Adenylate Kinase–Catalyzed Phosphotransfer in the Myocardium
Increased Contribution in Heart Failure

Petras P. Dzeja, Konradas T. Vitkevicius, Margaret M. Redfield, John C. Burnett, Andre Terzic

Abstract—Although the downregulation of creatine kinase activity has been associated with heart failure, creatine kinase–deficient transgenic hearts have a preserved contractile function. This suggests the existence of alternative phosphotransfer pathways in the myocardium, the identity of which is still unknown. In this study, we examined the contribution of adenylate kinase–catalyzed phosphotransfer to myocardial energetics. In the isolated mitochondria/actomyosin system, which possesses endogenous adenylate kinase activity in both compartments, substrates for adenylate kinase promoted the rate and amplitude of actomyosin contraction that was further enhanced by purified adenylate kinase. Inhibition of adenylate kinase activity diminished both actomyosin contraction and mitochondrial respiration, which indicated reduced energy flow between mitochondria and myofibrils. In intact myocardium, the net adenylate kinase–catalyzed phosphotransfer rate was 10% of the total ATP turnover rate as measured by 18O-phosphoryl labeling in conjunction with gas chromatography and mass spectrometry. In pacing-induced failing heart, adenylate kinase–catalyzed phosphotransfer increased by 134% and contributed 21% to the total ATP turnover. Concomitantly, the contribution by creatine kinase dropped from 89% in normal hearts to 40% in failing hearts. These phosphotransfer changes were associated with reduced levels of metabolically active ATP but maintained overall ATP turnover rate. Thus, this study provides evidence that adenylate kinase facilitates the transfer of high-energy phosphoryls and signal communication between mitochondria and actomyosin in cardiac muscle, with an increased contribution to cellular phosphotransfer in heart failure. This phosphotransfer function renders adenylate kinase an important component for optimal myocardial bioenergetics and a compensatory mechanism in response to impaired intracellular energy flux in the failing heart. (Circ Res. 1999;84:1137-1143.)

Key Words: heart failure □ bioenergetics □ adenylate kinase □ creatine kinase □ mitochondria □ actomyosin

In cardiomyocytes, the generation of ATP primarily occurs in the mitochondria, which are separated from ATP-consuming sites in the myofibrils.1 To ensure communication between sites that generate and use ATP, it has been hypothesized that cells rely on phosphotransfer networks that facilitate the transfer and distribution of energy-rich phosphoryls between cellular compartments in a kinetically and thermodynamically efficient manner.2–7 Several studies have indicated that most phosphoryls are transferred through multiple creatine kinase–catalyzed phosphoryl exchanges that involve creatine phosphate and ATP.8–11

In fact, this enzyme is essential in supporting cardiac bioenergetics,5,7,10,12 and a decrease in energy reserve due to abnormalities in creatine kinase may contribute to heart failure.13–15 However, neither the suppression of creatine kinase activity7 nor the disruption of genes that encode the cytosolic and mitochondrial creatine kinase isoforms8,16–20 produce overt ventricular dysfunction. This suggests that alternative phosphotransfer routes may also support cardiac function. The identity of such phosphotransfer systems and their contribution to myocardial bioenergetics in the normal or failing heart remain unknown.

A candidate enzyme is adenylate kinase,4,21–23 which in skeletal muscle can transfer from 3% to 23% of the high-energy phosphoryls, which depends on the functional load of the muscle.8,22,24 This enzyme catalyzes the reversible reaction 2ADP→ATP+AMP and may process metabolic signals associated with ATP use,2,4,21,26,27 In this case, adenylate kinase has been implicated in the regulation of metabolically sensitive ion channels and transporters,3,28–30 In addition, disruption of the adenylate kinase gene impedes the export of ATP from the mitochondria.31 Although in the heart, isoforms of this enzyme are found in the mitochondria, cytosol, and membranes,21,28,32 it is unknown whether adenylate kinase contributes to the delivery of ATP to ATP-consuming sites.

