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

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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 hearts. Postischemic AK1 knockout hearts maintained relative contractile force 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 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-
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.

**AK1 knockout mice.** AK1 knockout mice were generated from embryonic stem cells carrying a replacement mutation in the AK1 gene (17). Inactivation of AK1 expression was achieved by homologous DNA recombination, with a HygroB-cassette vector used to replace the entire exon 3–5 region in the AK1 gene (16, 30). Male homozygous AK1 knockout (AK1−/−) 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 AK1 knockdout 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 determined from 31P NMR spectra were corrected for nuclear broadening factor of 0.3 Hz. Phase and baseline were automatically corrected, and peak integrals were determined with a built-in integration routine (Xylinmr 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 β-phosphoryls of ATP and ADP were transferred to glycerol by a combined catalytic action of adenylyl kinase and glycerokinase. 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, 18O, 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 P, with each act of ATP hydrolysis and the subsequent distribution of 18O-labeled phosphoryls among ATP (6, 13, 14). 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 leupetin, 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) 100 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. 1, A 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
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 ± 1.1 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 remodelled 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 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 protein⁻¹·min⁻¹ in wild-type and AK1 knockouts, respectively (P > 0.05, n = 7 in each group). 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 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 (P > 0.05) before ischemia and 10,300 ± 2,700 and 12,500 ± 2,100 mmHg/min (P > 0.05) after ischemia in wild-type and AK1 knockout hearts, respectively (n = 7 in both groups; Fig. 3D). In fact, functional recovery
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 18O-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

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**Fig. 3.** Phosphotransfer dynamics and postischemic contractile recovery in AK1-KO heart. A: reduction of total adenylate kinase (AK) activity vs. AK flux in AK1-KO hearts as % of WT. B: creatine kinase (CK) flux determined by 18O-assisted 31P NMR in WT and AK1-KO hearts, expressed in nmol of CrP produced. C: total ATP turnover as the sum of 18O incorporated into cellular high-energy phosphoryls in WT and AK1-KO hearts. D: rate-pressure product (RPP), an indicator of cardiac contractile performance, in WT and AK1-KO hearts before (open bars) and after (filled bars) ischemia-reperfusion.
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 posts ischemic 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 adenosine 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.

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