Activation time of myocardial oxidative phosphorylation in creatine kinase and adenylate kinase knockout mice

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Gustafson, Lori A., and Johannes H. G. M. Van Beek. Activation time of myocardial oxidative phosphorylation in creatine kinase and adenylate kinase knockout mice. Am J Physiol Heart Circ Physiol 282: H2259–H2264, 2002. —Our goal was to determine whether mice genetically altered to lack either creatine kinase (M/MtCK) or adenylate kinase (AK) show altered properties in the dynamic regulation of myocardial oxygen consumption (MV02). We measured contractile function, oxygen consumption, and the mean response time of oxygen consumption to a step increase in heart rate [i.e., mitochondrial response time (tmito)] in isolated Langendorff-perfused hearts from wild-type (n = 6), M/MtCK (n = 6), and AK (n = 4) mice. Left ventricular developed pressure was higher in M/MtCK hearts (88.2 ± 6.8 mm Hg) and lower in AK hearts (46.7 ± 9.4 mm Hg) compared with wild-type hearts (60.7 ± 10.1 mm Hg) at the basal pacing rate. Developed pressure fell slightly when heart rate was increased in all three groups. Basal MV02 at 300 beats/min was 19.1 ± 2.4, 19.4 ± 1.5, and 16.3 ± 1.9 μmol·min−1·g dry wt−1 for M/MtCK, AK, and wild type, respectively, which increased to 25.5 ± 3.7, 25.4 ± 2.6, and 22.0 ± 2.6 μmol·min−1·g−1 when heart rate was increased to 400 beats/min. The tmito was significantly faster in M/MtCK hearts: 3.0 ± 0.3 s versus 7.3 ± 0.6 s for M/MtCK, AK, and wild-type hearts, respectively. Our results demonstrate that MV02 of M/MtCK hearts adapts more quickly to an increase in heart rate and thereby support the hypothesis that creatine kinase acts as an energy buffer in the cytosol, which delays the energy-related signal between sites of ATP hydrolysis and mitochondria.

Oxygen consumption; mitochondria; metabolic wave

IN HEART AND SKELETAL muscle, a step increase in work does not result in an immediate step increase in oxygen consumption. A certain delay occurs between increased work and increased oxygen consumption, representing the time necessary for the signal from the site of ATP hydrolysis, the myofibril, to travel to the mitochondria. A measure of this delay time has been termed “the mitochondrial response time of oxygen consumption” (tmito) (28, 29). In skeletal muscle, the dynamic response of mitochondrial oxygen consumption has a time constant >30 s, but in the heart, the time constant is much quicker, ~5–10 s.

With the use of nuclear magnetic resonance (NMR) techniques, it has been found that global, intracellular concentrations of phosphocreatine (PCr), inorganic phosphate, and ADP do not always change during increases in oxygen consumption (1, 2). However, the delay of 5–10 s, which has been measured for the adaptation of oxygen consumption to ATP hydrolysis, implies local, transient decreases in high-energy phosphates. Thus the response time of mitochondrial oxygen consumption suggests metabolic compartmentation of the cytosol and lends support for the metabolic wave theory, which postulates that oscillations of local concentrations of ADP and creatine are propagated through cytosolic compartments and are mediated through the enzymes creatine kinase (CK) and adenylate kinase (AK) (19, 20).

CK is a family of isoenzymes that catalyze the transfer of high-energy phosphates between PCr and ATP, thus keeping cellular ATP-to-ADP ratios balanced and the ATP pool highly charged. The role of CK in the regulation of phosphotransfer remains complex and poorly understood. A “PCr shuttle” has been hypothesized (3, 13, 33), where PCr and creatine provide a spatial buffer for the maintenance of optimal cellular ATP-to-ADP ratios, and where specialized CK isoenzymes are compartmentalized in the mitochondria and near the myofibrils. On the other hand, however, there is also support for the concept that CK catalyzed phosphotransfer operates in equilibrium as a “metabolic capacitor,” thus allowing the free diffusion of ATP and ADP to act as the energy transfer mechanism (16, 22, 24).

However, CK is not the only enzyme involved in phosphotransfer. Whereas several studies (7, 15, 19, 20, 34) have indicated that the most phosphoryls are transferred through multiple CK-catalyzed phosphoryl exchanges, disruption of either the mitochondrial or cytosolic CK genes does not result in overt ventricular dysfunction (21, 31). Such robustness suggests alternative phosphotransfer routes; i.e., AK or hexokinase. AK catalyzes the reversible reaction 2ADP ⇔ ATP +
AMP and can transfer ~10% of high-energy phosphoryls in skeletal muscle (7, 35). Indeed, it was recently shown (6) that AK-catalyzed phosphotransfer increased 134% in failing hearts, thus suggesting that AK may play a compensatory role in heart failure. Hexokinase catalyzes the reaction glucose + ATP ↔ G-6-P + ADP and may play a role in the control of mitochondrial oxidative phosphorylation by providing for the regeneration of the ADP signal (5).

