiac functional adaptability; High-energy phosphate

1. Introduction

It is a well-recognized clinical condition that myocardial ischemia can lead to infarction of the heart with subsequent scar formation and loss of contractile function. Congestive heart failure is one sequel of myocardial infarction, and is reflected by deterioration of cardiac function and poor survival and prognosis. Myocardial infarction with loss of function in the infarcted region imposes an increased mechanical load on the non-infarcted region. The subsequent hemodynamic course and lifespan of a subject with myocardial infarction largely depend on the ability of the non-infarcted region to remodel, adapt, and compensate for the loss of the infarcted region. As a result, the remaining viable myocardium in chronically infarcted hearts is remodeled, not only morphologically and functionally, but also biochemically (Dhalla et al., 1996; Laser et al., 1996; Zhang et al., 1996; Swynghedauw, 1999). Under normoxic conditions, cardiac myocytes produce most of their ATP by oxidative phosphorylation, making the regulation of this process central to balancing cardiac energy metabolism (Opie, 1991). It is estimated that approximately 60–70% of myocardial energy is obtained from the oxidation of fatty acids. However, during ischemia or hypoxia, the depleted oxygen supply suppresses β-oxidation of fatty acids, resulting in accumulation of harmful fatty acid metabolism intermediates, such as long-chain acylcarnitine and long-chain acyl CoA, without an effective increase in ATP production (Opie, 1979). In addition, the ischemic myocardium shifts its energy source from β-oxidation to glycolysis (Depre et al., 1999). Therefore, the inhibition of accumulation of harmful fatty acid

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metabolism intermediates and the facilitation of glucose utilization may be beneficial under specific conditions in which the oxygen supply is limited.

3-(2,2,2-trimethylhydrazinium) propionate (MET-88) is an inhibitor of γ-butyrobetaine hydroxylase that catalyzes the synthesis of carnitine from γ-butyrobetaine, and decreases the intracellular levels of long-chain acylcarnitine and long-chain acyl CoA (Simkhovich et al., 1988). We reported that MET-88 protected cardiac function with a reduced accumulation of harmful fatty acid metabolites in isolated perfused rat hearts under hypoxia (Asaka et al., 1998), and improved myocardiac energy metabolism in the ischemic dog heart (Kirimoto et al., 1996). In addition, it was suggested that MET-88 facilitates glucose utilization (Asaka et al., 1998; Yonekura et al., 1999). Thus, MET-88 may show a beneficial effect on the impaired myocardium under ischemic conditions. In fact, MET-88 protected against left ventricular dysfunction in isolated heart preparations from rats with congestive heart failure following myocardial infarction (Aoyagi et al., 1997). However, the effects of MET-88 have not yet been clarified with respect to such aspects as survival and ventricular remodeling, which are important parameters in congestive heart failure. The present study was designed to make this clarification.

2. Materials and methods

2.1. Induction of congestive heart failure

Myocardial infarction was produced according to a slight modification of the method published by several laboratories (Selye et al., 1960; Johns and Olson, 1954). Briefly, male Sprague–Dawley rats (Clea, Tokyo, Japan) ranging in age from 7 to 10 weeks were anesthetized with ether, and thoracotomy was performed. The left anterior descending coronary artery was ligated approximately 2 mm from its origin with a silk suture (5–0), and the thorax was immediately closed. Sham-operated (sham) rats were treated similarly, with the exception of coronary artery ligation. The congestive heart-failure rats were given orally vehicle (control) or MET-88 at 100 mg/kg for 181 (survival study) or 20 days (other studies). The administration was started from the 2nd day after surgery to avoid the possibility that the compounds could influence infarct size. For the histological study, we added a group treated with MET-88 at 50 mg/kg and one treated with captopril at 20 mg/kg. For the hemodynamic study, we also used MET-88 at 50 mg/kg.