Therefore, we examined the contribution of adenylate kinase to the transfer of energy-rich phosphoryls in an isolated mitochondrial/actomyosin system and in intact car-
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diac muscle. We report that adenylate kinase accelerates energy transfer and promotes communication between mitochondria and actomyosin, with an increased contribution to cellular phosphotransfer in the failing heart. This indicates a previously unrecognized function for this enzyme in cardiac muscle.

Materials and Methods

Isolated Actomyosin/Mitochondrial System

Mitochondria and actomyosin were isolated from rabbit (Kaunas Medical Academy, Kaunas, Lithuania) hearts.33,34 Mitochondria (at 0.08 mg of protein per mL) were coinoculated with actomyosin (0.5 mg/mL) in (mmol/L): sucrose 300, Tris-HCl 10 (pH 7.4), KH₂PO₄ 5, MgCl₂ 3, α-ketoglutarate 4, and EGTA-Ca²⁺ 3. To maintain a steady flow of high-energy phosphoryls between mitochondria and actomyosin, conditions were chosen to match ATP consumption by actomyosin ATases with ATP production by mitochondria. The amount of actomyosin was optimal for spectrophotometric recording of contraction, which was based on changes in absorbance as an indicator of actin-myosin interaction.35 Contraction was initiated by 100 μmol/L ADP or 10 μmol/L ATP and expressed as an increase in absorbance at 540 nm.34 Mitochondrial respiration was measured by O₂ electrodes.33 The respiratory control ratio of mitochondria-oxidizing NAD-dependent substrates was 6.98 ± 0.41 and 11.10 ± 0.62 (n = 10) with and without Mg²⁺, respectively.

Heart Failure

To induce heart failure, dogs were paced at incremental rates during 38 days.36,37 Ventricular pacing was initiated at 180 bpm for 10 days. The rate was increased at 7-day intervals to 200, 210, 220, and 240 bpm. This protocol results in severe heart failure with systolic and diastolic dysfunction; reduced ejection fraction, blood pressure, and cardiac output; and elevation in left ventricular filling pressure.36,37 Samples that contained phosphoryls of γ-ATP, β-ATP, β-ADP, inorganic phosphate, and creatine phosphate were purified and quantified with HPLC.30 The γ-phosphoryl of ATP was transferred to glycero kinase, and the β-phosphoryls of ATP and ADP were transferred to glycero by a combined catalytic action of adenylate kinase and glycero kinase. The phosphoryl of creatine phosphate was transferred to γ-ATP by creatine kinase and then to glycero with glycero kinase. Samples and the net rate of adenylate kinase catalyzed phosphotransfer was estimated from the initial rate of appearance of creatine phosphate species with γ-O-labeled phosphoryl.35 The net rate of creatine kinase–catalyzed phosphotransfer was determined from the initial rate of appearance of creatine phosphate species with γ-O-labeled phosphoryl.35

Statistical Analysis

Data are expressed as mean±SEM. Student t test for unpaired samples was used for statistical analysis, and a difference at P<0.05 was considered significant.

Results

Adenylate Kinase–Mediated Communication Between Isolated Actomyosin and Mitochondria

Isolated mitochondrial (n=5) and actomyosin (n=10) preparations possessed endogenous adenylate kinase activity of 469±49 and 83±5 nmol ATP=min⁻¹·mg⁻¹ protein, respectively, and were coincubated at protein concentrations in which the mitochondrial/myofibrillar ratio of adenylate kinase activity was 1:1. When ADP, a substrate of adenylate kinase and mitochondria, was added to the mitochondrial/actomyosin system, actomyosin contraction was promoted, as observed by increased absorbance of the actomyosin complex (Figure 1A, curve 1). Treatment with Ap₅A slowed the development and decreased the amplitude of actomyosin contraction (Figure 1A). The rate and amplitude of contraction were 150±25 absorbance units (AU)=min⁻¹ and 0.089±0.004 AU in the absence versus 21±3 AU=min⁻¹ and 0.015±0.001 AU in the presence of Ap₅A (n=10; Figure 1B). Values measured in the presence of Ap₅A were significantly lower than those obtained in the absence of the