Recent studies (30–32) performed with CK-inhibited hearts and CK-knockout mice show that normal workloads and high-energy phosphate levels can be maintained without active CK. However, contractile reserve is compromised in the hearts, with the evidence pointing to elevated ADP levels causing myofibrillar dysfunction (14, 26). Furthermore, energy transfer is delayed in stunned myocardium and it has been proposed that this was due to a decrease in CK activity (36). However, experiments performed in our laboratory show that graded pharmacological inhibition of CK produced a quickening, instead of a slowing, of the mitochondrial response time to a step in heart rate, suggesting that the energy metabolic signaling function of CK can be successfully bypassed by metabolite diffusion (11). In addition to CK, glycolysis buffers energetic signaling and results in slowed activation of oxidative phosphorylation (8, 10, 11, 28), in particular in stunned myocardium (37).

It was the purpose of this study to examine the role of CK- and AK-catalyzed phosphotransfer in the regulation of myocardial oxidative phosphorylation. We studied the myocardial mitochondrial response time to a step in pacing rate in isolated, Langendorff-perfused hearts obtained from mice, in which both the mitochondrial and the cytosolic (M) isoforms of the CK gene have been disrupted (M/MtCK−/−), and from mice in which the AK1 (cytosolic) isoform (25) of the AK gene has been disrupted (AK−/−). We show that the mitochondrial response time to a step in heart rate is not altered by disruption of the AK gene but that disruption of the mitochondrial and cytosolic genes encoding for CK leads to a considerable quickening of the activation of oxidative phosphorylation.

**METHODS AND MATERIALS**

**Animals.** M/MtCK−/− and AK−/− mice were generated and provided by the laboratory of Dr. B. Wieringa (University of Nijmegen, The Netherlands) (23) and were a gift from Dr. Klaas Nicolay (University of Utrecht, The Netherlands) and Dr. Arnold de Haan (Vrije Universiteit, Amsterdam, The Netherlands). The mutant mice had a mixed inbred background (C57BL6 and 129/Sv), whereas wild-type mice were of the C57/BL6 strain. Male and female mice weighing between 21 and 28 g were studied. The Institutional Animal Care and Use Committee of the Vrije Universiteit approved all experiments.

**Experimental preparation.** Each mouse was anesthetized with 2,2,2-tribromo-ethanol (0.4 g/kg ip; Avertin). A tracheotomy was performed and the mouse was artificially ventilated with 100% O2. After the thorax was opened, heparin (200 IU) was injected intravenously. The aorta was cannulated in situ with a blunted and grooved 20-gauge needle and perfusion was initiated before excision of the heart. The hearts were Langendorff-perfused at 37°C with Tyrode solution containing (in mM) 128.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.05 MgCl2, 20.2 NaHCO3, 0.42 NaH2PO4, and 11 glucose, and gassed with 95% O2–5% CO2. Adenosine (10 μM) was added to the perfusate to ensure maximal vasodilation of the preparation. Constant flow was maintained by pump perfusion and was set to achieve a perfusion pressure of 80–100 mmHg, resulting in a coronary flow varying between 1.1 and 1.7 ml/min. A small, flanged length of polyethylene (PE)-100 tubing was placed through the apex of the left ventricle to ensure drainage of Thebesian flow. A fluid-filled line attached to a latex balloon was introduced into the left ventricle and connected to a Statham P23 Db pressure transducer (Statham Instruments) for the measurement of left ventricular (LV) pressure. Fluid was injected via the line into the balloon by a microsyringe until end-diastolic pressure was 5–10 mmHg. LV developed pressure (LVDP) was calculated by subtracting end-diastolic pressure from systolic pressure. The pulmonary artery was cannulated with PE-100 tubing, and the venous effluent was drawn across a fast Clark-type oxygen electrode (time constant 1 s) at a rate of 1 ml/min for the measurement of coronary venous oxygen tensions. The heart was submerged and electrically stimulated via an electrode attached to the metallic aortic cannula and an electrode placed in the submersion bath. The basal pacing rate was set to 300 beats/min. Data were sampled at 100 Hz, or at 1,000 Hz for the determination of rise in developed pressure over time (dP/dt), and were recorded online with the use of a personal computer.