2.2. Survival study

Rats were housed in clear stainless-steel cages (up to a maximum of two rats per cage) and placed according to therapy assignment and time of entry into the study. All animals were allowed free access to food and the assigned therapy (water or MET-88). The concentration of MET-88 solution was adjusted once a week so that the daily intake approximated 100 mg/kg/day. Each animal was followed until death or for up to 181 days after the treatment procedure.

2.3. Histological study

On the 22nd day after surgery, the hearts were arrested in diastole by infusion with a saturated solution of KCl into the femoral vein under ether anesthesia. The left ventricle (including the interseptum) was fixed in 10% phosphate-buffered formalin, and cut from apex to base into four transverse slices (interval: 2–3 mm). Sections of 50-μm thickness were cut and stained with Masson's trichrome. Left ventricular, infarct, and cavity areas, as in photomicrographs of each section, were measured with a computerized planimeter (Olympus, Tokyo, Japan). Left ventricular cavity volume was approximated by summing the cavity volumes of the transverse slices, which were calculated from the cavity area and the thickness of each transverse slice. Infarct size was expressed as a percentage of the left ventricular area.

2.4. Hemodynamic study

The hemodynamic study was performed according to the method reported on by Pfeffer et al. (1979). Rats were anesthetized with ether on the 22nd day after surgery. Polyethylene catheters were inserted into the right carotid artery, right jugular vein, and femoral vein and connected to transducer systems (right carotid artery, MPU-0.5A; right jugular vein, LPU-0.1A; Nihon Kohden). Left ventricular and right atrial pressure were measured with an amplifier (AP-620G, Nihon Kohden) connected to these transducers. The peak rate of rise of left ventricular pressure (peak left ventricular $+dP/dt$, peak left ventricular $-dP/dt$) was measured with a differentiator (ED-601G,

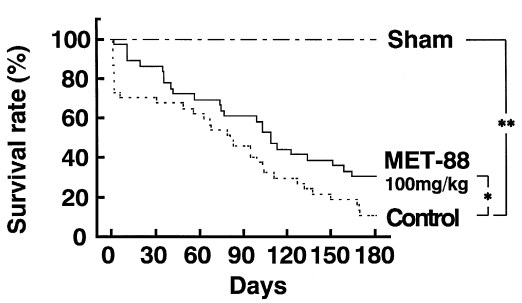

Fig. 1. Cumulative percent survival of sham, MET-88-untreated (control), and MET-88-treated heart-failure rats for 181 days. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. heart-failure control rats.

Table 1
Effects of MET-88 and captopril on body weight, ventricular weight and infarct size in rats with congestive heart failure
Data are shown as means ± S.E.M.

Parameter	Sham	Heart failure			
		Control	MET-88 50 mg/kg	MET-88 100 mg/kg	Captopril 20 mg/kg
n	13	23	23	20	25
Body weight (g)	390 ± 9	373 ± 6	379 ± 5	373 ± 7	363 ± 5
Right ventricular weight (mg)	212 ± 9[a]	301 ± 20	300 ± 20	279 ± 20	277 ± 18
Left ventricular weight (mg)	744 ± 24	694 ± 15	707 ± 11	691 ± 15	643 ± 11[a]
Infarct size (%)	–	26.5 ± 1.7	25.3 ± 2.0	25.3 ± 2.1	24.7 ± 2.1

[a] $P < 0.05$ vs. heart-failure control rats.

Nihon Kohden). Heart rate was measured with a tachometer (AT-600G, Nihon Kohden). Cardiac functions were first measured under basal conditions. Next, the measurement was performed under preload stress (volume load), i.e., warmed (37°C) saline was infused into the femoral vein at a rate of 40 ml/min/kg for 45 s with an infusion pump (STC-521, Terumo, Tokyo, Japan). Right atrial pressure was then measured during the infusion. Finally, data were collected under conditions of afterload stress (pressure load). In this case, the rats were artificially ventilated with a respirator, and thoracotomy was performed. The ascending aorta was occluded for 3 s with a suture placed around the aorta. Left ventricular pressure, peak left ventricular $+dP/dt$, and peak left ventricular $-dP/dt$ were measured during the aortic occlusion.

