Bioenergetic protection of failing atrial and ventricular myocardium by vasopeptidase inhibitor omapatrilat

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Bioenergetic protection of failing atrial and ventricular myocardium by vasopeptidase inhibitor omapatrilat. Am J Physiol Heart Circ Physiol 290: H1686–H1692, 2006. First published December 9, 2005; doi:10.1152/ajpheart.00384.2005.—Deficient bioenergetic signaling contributes to myocardial dysfunction and electrical instability in both atrial and ventricular cardiac chambers. Yet, approaches capable to prevent metabolic distress are only partially established. Here, in a canine model of tachycardia-induced congestive heart failure, we compared atrial and ventricular bioenergetics and tested the efficacy of metabolic rescue with the vasopeptidase inhibitor omapatrilat. Despite intrinsic differences in energy metabolism, failing atria and ventricles demonstrated profound bioenergetic deficiency with reduced ATP and creatine phosphate levels and compromised adenylyl kinase and creatine kinase catalysis. Depressed phosphotransfer enzyme activities correlated with reduced tissue ATP levels, whereas creatine phosphate inversely related with atrial and ventricular load. Chronic treatment with omapatrilat maintained myocardial ATP, the high-energy currency, and protected adenylyl and creatine kinase phosphotransfer capacity. Omapatrilat-induced bioenergetic protection was associated with maintained atrial and ventricular structural integrity, albeit without full recovery of the creatine phosphate pool. Thus therapy with omapatrilat demonstrates the benefit in protecting phosphotransfer enzyme activities and in preventing impairment of atrial and ventricular bioenergetics in heart failure.

Methods

Procedures were designed in accordance with the National Institutes of Health guidelines and approved by the Mayo Institutional Animal Care and Use Committee. Experimental heart failure. CHF was produced in 18 male dogs by progressive rapid right ventricular pacing (46, 47, 55). Eight out of 18 dogs received omapatrilat therapy (CHF + OMA group). The remaining 10 dogs received no pharmacotherapy (CHF group). Dogs were anesthetized with thiopental sodium (20 mg/kg) and isoflurane (0.5–2.5%) and ventilated. An epicardial pacing lead was placed on the right ventricle via thoracotomy and connected to a generator (Medtronic Legacy 8161/8165). After a 2-wk long recovery, incremental ventricular pacing was imposed at 180 beats/min for 14 days, followed by pacing at 200, 220, and then 240 beats/min for 1 wk each for a total of 5 wk of pacing. In the treated group, omapatrilat (10 mg/kg, twice a day orally) was initiated at the start of the pacing protocol. Six normal dogs served as controls.

ECHOCARDIOGRAPHY. Cardiac ultrasound was performed in sinus rhythm in the conscious standing state. Left ventricular dimension (LVD) and ejection fraction were measured by two-dimensional-guided M-mode (47, 55). The LVD index, left atrial area index, and the mitral regurgitant jet size were measured from two-dimensional...
images in the parasternal long- and short-axis views. Images were digitally acquired and analyzed off-line (GE system 5, Horten, Norway). Five averaged measurements are reported for each parameter.

**Hemodynamics.** To record hemodynamic parameters, the pacemaker was turned off. Dogs anesthetized with pentobarbital sodium (25 mg/kg in the control group and 12.5 mg/kg in the heart failure groups, supplemented with 50-mg bolus titrated to effect) were intubated and ventilated. A Swan-Ganz catheter was advanced into the pulmonary artery via the right external jugular vein. Pressures and cardiac output readings were repeated at least three times and averaged. A high-fidelity Millar catheter was advanced into the left ventricle via the right femoral artery. Left ventricular pressure recordings were stored digitally and analyzed off-line (Sonoview, London, Canada). The femoral artery pressure was measured and monitored throughout.

**Bioenergetic profiling.** After sternotomy was completed, the pericardium was opened, and atrial biopsies were obtained from the right and left atrial appendages and free walls, whereas a ventricular biopsy was obtained from the anterior left ventricular wall. For atrial biopsies, tissue to be harvested was surrounded with sutures, and samples snipped with sutures were tightened to achieve hemostasis. For ventricular biopsies, an electrical biopsy drill (4 mm in diameter) was used to penetrate the anterior wall and to secure the whole thickness of the ventricular tissue (8). Tissue samples were immediately frozen in liquid nitrogen, were done by using the Hydragel ISO-CK K20 kit and quantitation of CK isoforms in myocardial extracts, stored in liquid nitrogen, were done by using the Pearson correlation coefficient if data were normally distributed or the Spearman correlation coefficient if they were not. A P value <0.05 was predetermined.