**Measurement of the response time of oxygen consumption.** The mean venous response time (ttrp) is determined from the time course of the venous oxygen tension to a step in heart rate. To derive the true tinito, i.e., the response time of oxygen consumption, the mean time necessary for oxygen diffusion and vascular transport, ttransp, is subtracted from ttrp (tinito = ttrp − ttransp). The ttransp was experimentally determined in this study from the venous oxygen response to a small step (<10%) in perfusion flow (29). In addition, it is necessary to adjust the response time for a delay in the rate-pressure product (tRPP), because a step in heart rate does not result in an immediate step in functional performance. The delayed RPP results in a negative tRPP, giving tinito = ttrp − tRPP. This procedure has been previously fully described for rabbit heart (28, 29) and recently for the mouse heart (10). Oxygen consumption (μmol·min⁻¹·g dry wt⁻¹) was calculated as the product of the flow collected from the pulmonary artery and the arteriovenous oxygen concentration difference.

**Experimental protocol.** The response time of oxygen consumption was studied during steps in pacing frequencies, from either 300 to 360 beats/min, or from 300 to 400 beats/min, in six wild-type, four AK−/−, and six M/MtCK−/− mice. Statistical analysis: Data are presented as means ± SE and were analyzed statistically using one-way analysis of variance and linear regression analysis. Differences were considered statistically significant at P < 0.05.

**RESULTS**

**Mechanical performance.** Heart weights were significantly higher for the M/MtCK−/− group, and lower for the AK−/− group, compared with the wild-type group (183.8 ± 10.4 and 114.3 ± 2.0 vs. 138.3 ± 5.3 mg for control) (Table 1). This difference was maintained when heart weight was expressed as milligrams per gram of mouse weight. The amount of coronary effluent...
that could be collected from the pulmonary artery (expressed in milliliters coronary flow·min⁻¹·g heart wt⁻¹) was similar for all three groups: 9.0 ± 1.5 (M/MtCK⁻/⁻) and 9.5 ± 1.8 (AK⁻/⁻) versus 9.4 ± 1.0 ml for control (Table 1). Systolic LVDP (Fig. 1) was highest in the M/MtCK⁻/⁻ hearts, 88.2 ± 6.8 mmHg at 300 beats/min (P < 0.05), which fell to 74.8 ± 6.1 and 69.7 ± 6.1 mmHg when pacing was set to 360 and 400 beats/min, respectively. A similar fall in LVDP was exhibited by hearts, although MVO₂ was higher for all three groups (65.4 ± 3.8, 68.3 ± 3.7, and 66.6 ± 6.5% for the wild-type, M/MtCK⁻/⁻, and AK⁻/⁻ mice, respectively). In contrast, there was no difference between the three groups of the ratio of dP/dtmax to dP/dtmin during the recovery stage from a higher pacing rate back to the basal heart rate (65.4 ± 3.8, 68.3 ± 3.7, and 66.6 ± 6.5% for the wild-type, M/MtCK⁻/⁻, and AK⁻/⁻ mice, respectively).

Oxygen consumption. RPP, which is the product of LVDP and the pacing rate, did not increase significantly for either the wild-type, M/MtCK⁻/⁻, or AK⁻/⁻ hearts when pacing steps were induced from 300 to 360 beats/min or from 300 to 400 beats/min (Fig. 3). M/MtCK⁻/⁻ hearts, however, exhibited consistently higher RPPs compared with the wild-type hearts (P < 0.05). The significantly higher RPPs found in the M/MtCK⁻/⁻ hearts, however, did not result in higher oxygen consumption compared with the wild-type hearts, although MV O₂ was higher for all three groups at 400 beats/min, compared with 300 beats/min. Thus the M/MtCK⁻/⁻ hearts achieved higher levels of pressure development (i.e., RPP) (P < 0.05) at similar levels of myocardial oxygen consumption (MV O₂), as represented in Fig. 4.

Response time. The mitochondrial response to a step in pacing rate (300 to 360 or 300 to 400 beats/min) was 8.0 ± 0.4 s for wild-type hearts, 3.0 ± 0.3 s for M/MtCK⁻/⁻, and 7.3 ± 0.6 s for AK⁻/⁻ hearts (Fig. 5). The size of the pacing step (either 60 or 100 beats/min) did not have an effect on the mitochondrial response time and was therefore grouped for each heart.