2.5. Biochemical study

The rats were anesthetized with ether and ventilated. After thoracotomy, the myocardium was rapidly frozen by

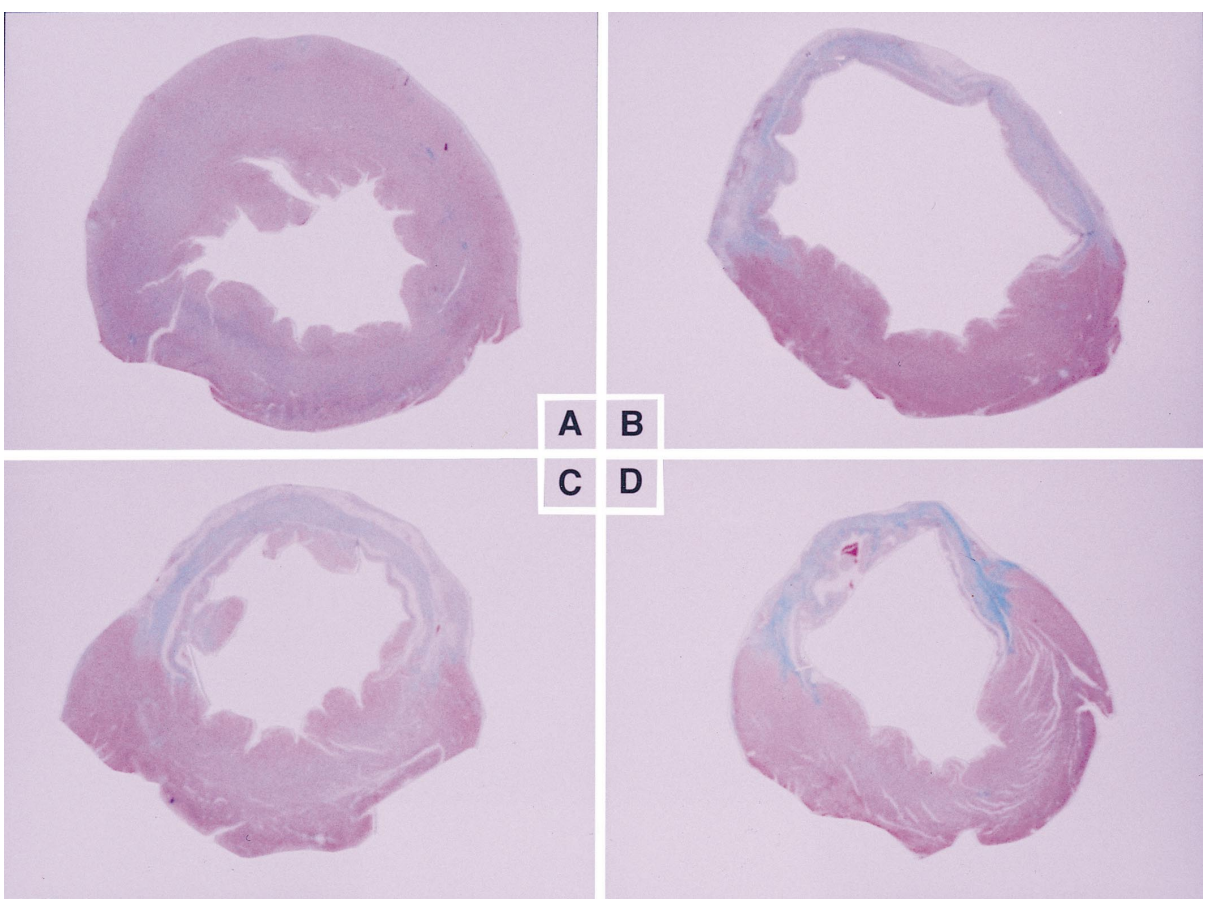

Fig. 2. Typical photomicrographs of the left ventricle from sham and congestive heart-failure rats. (A) sham rat; (B) heart-failure control rat; (C) heart-failure rats given MET-88 at 100 mg/kg; (D) heart-failure rats given captopril at 20 mg/kg.