**RESULTS**

**Atrial versus ventricular bioenergetic profiles.** Catalytic activities of the major phosphotransfer enzymes, CK and adenylate kinase, were consistently higher in the normal left ventricle compared with the normal atrium, i.e., by 2.8- and 1.6-fold, respectively (n = 6; P < 0.001, Table 1). The normal ventricular myocardium also had a higher CK over adenylate kinase activity ratio compared with normal atria, i.e., 32 versus 19, respectively, indicating a larger contribution of CK-catalyzed phosphotransfer supporting ventricular energy metabolism. Ventricular compared with atrial myocardium displayed a higher mitochondrial CK (CKmit) isoform fraction, 13 ± 1% versus 5 ± 1%, respectively (P < 0.01). Conversely, atrial myocardium compared with ventricular tissue contained higher creatine kinase CK-BB and CK-MB isoform fractions, 3 ± 1% and 16 ± 1% versus 1 ± 1 and 8 ± 1%, respectively (P < 0.01). Myocardial ATP, GTP, CrP, and total creatine levels were higher in left ventricular biopsies than in corresponding atrial samples, by 1.9-, 1.4-, 1.9-, and 1.8-folds, respectively (P = 0.003, 0.001, 0.003, and 0.001; Table 1). In addition, ATP-to-ADP, GTP-to-GDP, and CrP-to-Pi ratios were all higher in the left ventricle compared with atrial tissue, indicating overall a higher ventricular energetic potential.

Taken together, higher CK and adenylate kinase activities and elevated ATP-to-ADP and CrP-to-Pi ratios in the ventricular myocardium may indicate efficient metabolic cycling between ATP-generating and ATP-consuming sites compared with atrial muscle. Thus direct comparison of atria versus ventricles indicates unique energetic profiles of cardiac chambers, with ventricles characterized by an apparently more robust energetic potential and phosphotransfer ability to control ATP-to-ADP ratios in subcellular locales.

**Altered bioenergetics in failing myocardium.** Chronic pacing induced significant left ventricular systolic dysfunction, left ventricular dilatation and hypertrophy, as well as left atrial dilatation, typical signs of heart failure (Table 2). As a consequence, left ventricular end-diastolic filling pressure (LVEDP) increased by 19.2 ± 20.3 mmHg. A significant decrease in peak rate of LV pressure change (dP/dt peak) and time constant of LV relaxation (τ, ms) were noted in CHF and CHF + Oma groups compared with the controls (Table 2).

**Table 2. Effects of omapatrilat on hemodynamic and structural remodeling in heart failure**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>CHF</th>
<th>CHF + Oma</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>4.2 ± 1.5</td>
<td>20.4 ± 1.5†</td>
<td>14.3 ± 2.2†</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>153 ± 7</td>
<td>119 ± 7†</td>
<td>110.0 ± 2.4†</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4.9 ± 2.0</td>
<td>28.1 ± 5.1†</td>
<td>22.1 ± 2.0†</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>2.783 ± 275</td>
<td>1.675 ± 191†</td>
<td>1.332 ± 53†</td>
</tr>
<tr>
<td>τ, ms</td>
<td>28.1 ± 3.3</td>
<td>72.4 ± 20.8†</td>
<td>78.0 ± 6.9†</td>
</tr>
<tr>
<td>LVES</td>
<td>0.66 ± 0.03</td>
<td>0.31 ± 0.04†</td>
<td>0.29 ± 0.02†</td>
</tr>
<tr>
<td>LA area index, mm2/kg</td>
<td>0.60 ± 0.04</td>
<td>0.91 ± 0.06†</td>
<td>0.71 ± 0.04*</td>
</tr>
<tr>
<td>LV dimension index, mm/kg</td>
<td>0.18 ± 0.01</td>
<td>0.21 ± 0.01†</td>
<td>0.19 ± 0.01*</td>
</tr>
<tr>
<td>LV mass index, g/kg</td>
<td>4.2 ± 0.2</td>
<td>5.0 ± 0.2†</td>
<td>4.3 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. CHF, congestive heart failure; Oma, omapatrilat; PCWP, pulmonary capillary wedge pressure; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; +dP/dt, peak rate of LV pressure change; τ, time constant of LV relaxation; LVEF, LV ejection fraction; LA, left atrial. *P < 0.05, CHF + OMA vs. CHF; †P < 0.05 vs. control.
and pulmonary capillary wedge pressure (PCWP) were significantly increased in the CHF group (Table 2). Mild to moderate mitral regurgitation was also uniformly observed, characteristic of overt heart failure. Atrial and ventricular biopsies from failing hearts displayed profound bioenergetic deficiencies, characterized by a reduced phosphotransfer capacity coupled with a significant nucleotide imbalance (Figs. 1 and 2). The main energy reserve molecule CrP was markedly decreased by 70% in failing atrial and ventricular specimens ($P < 0.05$, Figs. 1 and 2). This reduction was accompanied by depletion of total creatine content by 48% and 55% in atria and ventricles, respectively ($P < 0.05$). The CrP-to-creatine ratio, which in control atria and ventricle was $1.1 \pm 0.1$ and $1.3 \pm 0.1$, in heart failure was reduced to $0.6 \pm 0.1$ and $0.5 \pm 0.1$ ($P < 0.01$), respectively (Table 1). The principal energetic currency ATP was also reduced by 35% ($P < 0.05$) in failing atria and by

