Adenylate kinase AK1 knockout heart: energetics and functional performance under ischemia-reperfusion

DARKO PUCAR,1 PETER BAST,1 RICHARD J. GUMINA,1 LYNETTE LIM,1 CARMEN DRAHL,1,2 NENAD JURANIC,2 SLOBODAN MACURA,2 EDWIN JANSSEN,3 BE WIERINGA,3 ANDRE TERZIC,1 AND PETRAS P. DZEJA1

1Division of Cardiovascular Diseases, Departments of Medicine and Molecular Pharmacology and Experimental Therapeutics, and 2Department of Biochemistry and Molecular Biology, Mayo Clinic, Mayo Foundation, Rochester, Minnesota 55905; and 3Center for Molecular Life Sciences, University Medical Center, University of Nijmegen, Nijmegen 6500, The Netherlands

Received 12 February 2002; accepted in final form 3 April 2002

Pucar, Darko, Peter Bast, Richard J. Gumina, Lynette Lim, Carmen Drahl, Nenad Juranic, Slobodan Macura, Edwin Janssen, Be Wieringa, Andre Terzic, and Petras P. Dzeja. Adenylate kinase AK1 knockout heart: energetics and functional performance under ischemia-reperfusion. Am J Physiol Heart Circ Physiol 283: H776–H782, 2002. First published April 11, 2002; 10.1152/ajpheart.00116.2002.—Deletion of the major adenylate kinase AK1 isoform, which catalyzes adenine nucleotide exchange, disrupts cellular energetic economy and compromises metabolic signal transduction. However, the consequences of deleting the AK1 gene on cardiac energetic dynamics and performance in the setting of ischemia-reperfusion have not been determined. Here, at the onset of ischemia, AK1 knockout mice hearts displayed accelerated loss of contractile force compared with wild-type controls, indicating reduced tolerance to ischemic stress. On reperfusion, AK1 knockout hearts demonstrated reduced nucleotide salvage, resulting in lower ATP, GTP, ADP, and GDP levels and an altered metabolic steady state associated with diminished ATP-to-Pi and creatine phosphate-to-Pi ratios. Postischemic AK1 knockout hearts maintained ~40% of β-phosphoryl turnover, suggesting increased phosphotransfer flux through remaining adenylate kinase isoforms. This was associated with sustained creatine kinase flux and elevated cellular glucose-6-phosphate levels as the cellular energetic system adapted to deletion of AK1. Such metabolic rearrangements, along with sustained ATP-to-ADP ratio and total ATP turnover rate, maintained postischemic contractile recovery of AK1 knockout hearts at wild-type levels. Thus deletion of the AK1 gene reveals that adenylate kinase phosphotransfer supports myocardial function on initiation of ischemic stress and safeguards intracellular nucleotide pools in postischemic recovery.

energy metabolism; adenine nucleotides; glycolysis; phosphotransfer; oxygen-18 phosphoryl labeling; phosphorus-31 nuclear magnetic resonance

MAINTENANCE OF OPTIMAL CARDIAC function requires precise control of cellular nucleotide ratios and high-energy phosphoryl fluxes (11, 22, 30, 32, 33, 36, 40). Within the cellular energetic infrastructure, adenylate kinase has been recognized as an important phosphotransfer enzyme that catalyzes adenine nucleotide exchange (ATP + AMP ↔ 2ADP) and facilitates transfer of both β- and γ-phosphoryls in ATP (9, 15, 24, 25, 43). In this way, adenylate kinase doubles the energetic potential of ATP as a high-energy-phosphoryl carrying molecule and provides an additional energy source under conditions of increased demand and/or compromised metabolic state (13–15, 31, 35, 42). By regulating adenine nucleotide processing, adenylate kinase has been implicated in metabolic signal transduction (12, 15, 27). Indeed, phosphoryl flux through adenylate kinase has been shown to correlate with functional recovery in the metabolically compromised heart (30) and to facilitate intracellular energetic communication (3, 9, 13).