Phosphoryl Flux in Intact Heart Muscle

ATP turnover and phosphoryl flux through adenylate and creatine kinases were measured in intact cardiac muscle with the ³²O-phosphoryl-labeling technique.8 This procedure is based on the incorporation of a ³²O atom in inorganic phosphate with each act of ATP hydrolysis and the distribution of ³²O-labeled phosphoryls among high-energy phosphoryl-carrying molecules that depend on the flux via specific phosphotransfer reactions.24,25 Therefore, a method to quantify flux through individual enzymatic pathways is to monitor the kinetics of ³²O-phosphoryl–oxygen exchange.22 Ventricular slices (1- to 2-mm thick) were washed 3 times, preincubated (15 minutes, 37°C) in oxygenated Krebs-Henseleit buffer (118 mmol/L NaCl; 4.7 mmol/L KCl; 1.8 mmol/L CaCl₂; 1.2 mmol/L MgCl₂; 0.5 mmol/L EDTA; 25 mmol/L NaHCO₃; 11 mmol/L glucose; and 10 mmol/L insulin, pH 7.45), and transferred into 95% O₂/5% CO₂ saturated buffer, in which 0% to 30% of water was replaced with 18O-containing water. After 1, 2, 4, and 6 minutes of incubation, slices were freeze-clamped, pulverized in mortar with liquid N₂, and extracted.8 Cellular ATP, ADP, inorganic phosphate, and creatine phosphate were purified and quantified with HPLC.30 The ³²O-enrichment of phosphoryls in glycerol 3-phosphates was determined with a Hewlett-Packard 5980B gas chromatograph-mass spectrometer operated in the select ion-monitoring mode. Mass ions (m/z) of 357, 359, 361, and 363 that corresponded to phosphoryl species of ³²O₆O, ³²O₅O, ³²O₄O, and ³²O₃O were monitored. The trimethylsilyl derivative of orthophosphate yielded mass ions (m/z) of 299, 301, 303, 305, and 307 that corresponded to species of orthophosphate that contained 0, 1, 2, 3, and 4 atoms of ³²O. The percentage of nucleotide phosphoryl oxygen replaced by ³²O was calculated as [(% ³²O₆O+2(% ³²O₅O)+3(% ³²O₄O))/135(% ³²O₆O)×H₂O].30 Total cellular ATP turnover was estimated from the sum of the total number of ³²O atoms that appeared in phosphoryl-containing metabolites and orthophosphate.9,24 The net rate of adenylate kinase–catalyzed phosphotransfer was estimated from the initial rate of appearance of ³²O-containing β-phosphoryls in ADP and ATP.8 The net rate of creatine kinase–catalyzed phosphoryl transfer was determined from the initial rate of appearance of creatine phosphate species with ³²O-labeled phosphoryls.8,24
adenylate kinase inhibitor (P<0.001). The addition of exogenous, purified, adenylate kinase (7 U/mL) significantly (P<0.001) accelerated the rate (325 ± 49 AU⁻³ · min⁻¹) and increased the amplitude (0.138 ± 0.006 AU) of contraction (Figure 1A and 1B); both effects were inhibited by Ap 5 A (not shown). Thus, adenylate kinase activity promotes actomyosin contraction, which could be due to local regeneration of ATP and/or increased transfer of ATP from mitochondria to myosin ATPases.