Fig. 1. Omapatrilat (Oma) treatment prevents congestive heart failure (CHF)-induced reduction in atrial myocardial creatine kinase (CK; $A$) and adenylate kinase (AK; $B$) activities and ATP levels (C) with limited effect on creatine phosphate (CrP) levels (D). *$P < 0.05$ vs. control; #$P < 0.05$ vs. CHF.

Fig. 2. Oma treatment prevents CHF-induced decline in left ventricular (LV) myocardial CK (A) and AK (B) activities and ATP levels (C) yet does not restore CrP levels (D). *$P < 0.05$ vs. control; #$P < 0.05$ vs. CHF.
23% ($P < 0.05$) in failing ventricles. Myocardial GTP levels remained unchanged, indicating a preserved biosynthetic capacity in atria and ventricles. Ventricular adenylate kinase and CK activities, however, were diminished by 38% ($P < 0.05$) and 42% ($P < 0.01$), respectively, compared with normal hearts. Atrial adenylate kinase and CK activities in failing hearts were lower by 21% ($P < 0.05$) and 23% ($P = 0.09$), respectively, compared with normal hearts. In heart failure, in atrial and ventricular myocardium, the CK$_{mt}$ isom form fraction dropped to 3 ± 1% and 9 ± 1% of total CK activity, a reduction of 41% and 34% ($P < 0.01$), respectively, whereas the CK-MB fraction increased to 22 ± 1% and 13 ± 1%, respectively. Thus heart failure is associated with a significant bioenergetic deficit and remodeling of CK isoform composition in both atrial and ventricular chambers.

**Omapatrilat prevents maladaptive remodeling and preserves high-energy substrates and phosphotransfer capacity in heart failure.** Chronic treatment with the vasopeptidase inhibitor omapatrilat significantly reduced atrial and ventricular remodeling, indicated by the attenuation of left ventricular and left atrial dilation, as well as by the reduction of myocardial hypertrophy (Table 2). Hemodynamics, measured at the trough level of omapatrilat treatment 12 h after the last dose, were similar in the treated and untreated groups (Table 2). When compared with the nontreatment of failing hearts, chronic omapatrilat treatment protected myocardial levels of ATP and the catalytic activities of adenylate kinase and CK in both atria and ventricles (Figs. 1 and 2). In omapatrilat-treated hearts, these bioenergetic parameters remained essentially at control levels despite the imposed load of heart failure, suggesting a significantly lower presence of damaging factors and/or an improved protection from protease activity or oxidant injury (5, 28, 37). In fact, high-energy ATP levels and the phosphotransfer catalytic activities of adenylate kinase and CK, which were closely paralleled, were substantially reduced in heart failure but significantly improved by omapatrilat therapy (Fig. 3, A and B). Despite omapatrilat treatment, however, reduction in CrP levels was still persistent, although CK activity per se was fully maintained. This was associated with poor recovery of the CK$_{mt}$ isom form fraction, to 4.1 ± 0.4% in atria and to 9.9 ± 0.8% in ventricles, not significantly different from untreated failing hearts. The total myocardial creatine content was also only partially improved with omapatrilat treatment and remained lower by 30% and 45% ($P < 0.05$) in atria and ventricles, respectively, compared with values in corresponding heart chambers from controls (Table 1). A persistent deficit in creatine metabolism, after omapatrilat treatment, may indicate a possibly dysfunctional creatine transporter (50) and/or CK-adenine nucleotide translactor functional interaction at the mitochondrial site, which would compromise CrP generation despite the recovery of total CK activity (25). Along with this suggestion are lower atrial and ventricular CrP-to-creatine ratios, which after omapatrilat treatment were 0.7 ± 0.1 and 0.6 ± 0.1, respectively ($P < 0.05$). Because cellular ATP levels recovered to near control values, this suggests that the mitochondrial content and the ATP production capacity were maintained with omapatrilat treatment. CrP levels were inversely proportional to PCWP and LVEDP, indicating that this bioenergetic parameter reflects myocardial energy consumption and energy delivery through the CK-CrP shuttle, depending on the degree of atrial or ventricular load (Fig. 3, C and D). Thus, although it may fall short in supporting in its entirety the whole bioenergetic system of a cardiomyocyte, chronic treatment with the vasopeptidase inhibitor omapatrilat prevents the impairment of several critical atrial and ventricular energetic parameters characteristic of the failing heart.