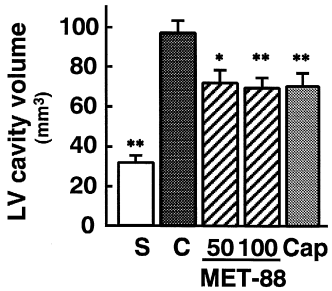

Fig. 3. Left ventricular cavity volume of rats with congestive heart failure treated with MET-88 and captopril. LV, left ventricular; S, sham rats ($n = 13$); C, heart-failure control rats ($n = 23$); MET-88 50, heart-failure rats given MET-88 at 50 mg/kg ($n = 23$); MET-88 100, heart-failure rats given MET-88 at 100 mg/kg ($n = 20$); cap, heart-failure rats given captopril at 20 mg/kg ($n = 25$); data are shown as means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. heart-failure control rats.

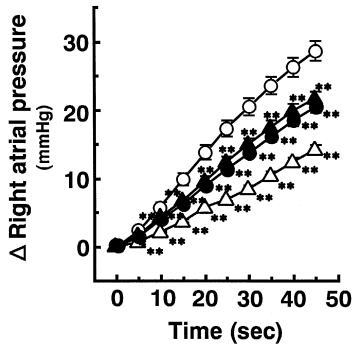

Fig. 4. Effects of MET-88 on the changes in the rise in right atrial pressure under preload stress in rats with congestive heart failure. Open triangles, sham rats ($n = 16$); open circles, heart-failure control rats ($n = 29$); closed triangles, heart-failure rats given MET-88 at 50 mg/kg ($n = 30$); closed circles, heart-failure rats given MET-88 at 100 mg/kg ($n = 23$); Data are shown as means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. heart-failure control rats.

pouring liquid nitrogen into a column surrounding the myocardium (column-freezing method: Sanbe et al., 1993). The isolated frozen myocardium was separated under liquid nitrogen cooling into three portions, i.e., scar, remaining left ventricle (including interseptum), and right ventricle, with an electric mini-drill. The frozen remaining left ventricle was pulverized and homogenized in 0.6 M perchloric acid. The homogenate was centrifuged at 15,000 rpm for 5 min at 4°C, and the supernatant was sampled for determination of tissue high-energy phosphates (AMP, ADP, and ATP) and lactate.

The levels of high-energy phosphates were measured according to the method of Sellevold et al. (1986) using high-performance liquid chromatography (LC-4A: Shimadzu, Kyoto, Japan). To estimate the energy state of the myocardial cell, we calculated the energy charge potential (ECP) according to the following equation

$$\mathrm{ECP} = (\mathrm{ATP} + 0.5\,\mathrm{ADP}) / (\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP}).$$

Lactate levels were measured spectrophotometrically with a commercial kit (Sigma 826-B, St. Louis, MO, USA).

2.6. Statistical analysis

All values are expressed as means ± S.E.M. One-way analysis of variance was used to assess the effects of multiple comparisons, followed by two-sided Dunnett's comparison test. Willcoxon's comparison test was used for the Kaplan–Meier survival curves.

3. Results

3.1. Survival study

Fig. 1 shows the cumulative percent survival of sham, MET-88-untreated (control), and MET-88-treated heart-failure rats. The initial number of animals in each group was 10, 37, and 36, respectively. In heart-failure control rats, the median 50% survival occurred on Day 79, and in the group given MET-88 at 100 mg/kg, the median 50% survival was significantly prolonged by 24 days (Day 103).

Table 2
Effects of MET-88 on cardiac function under the basal condition in rats with congestive heart failure
Data are shown as mean ± S.E.M.