To distinguish between these 2 interrelated functions of adenylate kinase, the local regeneration of ATP versus the spatially-directed transfer of nucleotides between mitochondria and actomyosin, we examined whether adenylate kinase activity promotes contraction under conditions in which ATP regeneration is minimal and diffusional limitations exist for adenine nucleotide exchange between mitochondria and actomyosin. In the absence of mitochondria, when actomyosin was induced to contract by the addition a low concentration of ATP (10 μmol/L), the rate and amplitude of contraction were 167 ± 6 AU⁻³ · min⁻¹ and 0.051 ± 0.003 AU (n=5; Figure 2A). In the continued absence of mitochondria and presence of Ap 5 A, the rate and amplitude of contraction were 161 ± 6 AU⁻³ · min⁻¹ and 0.043 ± 0.002 AU (n=8; Figure 2A). Values obtained in the absence and presence of adenylate kinase inhibitor were not significantly different (P>0.05), which suggested that in the absence of mitochondria and at a low concentration of ATP, the ATP-regenerating function of adenylate kinase is minimal. Additional mitochondria in the continued presence of Ap 5 A accelerated the rate of contraction to 216 ± 5 AU⁻³ · min⁻¹ and increased the amplitude of contraction to 0.065 ± 0.002 AU (P<0.001; Figure 2), which suggested that to some extent energy can be transferred by simple diffusion or other phosphotransfer systems in the absence of adenylate kinase-catalyzed phosphotransfer. In the presence of mitochondria but absence of Ap 5 A, the rate and amplitude of contraction were 282 ± 9 AU⁻³ · min⁻¹ and 0.086 ± 0.003 AU (n=8); these values are significantly higher than those measured in the presence of Ap 5 A (P<0.001; Figure 2A). Thus, in the reconstituted system, adenylate kinase promotes the delivery of ATP from mitochondria to actomyosin ATPases even in the absence of a major effect on ATP regeneration.

To assess whether adenylate kinase contributes to feedback communication between actomyosin and mitochondria, mitochondrial respiration was measured in the absence and presence of adenylate kinase.
presence of adenylate kinase inhibition. In the absence of Ap3A, mitochondria respired at 203±10 nanoatoms (natom) \(O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\). This value is between values that correspond to state 4 (49±3 natom \(O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\)) and state 3 (351±27 natom \(O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\)) of the mitochondrial respiratory rate determined in the absence and presence of ADP (200 \(\mu\text{mol/L}\)), respectively. This indicates that mitochondria participate in the regeneration of ATP consumed by myosin ATPases. The addition of Ap3A (50 \(\mu\text{mol/L}\)) reduced mitochondrial respiration to 172±10 natom \(O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\) (n=5), which was significantly lower than the value obtained in the absence of the adenylate kinase inhibitor (\(P<0.001\)). Thus, inhibition of adenylate kinase compromised communication between actomyosin and mitochondria.

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The contribution of adenylate kinase to cellular phosphotransfer was assessed in myocardial samples with \(^3\)H-phosphoryl labeling. The kinetics of \(^3\)H-labeled phosphoryl appearance in \(\gamma\)-ATP reflects cellular ATP turnover rate.\(^{24}\) The total ATP turnover rate, which is determined from the sum of \(^3\)H atoms inserted into phosphoryl-containing cellular metabolites, was 37.5±1.4 nmol ATP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\) (n=5; Figure 3A and 3B). Adenylate kinase catalyzes \(\beta\)-ATP \(^3\)H-labeling.\(^8\) The net adenylate kinase–catalyzed phosphotransfer rate in the direction of ADP formation was 3.72±0.3 nmol ADP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\) (n=5; Figure 3A and 3B). This value corresponds to \(\approx 10\%\) of the total ATP turnover rate. The rate of \(^3\)H-labeling of creatine phosphate reflects creatine kinase–catalyzed phosphotransfer.\(^8\) The net creatine phosphate–catalyzed phosphotransfer rate was 32.4±1.6 nmol CrP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\) (n=5) or 89% of the total ATP turnover rate (Figure 3A and 3B). Thus in intact myocardium, adenylate kinase can transfer high-energy phosphoryl groups that contribute to cellular phosphotransfer.