![Fig. 3](http://ajpheart.physiology.org/)

**Fig. 3.** Linkage among bioenergetic parameters and between bioenergetics and myocardial performance in atria and ventricle. **A** and **B**: correlation between LV ATP concentration and CK and AK activities (**B**). **C** and **D**: correlation between pulmonary capillary wedge pressure (PCWP) and atrial CrP and between LV end-diastolic pressure (LVEDP) and LV CrP in control, CHF, and treated (Oma) CHF dogs. **P < 0.01.**
This study shows that heart failure produces significant bioenergetic deficits in atria and ventricles, preventable, to a large extent, by chronic treatment with the vasopeptidase inhibitor omapatrilat. Although the normal ventricle exhibited a higher energetic potential, compared with the atrium that was characterized by a lower energetic potential and a lower phosphotransfer capability to control ATP-to-ADP ratios, both myocardial chambers responded to heart failure with a severe but overall similar maladaptive rearrangement in cell bioenergetics. Yet, ATP levels and phosphotransfer enzyme activities in both atria and ventricles were effectively protected by omapatrilat, indicating that this prototypic vasopeptidase inhibitor is useful in maintaining metabolic homeostasis in heart failure.

This study compared side-by-side atrial and ventricular energetic metabolism in a large animal model. Myocardial ATP, CrP, total creatine, and phosphotransfer enzymes in the normal ventricle were found twofold higher compared with those in the normal atrium. Ventricular myocardium displayed a higher CK_{max} fraction compared with atrial tissue. Conversely, atrial myocardium contained higher CK-BB and CK-MB isofrom fractions in line with previous reports (1, 26). This is in accord with the notion that the vigorous contractility of the ventricle, compared with that of a rather more passive atrial chamber with a thinner wall, requires a higher rate of energy supply to fulfill a sustained pumping function. Moreover, tissue content of Pi in the ventricle was less than half of that in the atrium, reflecting a requirement for a higher phosphorylation potential and energy of ATP hydrolysis (7, 29, 49) to support ventricular contractility.

The syndrome of heart failure, induced here by rapid ventricular pacing, is a generalized model of dilated cardiomyopathy in the intact animal, characterized by atrial and ventricular dilatation with severely depressed left ventricular systolic function and increased left ventricular filling pressure (8, 55). With the challenge of heart failure, profound bioenergetic deficits were manifested in the ventricle by the reduced activities of phosphotransfer enzymes, depletion of high-energy phosphoryls, and reduction in cellular energetic potential. This is in accord with reports that demonstrate ventricular energetic abnormalities as a metabolic denominator of cardiovascular disease (11, 12, 15, 18, 20, 21, 29, 30, 49). In cardiomyopathy, the increase in wall stress (dilatation) and muscle mass (hypertrophy) raises the myocardial energy demand while reducing the availability of myocardial energy supply due to increased intercapillary distance, fibrosis, and myocyte diameter (22, 32).

As reported in cardiomyopathic models, the energy reserve stored in the form of CrP, a key component of the high-energy phosphoryl pool, is first depleted to preserve ATP that is directly utilized for myocardial contraction and electrical integrity (18, 30, 31). In fact, the present study indicates that CrP, rather than ATP levels per se or the activities of the phosphotransfer enzymes CK and adenylate kinase, inversely relates to myocardial wall stress of atrial and ventricular chambers, indicating that this metabolic marker reflects altered energy consumption and CK flux associated with myocardial load (17, 56). Here we demonstrate the profile of bioenergetic distress in failing atria, with induced changes directionally comparable with those observed in ventricles in accord with previous reports (26). Depletion of ATP linearly corresponded to a marked reduction of CK and adenylate kinase activities in both failing atria and ventricles, indicating simultaneous and parallel impairment in total ATP content and in the capacity of high-energy phosphoryl transfer. This observation supports the notion that reduction of CK and adenylate kinase activities, two enzymes that facilitate ATP delivery and promote removal of ADP, Pi, and protons from cellular ATPases, engages the failing myocardium into a state of phosphotransfer deficit. The mechanisms responsible for ATP reduction in the failing myocardium may involve depletion of the adenine nucleotide pool in a compensatory role to sustain the phosphotransfer potential and the ATP-to-ADP ratio (43). Ultimately, however, the inability of the failing myocardium to sustain ATP and CrP levels leads to depletion of intracellular high-energy phosphoryls (11, 16, 20, 53), contributing to organ failure.