Isoforms of adenylate kinase are found in mitochondria, cytosol, and cellular membranes, creating an integrated phosphotransfer network (15, 16, 38). An intimate relationship of adenylate kinase with mitochondrial respiration, myofibrillar and membrane ATPases, as well as metabolic sensors such as ATP-sensitive K+ channels, contributes to efficient regulation of energy metabolism, membrane excitability, and cell contraction (3, 4, 12, 13, 29, 34, 44). Genetic deletion of the major adenylate kinase isoform, adenylate kinase 1 (AK1), reduces the cellular energetic economy and increases cell vulnerability to injury (17, 31). In humans, adenylate kinase deficiency caused by mutations in the AK1 gene has been associated with severe hemolytic anemia and psychomotor impairment (1). Although adenylate kinase has been considered an important stress-responsive element providing phosphotransfer support when other energetic systems are failing (10, 13, 14), the consequences of deleting the AK1 gene on cardiac energetics and functional performance after ischemia-reperfusion have not been determined.

Here, cellular phosphotransfer dynamics were determined using 18O-assisted 31P-NMR and mass spec-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
trometry in wild-type and AK1 knockout hearts in the setting of ischemia-reperfusion. Disruption of the AK1 gene accelerated loss of contractility on ischemia, followed by reduced nucleotide salvage and metabolic rearrangements in support of functional recovery on reperfusion.

**METHODS**

The investigation conformed to the National Institutes of Health guidelines regulating the care and use of laboratory animals (NIH Publication 5377-3, 1996) and was approved by the Institutional Animal Care and Use Committee.

**AKI knockout mice.** AKI knockout mice were generated from embryonic stem cells carrying a replacement mutation in the AKI gene (17). Inactivation of AKI expression was achieved by homologous DNA recombination, with a Hygrometer cassette vector used to replace the entire exon 3–5 region in the AKI gene (16, 30). Male homozygous AKI knockout (AKI/–/) mice were compared with age- and sex-matched wild-type controls.

**Heart perfusion.** In heparinized (50 U ip) and anesthetized (75 mg/kg pentobarbital sodium ip) wild-type and AKI knockout mice (40–50 g), hearts were excised and retrogradely perfused with a 95% O2-5% CO2-saturated Krebs-Henseleit (K-H) solution (in mM: 118 NaCl, 5.3 KCl, 2.0 CaCl2, 19 NaHCO3, 1.2 MgSO4, 11.0 glucose, 0.5 EDTA; 37°C) at a perfusion pressure of 70 mmHg. Hearts were paced at 400 beats/min. Hearts were perfused for 45 min and then subjected to 30 min of no-flow normothermic ischemia followed by a 30-min reperfusion. Left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), rate-pressure product (RPP), and heart rate were derived from the left ventricular pressure signal continuously monitored with a fluid-filled balloon-tipped pressure transducer (Harvard Apparatus). Time to contracture was defined as the time to a 3-mmHg increase in LVDP from baseline value.

**18O phosphoryl labeling.** After reperfusion, hearts were labeled with [18O]H2O, which was introduced for 30 s with the K-H buffer supplemented with 30% of [18O]-labeled H2O (Isotec) as described previously (30). Hearts were freeze-clamped, pulsed under liquid N2, and extracted in a solution containing 0.6 M HClO4 and 1 mM EDTA (30, 31). Protein content was determined with a DC Protein Assay kit (Bio-Rad). Extracts were neutralized with 2 M KOH and used to determine [18O] incorporation. Rapid [18O] labeling of P3, γ-ATP, and creatine phosphate (CrP) was determined with [18O]-assisted 31P NMR spectroscopy (30), whereas slow [18O] labeling of β-ADP and β-ATP was determined by mass spectrometry (14, 43).