**DISCUSSION**

The mitochondrial response to a step in MV O₂ reflects the processes involved in the interaction between energy utilization and energy supply. Transcytosolic signaling from the myofibril to the mitochondria is an inherent component of this interaction. It was the purpose, therefore, of this study to examine two components of high-energy phosphoryl transfer, which have been postulated to play primary roles in transcytosolic signaling. To achieve this, we examined t мito in

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**Table 1. Physiological data from wild-type, M/MtCK⁻/⁻, and AK⁻/⁻ mice**

<table>
<thead>
<tr>
<th></th>
<th>Mouse Weight, g</th>
<th>Wet Heart Weight, mg</th>
<th>Heart Wt/Mouse Wt, mg/g</th>
<th>Coronary Flow, ml/min</th>
<th>Coronary Flow, ml·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>23.3 ± 0.6</td>
<td>138 ± 5</td>
<td>6.0 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>CK⁻/⁻</td>
<td>24.7 ± 1.0</td>
<td>183 ± 1*</td>
<td>7.5 ± 0.1*</td>
<td>1.7 ± 0.3</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>AK⁻/⁻</td>
<td>25.9 ± 0.7*</td>
<td>114 ± 2*</td>
<td>4.4 ± 0.1*</td>
<td>1.1 ± 0.2</td>
<td>9.5 ± 1.8</td>
</tr>
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Values are means ± SE from 4 to 6 experiments. M/MtCK, mice genetically altered without creatine kinase; AK, adenylate kinase. *P < 0.05 vs. control.

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Fig. 1. Left ventricular (LV) developed pressure (LVDP) (in mmHg, means ± SE) for wild-type (●), adenylate kinase-altered (AK⁻/⁻) (■), or creatine kinase-altered (M/MtCK⁻/⁻) (▲) hearts. *P < 0.05 vs. wild type, +P < 0.05 vs. own 300 beats/min (bpm).
The major finding of the present study is that the mitochondrial response time to a step in MV \( \dot{O}_2 \) is 2–3 times faster in hearts that lack CK. In contrast to this, hearts lacking the cytosolic isoenzyme of AK showed a normal dynamic response to a step in pacing rate. The data from the M/MtCK\(^{-/-}\) hearts are in good agreement with previous findings using graded pharmacological inhibition of the flux through all CK isoenzymes. Harrison et al. (11), using iodoacetamide in rabbit heart to inhibit CK flux by 97%, have shown the mitochondrial response to become quicker: from \(~8\) s without inhibition to \(3\) s with inhibition. Previous biochemical assays performed on M/MtCK\(^{-/-}\) hearts indi-

![Fig. 2. Representative LV pressure (LVP) tracings from wild-type, AK\(^{-/-}\), or M/MtCK\(^{-/-}\) hearts for pacing steps made from 300 to 360 beats/min and back to 300 beats/min. Step to higher heart rate (300 to 360 beats/min) gave an initial dip in LVP, followed by a rise and then fall to a new steady state. Step back to basal heart rate resulted in a spike, followed by a return to the basal level of LVP. Note the delayed return to basal LVP in the M/MtCK\(^{-/-}\) hearts.](http://ajpheart.physiology.org)

![Fig. 3. Rate pressure products (RPP; mmHg/min) (A) and myocardial oxygen consumption (MV\( \dot{O}_2 \)) (mol·min\(^{-1} \cdot \) g dry wt\(^{-1} \)) (B) (means ± SE) for wild-type (solid bars), AK\(^{-/-}\) (gray bars), or M/MtCK\(^{-/-}\) (open bars) hearts. *\(P < 0.05\) vs. wild type, +\(P < 0.05\) vs. own 300 beats/min.](http://ajpheart.physiology.org)

![Fig. 4. Relationship between rate-pressure product (RPP) and MV\( \dot{O}_2 \) (means ± SE) for wild-type (●), AK\(^{-/-}\) (●), or M/MtCK\(^{-/-}\) (▲) hearts.](http://ajpheart.physiology.org)
cate residual myocardial CK activity to be \(~4\%\) (21); very similar to the degree of inhibition achieved by Harrison et al. (11) with infusion of 0.4 mM iodoacetamide. Both pharmacological inhibition and genetic ablation of CK thus result in faster activation of oxidative phosphorylation. This is compatible with the theory that CK buffers ADP and ATP near sites of ATP hydrolysis and thereby delays the transfer of the energy-related signal to the mitochondria.