Parameter	Sham	Heart failure		
		Control	MET-88 50 mg/kg	MET-88 100 mg/kg
n	16	29	31	23
Heart rate (bpm)	366 ± 8[a]	337 ± 7	341 ± 7	329 ± 7
Left ventricular pressure (mm Hg)	134 ± 5[b]	109 ± 3	112 ± 3	114 ± 4
Peak left ventricular $+dP/dt$ (mm Hg/s)	7155 ± 418[b]	4446 ± 231	4836 ± 259	4982 ± 248
Peak left ventricular $-dP/dt$ (mm Hg/s)	5528 ± 326[b]	3137 ± 162	3196 ± 147	3596 ± 159
Right atrial pressure (mm Hg)	0.78 ± 0.35[b]	4.48 ± 0.78	2.00 ± 0.54[b]	0.39 ± 0.13[b]

[a] $P < 0.05$ vs. heart-failure control rats.
[b] $P < 0.01$ vs. heart-failure control rats.

Table 3
Effects of MET-88 on the increase in right atrial pressure under preload stress in rats with congestive heart failure
Data are shown as means ± S.E.M.

Parameter	Sham	Heart failure		
		Control	MET-88 50 mg/kg	MET-88 100 mg/kg
n	16	29	30	23
Time to Δ10 mm Hg (s)	34.1 ± 1.3[a]	16.5 ± 1.1	22.0 ± 1.1[a]	23.3 ± 1.1[a]
Maximum right atrial pressure (mm Hg)	14.3 ± 0.6[a]	28.7 ± 1.4	21.8 ± 1.0[a]	20.5 ± 0.8[a]

[a] $P < 0.01$ vs. heart-failure control rats.

The final number of surviving rats in each group was 10, 4, and 11, respectively.

3.2. Histological study

Table 1 shows the body weight, left ventricular weight, right ventricular weight, and infarct size of each group on the 22nd day after surgery. Heart-failure control rats showed an increase in right ventricular weight compared with that from sham rats. There was a trend toward lower right ventricular weight in heart-failure rats treated with MET-88 at 100 mg/kg or captopril at 20 mg/kg. Treatment with MET-88 at 50 or 100 mg/kg had no effect on the left ventricular weight in heart-failure rats, whereas captopril at 20 mg/kg significantly decreased it. The infarct size was not significantly different among the four experimental heart-failure groups, indicating that MET-88 and captopril had no influence on infarct size, and the same results were also seen for the groups studied with respect to the other parameters.

Fig. 2 shows typical photomicrographs of Masson's trichrome-stained sections of the left ventricle from sham and heart-failure rats. On the 22nd day after ligation of the coronary artery, heart-failure control rats were characterized by thinning of the left ventricular wall in the infarcted region and expansion of the left ventricular cavity. The left ventricular cavity volumes are summarized in Fig. 3. The left ventricular cavity volume in heart-failure control rats was significantly greater than that in the sham rats. MET-88 at 50 and 100 mg/kg significantly reduced the increase in left ventricular cavity volume. Captopril at 20 mg/kg also significantly lessened the increased left ventricular cavity volume, with the magnitude of the reduction being equivalent to that achieved with MET-88.

3.3. Hemodynamic study

Cardiac functions under the basal condition are summarized in Table 2. Heart rate, left ventricular pressure, peak left ventricular $+dP/dt$, and peak left ventricular $-dP/dt$ decreased significantly and right atrial pressure increased significantly in heart-failure control rats compared with the values for the sham rats. MET-88 at 50 and 100 mg/kg significantly inhibited the rise in right atrial pressure.