**NMR spectroscopy.** 31P- and 1H-NMR spectroscopy was performed on heart extracts (30). Neutralized extracts were supplemented with internal standards for 31P and 1H spectroscopy (1,250 nmol methylene diphosphonate and 50 nmol 3-trimethylsilyl tetradecutosodium propionate), chelated with Chelex 100 resin (Sigma) for 1 h, concentrated to 0.1 mL, filtered, and reconstituted with 0.4 mL of D2O (Isotec). Samples were additionally cleaned with Chelex 100 resin at 4°C for 12 h. High-resolution 31P and 1H NMR spectra were acquired at 202.5 and 500 MHz, respectively, on a Bruker 11 T spectrometer (Avance) in 5-mm tubes at ambient temperature. For 31P spectra, signal accumulation (i.e., 36,000 scans) was run without relaxation delay (acquisition time 1.61 s) with a pulse width of 10 μs (53° angle). Proton decoupling (WALTZ-16 decoupling at 3 kHz radio frequency; pulse width of 506 μs for 31P) was applied during signal acquisition. Free induction decays (FIDs) were Fourier transformed after zero filling to 32 K and filtering with a line-broadening factor of 0.3 Hz. Phase and baseline were automatically corrected, and peak integrals were determined with a built-in integration routine (XwinNMR 2.5 software, Bruker). For 1H spectra, 128 scans were accumulated under fully relaxed conditions (12.8-s relaxation delay) with a pulse width of 9 μs (90° angle). FIDs were zero filled to 32 K and Fourier transformed without filtering. Phase and baseline were manually corrected, and peak integrals were determined with an integration routine (30).

**Mass spectrometry.** [18O] labeling of β-ADP and β-ATP was quantified by mass spectrometry (13, 14). Cellular ATP and ADP were purified by high-performance liquid chromatography (Hewlett-Packard 1100 series HPLC system) with a Mono Q HR 5/5 ion-exchange column (Pharmacia Biotech). The [18O] phosphoryls of ATP and ADP were transferred to glycerol by a combined catalytic action of adenylate kinase and glycero kinase. Samples that contained phosphoryls of β-ADP and β-ATP, as glycerol 3-phosphate, were converted to respective trimethylsilyl derivatives with Tri-Sil/BSA (Pierce) as the derivatization agent. The [18O] enrichment of phosphoryls in glycerol 3-phosphates was determined with a Hewlett-Packard 5973 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 16O, 18O1, 18O2, and 18O3 were monitored in trimethylsilyl derivatives of β-ADP and β-ATP (13, 17).

**Phosphotransfer rates.** The 18O labeling procedure is based on the incorporation of one 18O atom, provided from [18O]H2O, into P3, with each act of ATP hydrolysis and the subsequent distribution of [18O]-labeled phosphoryls among other high-energy phosphoryl-carrying molecules (6). Therefore, cellular ATP turnover, which directly reflects the ATP utilization rate, was estimated from the total number of [18O] atoms that appeared in phosphoryls of P3, γ-ATP, CrP, β-ADP, and β-ATP (6, 13, 14). Creatine kinase phosphotransfer rate was determined from the rate of appearance of CrP species containing [18O]-labeled phosphoryls by pseudolinear approximation (14, 30). Adenylate kinase phosphotransfer flux was determined from the rate of appearance of 18O-containing β-phosphoryls in ADP and ATP (14, 42).

**Metabolite levels.** Tissue levels of ATP, ADP, CrP, and lactate were quantified according to internal standards from 1H NMR spectroscopy, whereas P3, γ-ATP, CrP, and glucose-6-phosphate (G6P) were calculated from 31P NMR spectra (30). Levels determined from 31P NMR spectra were corrected for nuclear Overhauser enhancement and incomplete relaxation (30). Percentage recovery of 86% and 92% and hydrolysis of 3% and 1% were measured for CrP and ATP, respectively. Myocardial GTP, GDP, and AMP levels were measured with high-performance liquid chromatography (17).

**Enzyme activity.** Cardiac tissue was extracted with (in mM) 150 NaCl, 60 Tris-HCl (pH 7.5), 5 EDTA, and 1 phenylmethylsulfonyl fluoride with 10 μg/ml leupeptin, 1 μg/ml aprotinin, and 0.2% Triton X-100 and centrifuged (10 min, 8,000 g, 4°C). Adenylate kinase activity was measured with a Beckman DU 7400 spectrophotometer in (in mM) K+-acetate, 20 HEPEs (pH 7.5), 20 glucose, 4 MgCl2, 2 NADP+, 1 EDTA, 1 dithiothreitol, and 2 ADP, with 4.5 U/ml hexokinase and 2 U/ml glucose-6-phosphate dehydrogenase (10, 31).