Increased Contribution of Adenylate Kinase in Heart Failure

The kinetics of \(^18\)O-labeling of phosphoryls in \(\gamma\)-ATP, creatine phosphate, and \(\beta\)-ATP in failing hearts are presented in Figure 3C. The overall ATP turnover rate (42.1±1.5 nmol ATP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\), n=6) was essentially unchanged (\(P>0.05\)) when compared with controls (Figure 3A). Although the initial rate of \(^18\)O-labeled phosphoryl appearance in \(\gamma\)-ATP was similar in the 2 groups, subsequent \(^18\)O incorporation was reduced in the failing myocardium and approached saturation faster, which suggested that in heart failure, a smaller pool of ATP is involved in energy turnover (Figure 3A through 3C). Thus at 6 minutes of \(^18\)O-labeling, the pool size of labeled \(\gamma\)-ATP was lower by 20% (\(P<0.05\)) in the failing myocardium compared with normal myocardium (Figure 3A through 3C). The failing heart also displayed a marginal decrease in ATP levels to 25.7±2.3 nmol \(\cdot\text{mg}^{-1} \text{protein}\) (n=6) from 29.2±2.5 nmol \(\cdot\text{mg}^{-1} \text{protein}\) (n=5), which was found in normal hearts (\(P>0.05\)). There was a reduction (by 52%) in creatine kinase-catalyzed \(^18\)O-labeling of creatine phosphate to 17.4±0.7 nmol \(\cdot\text{mg}^{-1} \text{protein} \cdot \text{min}^{-1}\) (n=6; \(P<0.001\); Figure 3D). In failing hearts, the contribution of creatine kinase to the overall ATP turnover rate decreased to \(\approx 40\%\). But, adenylate kinase–catalyzed \(\beta\)-ATP \(^18\)O-labeling increased. The rate of adenylate kinase–catalyzed phosphotransfer increased to 8.71±0.4 nmol \(\cdot\text{mg}^{-1} \text{protein} \cdot \text{min}^{-1}\) (n=6) or 134% compared with controls (\(P<0.001\)). In the failing myocardium, the contribution of adenylate kinase to the overall ATP turnover doubled and was 21% of the total ATP turnover rate.

To assess whether changes in phosphoryl fluxes are related to altered enzyme activities, specific activities of adenylate

![Figure 3. Kinetics of high-energy phosphoryl exchange and metabolic flux in normal and failing myocardium. Kinetics of \(^18\)O-labeled phosphoryl appearance in \(\gamma\)-ATP, creatine phosphate (CrP), and \(\beta\)-ATP in the normal (A) and failing (C) myocardium. Average values of calculated rate of total cellular ATP flux expressed in nmol ATP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\), creatine kinase (CK) net flux (nmol CrP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\)), and adenylate kinase (AK)–catalyzed net phosphoryl transfer (nmol ADP \(\cdot\text{min}^{-1} \cdot \text{mg}^{-1} \text{protein}\)) in normal (B, n=5) and failing (D, n=6) myocardium. D, * indicates a significant (\(P<0.001\)) change compared with corresponding values in normal myocardium (B).](http://circres.ahajournals.org/lookup/doi/10.1161/01.CIR.95.6.1140)
and creatine kinase were measured. In control hearts (n=6), the activities of adenylate and creatine kinases were 537±18 and 2551±64 nmol · min⁻¹ · mg⁻¹ protein, respectively. In extracts from failing hearts (n=8), specific activities of adenylate and creatine kinases were 422±16 and 1791±42 nmol · min⁻¹ · mg⁻¹ protein, respectively. These are significant (P<0.001) decreases in specific activities when compared with controls: 21% for adenylate and 30% for creatine kinase. Thus, the increase in phosphoryl flux through adenylate kinase in failing hearts occurs despite a reduction in the total activity of this enzyme.