The changes in the rise in right atrial pressure under preload stress (volume load) are shown in Fig. 4. In heart-failure control rats, right atrial pressure increased markedly compared with that in the sham rats. Treatment with MET-88 at 50 and 100 mg/kg significantly inhibited the rise in right atrial pressure, and MET-88 was more effective at 100 mg/kg than at 50 mg/kg. The time to Δ10 mm Hg right atrial pressure, i.e., the time required for the rise in right atrial pressure to reach 10 mm Hg, was markedly shorter in heart-failure control rats than in the sham rats (Table 3). Treatment with MET-88 at 50 and 100 mg/kg significantly prolonged the time to Δ10 mm Hg right atrial pressure. Also, the maximum right atrial pressure under preload stress increased significantly in heart-failure control rats compared with the value for the sham rats. MET-88 at 50 and 100 mg/kg significantly inhibited the increase in maximum right atrial pressure.

Under afterload stress (pressure load), the left ventricular pressure in heart-failure control rats (191 ± 5 mm Hg) was significantly lower than that in sham rats (245 ± 4 mm Hg). However, there was no significant difference in left ventricular pressure between the heart-failure control rats

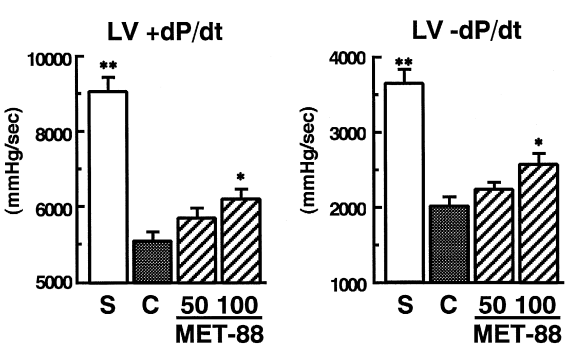

Fig. 5. Effects of MET-88 on peak LV $+dP/dt$ and peak LV $-dP/dt$ under afterload stress in rats with congestive heart failure. LV, left ventricular; S, sham rats ($n = 15$); C, heart-failure control rats ($n = 23$); MET-88 50, heart-failure rats given MET-88 at 50 mg/kg ($n = 27$); MET-88 100, heart-failure rats given MET-88 at 100 mg/kg ($n = 22$); data are shown as means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. heart-failure control rats.

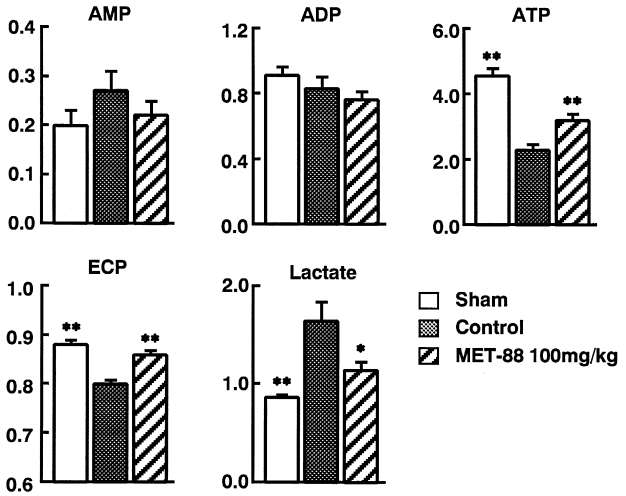

Fig. 6. Effects of MET-88 on myocardial high-energy phosphate and lactate levels in rats with congestive heart failure. ECP, energy charge potential; sham, sham rats ($n=7$); control, heart-failure control rats ($n=10$); MET-88, heart-failure rats given MET-88 at 100 mg/kg ($n=10$); units of AMP, ADP, and ATP are expressed as μmol/frozen tissue weight (g). Units of lactate are expressed as nmol/frozen tissue weight (g). Data are shown as means ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$ vs. heart-failure control rats.

and either MET-88-treated heart-failure rats (MET-88 at 50 mg/kg, 191 ± 4; MET-88 at 100 mg/kg, 199 ± 4 mm Hg). As shown in Fig. 5, the peak left ventricular $+dP/dt$ and peak left ventricular $-dP/dt$ under afterload stress decreased significantly in heart-failure control rats compared with the values for the sham rats. MET-88 at 100 mg/kg significantly attenuated the decreases in peak left ventricular $+dP/dt$ and peak left ventricular $-dP/dt$.