**Statistical analysis.** Data are expressed as means ± SE. Student’s t-test was used for statistical analysis. A difference of P < 0.05 was considered significant.
RESULTS

Deletion of the AK1 gene accelerates loss of cardiac contraction on ischemic challenge. AK1 knockout hearts have a reduced ability to maintain metabolic integrity under stress (31). Here, knockout of the AK1 gene was associated with faster loss of contractility on initiation of no-flow ischemia (Fig. 1A). On average, before ischemia LVDP was 73 ± 4 mmHg in the wild-type and 80 ± 6 mmHg in the AK1 knockout hearts. After ischemia, 9.1 ± 0.9 s (n = 7) was required to halve LVDP in the wild-type but only 6.6 ± 0.5 s (n = 7) was required in hearts lacking AK1 (P < 0.05), a reduction of ~30% (Fig. 1A and B). Throughout ischemia, the time to contracture (9.9 ± 1.7 and 10.2 ± 2.2 min, P > 0.05; n = 7 in both groups) and the maximal developed contracture (60 ± 6 and 65 ± 10 mmHg, P > 0.05; n = 7 in both groups) were comparable in wild-type and AK1-deficient hearts (Fig. 1, C and D). Thus deletion of the AK1 gene reduces the tolerance of heart muscle to sustain contractility on initiation of ischemic stress.

Altered metabolic profile in AK1-deficient heart after ischemia-reperfusion. Although in nonchallenged AK1 knockout hearts cellular nucleotide levels were maintained (31), here after ischemia-reperfusion injury, AK1-deficient hearts displayed lower levels of nucleotides compared with the wild-type (Fig. 2, A and B). Specifically, ATP levels were 13.5 ± 0.5 and 10.4 ± 0.8 nmol/mg protein (P < 0.05), whereas ADP levels were 3.5 ± 0.1 and 2.9 ± 0.2 nmol/mg protein (P < 0.05) in wild-type and AK1 knockout hearts, respectively (n = 7 in each group). Myocardial AMP content was rather similar in wild-type and AK1 knockout hearts: 0.50 ± 0.04 (n = 5) and 0.64 ± 0.23 (P > 0.05, n = 4) nmol/mg protein, respectively. However, the total adenine nucleotide content in postischemic AK1 knockout hearts was reduced by 20% (P < 0.05) compared with the wild type, indicating larger loss of the adenine nucleotide pool and compromised nucleotide salvage on reperfusion in the absence of AK1. Myocardial GTP and GDP levels were also lower in postischemic AK1 knockout hearts, i.e., 0.61 ± 0.08 vs. 0.34 ± 0.02 and 0.32 ± 0.04 vs. 0.23 ± 0.03 nmol/mg protein in wild-type (n = 5) and AK1 knockouts (n = 4), respectively (P < 0.05), thus pointing to a more generalized perturbation in cellular energetics and nucleotide metabolism in AK1 deficiency. In fact, the ATP-to-Pi and CrP-to-Pi ratios, sensitive indicators of the cellular energetic status (2, 30, 31), were both reduced, from 0.6 ± 0.1 to 0.3 ± 0.1 (P < 0.05) and from 2.1 ± 0.4 to 1.1 ± 0.2 (P < 0.05; Fig. 2C) in wild-type and AK1 knockout hearts, respectively (n = 7 in each group). Reduced CrP-to-Pi ratio in AK1-deficient hearts reflected a trend toward lower CrP levels (36.8 ± 1.7 vs. 44.1 ± 5.0 nmol/mg protein; P > 0.05) and significantly higher amounts of Pi (34.9 ± 2.2 vs. 23.8 ± 4.7 nmol/mg protein; P < 0.05) in the AK1 knockout vs. wild-type hearts. These results indicate that the major adenylate kinase isoform, AK1, is required for preservation of cellular high-energy phosphoryls and in particular for the safeguarding the