**Discussion**

In the heart, adenylate kinase has been implicated in the control of oxidative phosphorylation,26,33,40 export of high-energy phosphoryls from mitochondria,21 and regulation of ATP-sensitive K⁺ channels.3,28,41 In this study, we show that adenylate kinase also facilitates the transfer of high-energy phosphoryls and signal communication between mitochondria and actomyosin. This was demonstrated in an isolated actomyosin/mitochondria system and in intact myocardium. The contribution of adenylate kinase to the overall cellular ATP metabolic flux significantly increased during heart failure when creatine kinase–dependent phosphotransfer was compromised. Thus, this study establishes the role for adenylate kinase in supporting myocardial energy flux.

In the reconstituted actomyosin/mitochondrial system devoid of other ATP-regenerating and ATP-consuming cellular components,24 adenylate kinase–catalyzed phosphotransfer, which can occur in mitochondria and myofibrils,33,42 accelerated the rate and increased the amplitude of contraction. This effect was suppressed by ApoA,39 which has no direct effect on myosin ATPases or mitochondria.43,44 A dependence of contraction on adenylate kinase activity could be due to local regeneration of ATP and/or transfer of nucleotides between mitochondria and actomyosin.4,25 When conditions were able to minimize the ATP-regenerating function of adenylate kinase at low concentrations of ADP or ATP, the effect of adenylate kinase–catalyzed phosphotransfer required both actomyosin and mitochondria. In the absence of mitochondria, the ATP-regenerating function of adenylate kinase is limited by the accumulation of AMP.33,40 In the presence of mitochondria, AMP is rephosphorylated to ATP and transferred back to the myofibrils, which allows for continuous phosphoryl flux.4 Yet, in the absence of adenylate kinase activity, the exchange of energy-rich nucleotides by simple diffusion is insufficient to sustain a maximal rate of contraction. But, combined actomyosin/mitochondrial adenylate kinase activities promoted actomyosin contraction and mitochondrial respiration, which indicated an adenylate kinase–dependent flow of high-energy phosphoryls from mitochondria to actomyosin. These findings fulfill criteria for adenylate kinase–catalyzed phosphotransfer to process cellular signals associated with ATP production and use in cardiomyocytes, as proposed previously for noncardiac tissues.3,4,8,22,30

In intact cardiac muscle, the total ATP turnover rate (37.5 nmol ATP · min⁻¹ · mg⁻¹ protein) was obtained directly from 18O measurements and was within the range (27 to 54 nmol ATP · min⁻¹ · mg⁻¹ protein) of that calculated from O₂ consumption rates in resting dog myocardium.45,46 The net adenylate kinase-catalyzed phosphotransfer rate accounted for 10% of the total ATP turnover rate, compared with 3% to 5% in resting skeletal muscle,8,23 which suggested possible tissue-specific differences in the contribution of adenylate kinase to cellular phosphotransfer. In this experiment, creatine kinase phosphotransfer contributed 89% to the total ATP turnover rate. Thus, in the heart, the majority of high-energy phosphoryls are transferred through combined actions of creatine and adenylate kinases.

In the failing myocardium, the overall ATP turnover rate was similar to that in the normal heart, which supported the notion that ATP levels and ATPase activity may not change with the progression of disease.1 In fact, we did not observe a significant decrease in the ATP concentration in the failing myocardium. However, changes in the kinetics of γ-ATP 18O-labeling suggest that in heart failure a smaller pool of ATP is actively involved in cellular energetics. This can result from altered compartmentation and/or deficient phosphotransfer of adenine nucleotides.7,10,11 Present data show a reduction (by 52%) in creatine kinase–catalyzed phosphotransfer in pacing-induced heart failure and a decreased (from 89% to 40%) contribution of creatine kinase to the overall ATP turnover. This correlates with previous studies that have, in other models of heart failure, also implicated a deficiency in creatine kinase–catalyzed energy transfer.15,47 The new information provided by this study relates to the significance of adenylate kinase–catalyzed phosphotransfer that increased by ≈134% in heart failure. Consequently, the contribution of adenylate kinase to total ATP turnover rate doubled to 21%. Such increase in adenylate kinase–catalyzed phosphotransfer could support cellular energetics of a failing heart.