3.4. Biochemical study

Myocardial high-energy phosphate and lactate levels are shown in Fig. 6. In the heart-failure control rats, the myocardial ATP level in the viable left ventricle was significantly lower than that of sham rats. ECP was also significantly decreased in heart-failure control rats compared with that in the sham rats. MET-88 at 100 mg/kg significantly attenuated the decreases in the ATP level and the ECP in the myocardium. A significant increase in lactate level was found in the heart-failure control rats compared with the level for the sham rats, and this increase was attenuated by treatment with MET-88 at 100 mg/kg.

4. Discussion

The major findings of the present study were that MET-88, a γ-butyrobetaine hydroxylase inhibitor, (1) prolonged survival, (2) attenuated left ventricular chamber dilatation (ventricular remodeling), (3) augmented the adaptable capacity of cardiac function against increased load, and (4) improved cardiac energy states in rats with congestive heart failure following myocardial infarction.

Survival is the most important parameter in congestive heart failure. Only a few agents such as angiotensin-converting enzyme inhibitor, angiotensin II receptor antagonist, and endothelin receptor antagonist showed improvement of the survival in the same experimental model as used in this study (Pfeffer et al., 1985; Milavetz et al., 1996; Sakai et al., 1996). MET-88 significantly prolonged survival in heart-failure rats. However, MET-88 had no direct action on blood pressure or cardiac contractility, unlike the above-mentioned agents (Chiba et al., 1989; Nakano et al., 1999). Many experimental and clinical investigations have demonstrated that ventricular remodeling occurs after myocardial damage (Pfeffer and Braunwald, 1990; Ertl et al., 1993). Ventricular chamber dilatation is one of the features of ventricular remodeling. It has been reported that the degree of dilatation of the cardiac chamber correlates with both prognosis and survival rate of patients with myocardial infarction (White et al., 1987). Angiotensin-converting enzyme inhibitors such as captopril and enalapril are known to improve remodeling following myocardial infarction (McKay et al., 1986). Some clinical trials demonstrated that angiotensin-converting enzyme inhibitors showed favorable effects in patients with myocardial infarction (The CONSENSUS Trial Study Group, 1987; The SOLVD Investigators, 1991; Pfeffer et al., 1992). MET-88 suppressed dilatation of the left ventricular cavity induced by myocardial infarction. It is noteworthy that the effect of MET-88 on the attenuation of left ventricular chamber dilatation was almost equivalent to that of captopril at 20 mg/kg in this study. The inhibition of intrinsic production of angiotensin II interferes with the progression of disease (cardiac hypertrophy, etc.), and is considered to be one of the mechanisms of the beneficial effect exerted on remodeling by angiotensin-converting enzyme inhibitors (Childs et al., 1990). However, there is a difference between MET-88 and angiotensin-converting enzyme inhibitors, because MET-88 has no inhibitory effect on angiotensin-converting enzyme activity (Nakano et al., 1999).

Our hemodynamic studies indicated that cardiac function was impaired in heart-failure rats, and similar results were reported from a number of other studies (Pfeffer et al., 1979; Sanbe et al., 1993). Especially the elevated right atrial pressure in heart-failure rats introduces an increase in preload that can promote ventricular dilatation. MET-88 attenuated the increase in right atrial pressure in the heart-failure rats, which action may be involved in the reduction of ventricular dilatation. The rise in right atrial pressure under preload stress was increased in heart-failure rats compared with that in sham rats, indicating that cardiac function in the failing heart could not adapt against the load. As increasing load proceeds in a vicious circle that aids the progression of heart failure, it is important that

cardiac function be able to adapt against the increased load. Therefore, it is significant that MET-88 prevented the rise in right atrial pressure under preload stress. The same result was also observed in the hemodynamic study on the heart under afterload stress. We previously reported that MET-88 shifted the left ventricular end-systolic pressure–volume relationship in heart-failure rats toward that in sham rats in the isolated isovolumic heart taken from the same experimental model as used in this study (Aoyagi et al., 1997). These results indicate that MET-88 augments the adaptable capacity of cardiac function against the increased load.