Fig. 1. Accelerated loss of cardiac contraction on ischemia in AK1 knockout hearts. A: time course of the decline of contractility after initiation of no-flow ischemia (downward arrow) in wild-type (WT; *) and adenylate kinase AK1 knockout (AK1-KO; ▲) hearts. Double-headed arrows indicate time (t1/2) required for halving contraction in WT vs. AK1-KO after ischemia. LVDP, left ventricular developed pressure. B–D: average rate of loss in cardiac contraction, t1/2 (B), time to contracture (C), and maximal contracture (D) on ischemia in WT (n = 7) and AK1-KO (n = 7) hearts. *Significant difference between WT and AK1-KO hearts.
adenine and guanine nucleotide pools under ischemia-reperfusion. Although the total nucleotide pool was reduced, the ATP-to-ADP ratio, which reflects the kinetics and thermodynamics of energy-consuming processes (37, 38), was maintained, i.e., 3.82 ± 0.16 and 3.54 ± 0.12 nmol/mg protein (P > 0.05; n = 7 in each group) in wild-type and AK1 knockout hearts, respectively. Thus the cellular energetic system adapts to genetic deletion of AK1, albeit at a different metabolic steady state. After ischemia-reperfusion, the myocardium lacking AK1 responded with a significant increase in cellular G6P levels, from 1.8 ± 0.2 nmol/mg protein in the wild type (n = 7) to 4.1 ± 1 nmol/mg protein in the AK1 knockout (n = 7; P < 0.05), a 227% increase (Fig. 3D). As G6P is produced by phosphorylation of glucose by hexokinase, the very first enzyme in the glycolytic cascade, these data suggest an upregulation of glycolysis to compensate for the lack of AK1. Enhanced glycolysis was apparently coupled with accelerated Krebs cycle metabolism as lactate levels remained unchanged, i.e., 4.1 ± 0.6 and 4.8 ± 1.2 nmol/mg protein in wild type and AK1 knockouts, respectively (P > 0.05; n = 7 in each group). Thus under ischemia-reperfusion, hearts lacking AK1 display compromised nucleotide salvage and reduced adenine and guanine nucleotide pools, along with remodeled nucleotide and glycolytic metabolism.

Phosphotransfer dynamics and postischemic recovery in AK1 knockout hearts. In facilitating energetic communication between cellular sites of ATP production and consumption, adenylate kinase works in parallel with creatine kinase to jointly catalyze intracellular transfer of high-energy phosphoryls (9, 11, 13, 14, 34). After deletion of AK1, adenylate kinase activity was reduced from 1,180 ± 220 nmol ATP min⁻¹ mg protein⁻¹ in wild-type to 65 ± 2 nmol ATP min⁻¹ mg protein⁻¹ (P < 0.05, n = 5 in both groups) in AK1 knockout hearts, a 94% reduction (Fig. 3A; see also Ref. 29). However, the actual flux through the adenylate kinase system was 5.3 ± 0.9 and 2.2 ± 0.7 nmol mg⁻¹ protein⁻¹ min⁻¹ in wild-type and AK1 knockout hearts (P < 0.05, n = 7 in both groups), respectively, a reduction of only 58% (Fig. 3A). Thus deletion of the AK1 gene is associated with a rather maintained adenylate kinase phosphotransfer, suggesting a functional reserve in minor adenylate kinase isoforms, which are preserved in the heart after genetic deletion of AK1 (31). Moreover, energetic flux through the complementary phosphotransfer enzyme, creatine kinase, was not different between wild-type and AK1 knockout hearts (Fig. 3B). Creatine kinase flux was 240 ± 34 and 198 ± 20 nmol mg⁻¹ protein⁻¹ min⁻¹ in wild-type and AK1 knockouts, respectively (P > 0.05, n = 7 in both groups; Fig. 3B). Accordingly, total ATP turnover and contractile postischemic recovery were comparable in wild-type and AK1 knockout hearts. On average, total ATP turnover was 228 ± 25 and 210 ± 14 nmol mg⁻¹ protein⁻¹ min⁻¹ in wild-type and AK1 knockouts, respectively (P > 0.05, n = 7 in both groups; Fig. 3C). RPP was 29,200 ± 2,200 and 32,900 ± 2,500 mmHg min⁻¹ (P > 0.05) before ischemia and 10,300 ± 2,700 and 12,500 ± 2,100 mmHg min⁻¹ (P > 0.05) after ischemia in wild-type and AK1 knockout hearts, respectively (n = 7 in both groups; Fig. 3D). In fact, functional recovery

![Bar chart](http://ajpheart.physiology.org/)

**Fig. 2.** Reduced ability of AK1-KO hearts to maintain the cellular adenine nucleotide and high-energy phosphoryl pools on reperfusion. A and B: average cellular ATP (A) and ADP (B) levels in WT and AK1-KO hearts. C: ratio of creatine phosphate (CrP) to Pı levels in WT and AK1-KO hearts. D: cellular glucose-6-phosphate (G6P) levels in WT and AK1-KO hearts. *Significant difference between WT and AK1-KO hearts.