Compensation provided by adenylate kinase was only partial. The sum of phosphotransfers mediated through creatine and adenylate kinases contributed to ≈100% of the total ATP turnover rate in normal hearts but only 60% to 65% in failing hearts, which left a 35% to 40% phosphotransfer deficit. This term refers to a deficit in enzyme-mediated phosphotransfer in which remaining phosphoryls are delivered through a less-efficient mechanism, such as simple diffusion and/or the glycolytic pathway.3,4 Although in normal muscle an acute suppression of creatine kinase activity is well compensated through increased adenylate kinase phosphotransfer,8 the compensatory potential of adenylate kinase in diseased heart appears to be reduced. Previously, in ischemia-injured myocardium, a diminished activity of adenylate kinase has been reported to reduce the export of high-energy phosphoryls from mitochondria.48 The specific activity of adenylate kinase reflects the flux capacity of this enzyme. Therefore, a drop in flux capacity, as observed here in the failing myocardium, may limit the compensatory potential of adenylate kinase–dependent phosphotransfer. This could be due to a decrease in adenylate kinase expression and/or altered enzyme regulation. Because our preliminary results, which were performed with Western blot analysis to detect adenylate kinase level in ventricular samples, do not indicate a significant decrease in protein levels of adenylate kinase in heart failure, other mechanisms should be
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Figure 4. Adenylate kinase contribution to myocardial energy transfer and to communication between processes of ATP-generation and ATP-consumption by myosin ATPases. Adenylate kinase, present in mitochondrial and myofilamentary compartments, enables the transfer and makes available the energy of 2 high-energy phosphoryls, the β- and the γ-phosphoryls of a single ATP molecule. AMP, in this case, serves as a feedback signal to mitochondrial respiration that is amplified by the generation of 2 molecules of ADP at the mitochondrial intermembrane site. Within the intracellular environment of a cardiomyocyte, the transfer of ATP and ADP between ATP-production and ATP-consumption sites may involve multiple, sequential, phosphotransfer relays that result in a flux wave propagation and ligand conduction along clusters of adenylate kinase molecules.4 AK1 and AK2 indicate cytosolic and mitochondrial isoforms of adenylate kinase; o.m. and i.m., outer and inner mitochondrial membrane.

considered including changes in cardiac levels of ATP that may occur under metabolic stress.49

In summary, the current study, performed in the isolated mitochondria/actomyosin system and intact ventricular tissue, identifies a role for adenylate kinase in the transfer of energy and feedback communication between mitochondria and myofilaments in the heart. Although experimental systems permit the demonstration of phosphotransfer dynamics that are independent from variations due to muscle contraction, the functional integrity of cardiac muscle is only partially preserved, which warrants additional studies to establish the full role of adenylate kinase in a beating heart. A coordinated action of mitochondrial and cytosolic isoforms of adenylate kinase, which are encoded by separate genes and directed to different subcellular compartments,32 would provide a mechanism to transfer 2 high-energy phosphoryls within the ATP molecule (Figure 4). The exclusive property of adenylate kinase to transfer the energy of the β-phosphoryl of ATP, which is energetically equivalent to the commonly employed γ-phosphoryl, doubles the energetic potential of the ATP molecule and halves the diffusional resistance of the cytosol for energy transfer.45,50 Such phosphotransfer function renders adenylate kinase an important component for optimal myocardial bioenergetics. In particular, adenylate kinase could serve a compensatory role in heart failure. Thus, the promotion of adenylate kinase-mediated phosphotransfer could provide a novel strategy targeted at improving the energetic status of the failing heart.

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