The high-energy phosphate level in the remaining viable left ventricular tissue was decreased in the heart-failure rats, indicating derangement of the myocardial energy state (as evidenced by the decrease in the ECP value). The results of the present study are consistent with previous reports stating that the ATP level was decreased in heart-failure rats following myocardial infarction (Sanbe et al., 1993; Neubauer et al., 1995). As the alterations in high-energy phosphate levels in the myocardium are inversely correlated with the extent of left ventricular remodeling (Zhang and McDonald, 1995), the improvement of high-energy phosphate levels induced by MET-88 may have contributed to its beneficial effect on heart failure. It has been reported that MET-88 improved cardiac energy metabolism in the ischemic dog heart (Kirimoto et al., 1996), in isolated perfused rat hearts under hypoxia (Asaka et al., 1998), and in the isoproterenol-impaired rat heart (Simkhovich et al., 1988). These reports support the results of the present study. The limited oxygen supply suppresses β-oxidation of fatty acids during ischemia or hypoxia, resulting in accumulation of harmful fatty acid metabolism intermediates such as long-chain acylcarnitine and long-chain acyl CoA. Long-chain acylcarnitine and long-chain acyl CoA have been reported to affect adversely various enzymes such as Ca^{2+}-ATPase of the sarcoplasmic reticulum, Na^{+}, K^{+}-ATPase of the sarcolemma and adenine nucleotide translocase of mitochondria, all of which regulate cell function, resulting in cardiac dysfunction and derangement of the energy state (Shug et al., 1975; Wood et al., 1977; Lamers et al., 1984; Lopaschuk et al., 1983). Because MET-88 inhibits the accumulation of the harmful fatty acid metabolism intermediates, MET-88 can protect against damage to these enzymes. In addition, the ischemic myocardium shifts its energy source from β-oxidation to glycolysis. Under conditions of limited oxygen supply, it is important that the myocyte should efficiently utilize glucose oxidation, as β-oxidation of fatty acids is not available. A large number of studies have suggested that glycolysis protects cardiac myocytes from ischemic or hypoxic injury in vivo and in vitro (Eberli et al., 1991; Owen et al., 1990; Bekheit et al., 1993; Malhotra and Brosius, 1999). It has been also reported that ATP produced from the glycolytic pathway is important in the adjustment of the concentration of intracellular ions such as Ca^{2+} (Jeremy et al., 1992; Aasum et al., 1998). MET-88 increased the activity and the protein level of hexokinase in the viable myocardium in heart-failure rats following myocardial infarction (Yonekura et al., 1999). Also, MET-88 promoted glucose oxidation in isolated rat heart preparations under hypoxia (Asaka et al., 1998), suggesting an increase in pyruvate dehydrogenase activity. Thus, the reduction in lactate levels by MET-88 in this study may have resulted from an increase in pyruvate dehydrogenase activity. MET-88 has also been demonstrated to improve the cardiac mitochondrial function harmed by hypoxia/reperfusion and isoproterenol (Dhar et al., 1996; Hanaki et al., 1989). These results suggested that MET-88 could facilitate glucose utilization as the supply of energy. The beneficial effects of MET-88 in this study may have been due to the promotion of glucose utilization.

In conclusion, the present results indicated the beneficial effects of MET-88 in rats with congestive heart failure following myocardial infarction. The regulation of fatty acid oxidation and glucose utilization may be useful for treatment of congestive heart failure. Further studies are necessary to elucidate the mechanisms responsible for the beneficial effects of MET-88.

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