**AJP-Heart Circ Physiol • VOL 283 • AUGUST 2002 • www.ajpheart.org**
of AK1 knockout hearts tightly correlated with the ATP turnover rate \( r = 0.87, P < 0.05, n = 7 \) and creatine kinase flux \( r = 0.85, P < 0.05 n = 7 \) but not with the cellular ATP content \( r = 0.18, P > 0.05, n = 7 \), suggesting that energetic dynamics rather than nucleotide levels per se determine myocardial performance. Thus sustained creatine kinase flux and total ATP turnover rate, along with redistributed adenylate kinase phosphotransfer through minor isoforms, and enhanced glycolysis support recovery of AK1 knockout hearts on reperfusion.

**DISCUSSION**

Adenylate kinase is involved in multiple energetic and metabolic signaling processes (3, 4, 12, 15, 17); however, the role of this enzyme in metabolic and functional recovery of heart muscle in the setting of ischemia-reperfusion has not been determined. To directly assess the contribution of the major cytosolic adenylate kinase isoform, we took advantage of transgenic animals carrying a null mutation in the AK1 gene (17) and the versatility of the \( ^{18} \text{O} \)-phosphoryl labeling technique permitting the monitoring of intracellular phosphotransfer dynamics (6, 30).

Genetic deletion of AK1 removed all but 6% of total myocardial adenylate kinase activity, yet the intracellular adenylate kinase phosphotransfer flux was only halved in AK1 knockout hearts. This discrepancy between loss in total adenylate kinase enzymatic capacity and a more moderate reduction in net adenylate kinase metabolic flux indicates a high functional reserve of remaining adenylate kinase isoforms, such as AK2, still present in hearts after deletion of AK1 (17, 31, 38). Despite such reserve, however, reduced adenylate kinase-catalyzed phosphotransfer induced rearrangements in adenine nucleotide and glycolytic metabolism, shifting cellular energetics into an apparently new steady state. These metabolic alterations in AK1 knockout hearts were associated with accelerated loss of contractile force on myocardial ischemia while not limiting the reduced postischemic ATP turnover rate and contractile performance in the ischemia-reperfusion-compromised myocardium. Thus AK1-mediated phosphotransfer is an integral component in the response of the cellular energetic system to ischemia-reperfusion.

Despite comparable performance at baseline, AK1 knockout hearts displayed accelerated loss of contractile force on ischemia compared with the wild type. Such a deficit supports previous observations that adenylate kinase-catalyzed phosphotransfer is indeed activated on metabolic stress (13, 31). In fact, the ATP-regenerating function of adenylate kinase depends on total adenylate kinase activity (4, 24, 34, 35) and apparently could not be compensated by other phosphotransfer systems, reducing the tolerance of the myocardium to stress. Rapid deterioration of cardiac function on ischemia has been linked to changes in pH, \( P_i \), and ADP levels leading to reduced free energy of ATP hydrolysis, the thermodynamic determinant of cardiac contractility (11, 18, 37, 39). By regenerating ATP from ADP, adenylate kinase sustains the intramyofibrillar...
ATP-to-ADP ratio (4, 24), reducing the drop in free energy of ATP hydrolysis. Loss of this adenylate kinase function in the AK1 knockout thereby accelerates the decline in cardiac contractility at the onset of ischemia. This is in line with reports that have previously identified AK1 as an essential phosphotransfer enzyme in maintaining myocardial energetic homeostasis under metabolic challenge (13, 31). However, as ischemia progressed, time to contracture and the maximal developed contracture were similar in both wild-type and AK1 knockout hearts. This suggests that ionic and energetic determinants of heart muscle contractility at an advanced stage of ischemia eventually become compensated in AK1 knockout hearts. Such adaptation can be explained, at least in part, by the higher glycolytic potential of AK1-deficient hearts, because the activity of 3-phosphoglycerate kinase, a critical enzyme in glycolytic phosphotransfer, is upregulated after deletion of AK1 (31).