These findings reflect the dependence of cardiac performance not necessarily on concentrations of molecules carrying high-energy phosphoryls or on phosphotransfer enzyme activity but rather on the actual flux of high-energy phosphoryls (12, 20, 29, 30, 36, 53). Optimal function and communication between components of the bioenergetic cellular system are requisites for performance supported by complementation in phosphotransfer enzyme activity and intimate interaction of phosphotransfer proteins with cellular sites of metabolic sensing and/or transduction (12, 15, 35, 42, 59, 60). In failing hearts, a reduction in CK flux is associated with dysfunction in metabolic sensors despite a compensatory increase in adenylate kinase flux or glycolytic potential (15, 18, 45). Although a deficit in CK compromises energetic efficiency (16, 38), the plasticity in cellular bioenergetics maintains myocardial function, even at low ATP levels, providing that phosphotransfer systems are operational (19, 24). Here we demonstrate that the total phosphotransfer capacity correlates with myocardial CrP and ATP levels. This indicates that a higher CK and adenylate kinase-mediated catalysis is necessary to maintain high-energy phosphoryl-carrying pools, apparently through reporphorylation, thus preventing loss by degradation and/or through efflux pathways (34–36, 50). This is in accord with phosphotransferase enzyme knockout studies where genetic ablation of a single isofrom compromises the cellular adenine nucleotide content (34).

Significantly, we revealed a previously unrecognized aptitude of omapatrilat treatment to maintain ATP levels and the phosphoryl transfer function of CK and adenylate kinase in failing atria and ventricles. Such favorable outcome could, in principle, relate to the established omapatrilat-induced prevention of remodeling via vasopeptidase inhibition (7, 29), reducing oxidative stress and demands of myocardial mechanics for high-energy ATP. Organ failure gives rise to a vigorous neurohumoral response, exaggerated RAAS activity, insufficiency of the natriuretic peptide system, and an excess in circulating levels of catecholamines (51). The class of vasopeptidase inhibitors, exemplified by omapatrilat, suppresses RAAS while accentuating plasma natriuretic peptides through combined inhibition of the angiotensin-converting enzyme and vasopeptidase activity (4, 5, 51). Chronic intervention with omapatrilat reduced atrial and ventricular chamber dilatation and decreased myocardial mass, indicating a significant pressure and volume unloading effect in the setting of heart failure. Moreover, omapatrilat also preserved transcellular high-energy transfer by...
maintaining adenylate kinase and/or CK activity to compensate for the ATP requirements of the failing myocardium at the expense of energy reserve. Such metabolic benefit is further supported by the observation of improved myocardial ATP content in omapatrilat-treated versus untreated failing hearts. These findings are in line with reports that suggest an ability of angiotensin-converting enzyme inhibitors to attenuate oxidative damage and preserve heart mitochondrial function (9, 37). It has also been recognized that CK$_{\text{mit}}$ is among the most sensitive enzymes to oxidative damage (41) and was not fully protected by omapatrilat treatment. Poor protection of CK$_{\text{mit}}$ activity and of total creatine content could explain the uncompensated deficit in the creatine phosphate pool with therapy in heart failure. Despite the absence of full protection of the whole energetic system, the present findings reveal a distinct metabolic advantage of omapatrilat therapy and provide evidence for potential benefits that are beyond an exclusive action of the drug on neurohumoral regulation (27, 28, 54).

In summary, the present study demonstrates differences in the bioenergetic profiles of atrial versus ventricular myocardium in a large animal model and marked metabolic derangements in both chambers in the syndrome of heart failure. Omapatrilat, a multipotent vasopeptidase inhibitor, is here identified to possess bioenergetic antiremodeling effects, preserving myocardial phosphotransfer enzyme activities and ATP levels, while maintaining atrial and ventricular structural integrity under the stress of heart failure. This previously unrecognized benefit of omapatrilat on energy metabolism identifies a potentially useful therapeutic approach in preserving the myocardial energetic balance in the setting of heart failure.

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