In nonchallenged AK1 knockout hearts, cellular nucleotide levels are maintained (31). Here, after ischemia-reperfusion, AK1 knockout hearts had reduced ATP and ADP levels and lower ATP-to-Pi and CrP-to-Pi ratios, indicating that adenylate kinase is required for preservation of cellular high-energy phosphoryls, in particular for the maintenance of the adenine nucleotide pool. In the absence of the main adenylate kinase isoform, the capacity for resynthesis of ATP from ADP and the rate of nucleotide salvage, i.e., the AMP conversion to ADP, would be reduced because adenylate kinase catalysis is an essential step in adenine nucleotide metabolism (4, 17, 23). Also, decreased ADP levels in postischemic AK1-knockout hearts indicate greater consumption of this nucleotide by alternative phosphotransfer pathways, such as glycolysis. Reduced ATP-to-Pi and CrP-to-Pi ratios in the AK1-deficient myocardium would facilitate glycolysis through activation of phosphofructokinase (28). Activation of glycolysis in the AK1 knockout heart is indicated by the increase in G6P produced by phosphorylation of glucose by hexokinase, the very first enzyme in the glycolytic cascade. This, along with active creatine kinase phosphotransfer flux and maintained ATP-to-ADP ratio in AK1 knockout hearts, suggests an adaptive plasticity in the cellular energetic system to genetic deletion (15, 17). In fact, postischemic AK1 knockout hearts had sustained total ATP turnover, a global parameter reflecting the dynamics of cellular energetics. This is of significance because functional recovery of AK1 knockout hearts tightly correlated with the ATP turnover rate and creatine kinase flux but not with cellular ATP content. Indeed, the recovery of the injured myocardium closely depends on the energetic dynamics, including ATP turnover and phosphotransfer rates, rather than on static adenine nucleotide levels (18, 21, 26, 30, 37, 39). In this regard, comparable AMP levels observed in the wild-type and AK1 knockout hearts, under postischemic reperfusion, may reflect metabolic adjustment to the new steady state.

Wide metabolic rearrangements and significant cytoarchitectural adaptations are induced in transgenic animals lacking individual phosphotransfer enzymes such as creatine kinase (7, 22, 33, 41), which is tightly integrated with the adenylate kinase system (3, 14, 15, 34). Reorganization of cellular metabolism is believed to be essential in myocardial protection from hypoxia and/or ischemia, as observed in Sherpa hearts, which tolerate hypoxic stress well (20), or in preconditioned hearts where redistribution of cellular phosphotransfer increases tolerance to ischemic insult (30). Thus remodeling of the cellular energetic system in response to genetic and/or environmental challenge has common features and may have evolved as a general paradigm in metabolic adaptation to adverse conditions. Conversely, disruption of the intimate relationship of phosphotransfer enzymes with metabolic sensors, including the cardioprotective ATP-sensitive K+ channel (3, 5, 12, 19, 44), would result in defective signal transduction and reduced tolerance to stress. Ultimately, the actual phenotype of a phosphotransfer-deficient animal will depend on the net metabolic alterations and adaptations within the heart and other organs (17).

In summary, deletion of the AK1 gene compromises myocardial performance at the onset of ischemia and reduces adenine and guanine nucleotide salvage on reperfusion. These defects indicate a unique and nonredundant role for adenylate kinase in supporting cellular energetic homeostasis under ischemia-reperfusion. The myocardium partially adapted to reduction of AK1-catalyzed β-phosphoryl transfer through increased glycolytic metabolism and maintained adenine nucleotide dynamics. The requirement for such fail-safe alternate energetic mechanisms underscores the significance of adenylate kinase within the integrated cellular energetic network.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-64822, the American Heart Association, the Miami Heart Research Institute, the Bruce and Ruth Rappaport Program in Vascular Biology and Gene Delivery, Marriott Foundation, Hartz Foundation, NWO-GMW Program (901-01-095), and Nederlandse Kankerbestrijding KWF (KUN 98-1808). A. Terzic is an Established Investigator of the American Heart Association.

REFERENCES


6. Davis SM, Walseth TF, Deeg MA, Heyman RA, Graef RM, and Goldberg ND. Adenosine triphosphate utilization rates...