Deletion of AK1 has been shown to result in stress intolerance and disturbed muscle economy (12, 18). Furthermore, AK has recently been shown to facilitate delivery of mitochondrial signals to the cell membrane (4). That the data presented here show that ablation of the cytosolic isoenzyme of AK has no effect on the mitochondrial response is surprising yet may reflect that AK-catalyzed phosphotransfer only contributes to 10% of the total ATP turnover rate in intact, wild-type myocardium, as measured by \([^{18}\text{O}]\)phosphoryl-labeling techniques (6). Furthermore, Pucar et al. (18) have shown that AK2-catalyzed phosphoryl transfer is upregulated in AK1\(^{-/-}\) hearts, which may alleviate AK\(^{-/-}\) ablation effects. The AK\(^{-/-}\) hearts studied here showed similar contractile behavior as the wild-type hearts, except that, in addition to lower LVDP, the ratio of \(dP/dt_{\text{min}}\) to \(dP/dt_{\text{max}}\) was also lower (69% for AK\(^{-/-}\) vs. 83% for wild type, \(P < 0.05\)). A depression of this ratio may indicate slower relaxation kinetics compared with contraction kinetics. Because end-diastolic pressure, which can also be considered as indicative of relaxation kinetics, has been shown to rise with inhibition of glycolysis by iodoacetate acid (11), the slower relaxation kinetics found in the AK\(^{-/-}\) hearts may be due to the postulated link (35) between glycolysis and AK, coupled to ion pumps and/or channels. In contradiction to this hypothesis, however, is the observation that AK\(^{-/-}\) hearts have enhanced levels of the glycolytic enzyme 3-phosphoglycerate kinase along with normal basal nucleotide levels (18).

An interesting observation made in the M/MtCK\(^{-/-}\) hearts is the possibility of higher efficiency in the development of pressure, i.e., higher developed pressure with similar MVo2 compared with the wild-type hearts. This observation is in conflict with data presented by Saupe et al. (21), which showed that deletion of MCK and/or MtCK did not alter the relationship between RPP and MVo2. Because the measurement of energetic parameters was beyond the scope of this study, it is difficult to resolve these conflicting observations. One should note, however, that increases in work in Saupe et al. were achieved by elevations in perfusate calcium, at a constant pacing rate of 420 beats/min, compared with this study, in which the pacing steps were induced at constant calcium concentrations. Steeghs et al. (23) have found in skeletal muscle that metabolism in M/MtCK\(^{-/-}\) mice may rely more on glycolytic ATP generation. This conclusion was based on NMR findings as well as the histological observation that skeletal muscle and heart from M/MtCK\(^{-/-}\) mice contained large amounts of lipid droplets, suggesting a lowered ability for the aerobic oxidation of fatty acids. Because ATP yield per oxygen is higher with carbohydrate oxidation (9), one hypothesis is to be investigated for the enhanced performance per oxygen seen in the M/MtCK\(^{-/-}\) hearts is that the M/MtCK\(^{-/-}\) hearts have an enhancement of the glycolytic machinery. Biochemical assays of M/MtCK\(^{-/-}\) hearts and skeletal muscle, however, show no enhancement in the amounts of phosphofructokinase or glycer-aldehyde 3-phosphate dehydrogenase, two key glycolytic enzymes (21). In vitro measurements of glycolytic enzyme activity do not, however, exclude the possibility of an enhancement in vivo glycolytic flux in the M/MtCK\(^{-/-}\) hearts, compared with the wild-type or AK\(^{-/-}\) hearts.

Finally, our data show that both M/MtCK\(^{-/-}\) and AK\(^{-/-}\) hearts have sufficient contractile reserve to maintain RPP when pacing steps are made from 300 to 360 beats/min or from 300 to 400 beats/min. This finding of a normal contractile response was also observed by Saupe et al. (21) in MCK\(^{-/-}\) hearts, as well as M/MtCK\(^{-/-}\) hearts, yet is in contrast to studies in which pharmacological inhibition of CK activity to <5% diminished contractile reserve (27). The apparent difference between the genetic and pharmacological models may lie in the degree that the BB (cytosolic) isozyme is able to contribute because this isozyme is intact in the genetic knockout of M/MtCK\(^{-/-}\) (21).

A new observation is the maintained depression in M/MtCK\(^{-/-}\) hearts of systolic ventricular pressure after a return from the higher heart rate (i.e., 360 beats/ min) to the basal heart rate (300 beats/min). We speculate that this is due to an ADP inhibition of myosin ATPase, which has been shown to occur in guinea pig smooth muscle (17). NMR measurements performed on M/MtCK\(^{-/-}\) hearts show indeed a twofold higher ADP concentration during increased cardiac work (21).

In conclusion, the mitochondrial response time to a step in heart rate is two to three times faster in hearts where the mitochondrial and cytosolic isoforms of CK have been ablated. This is in contrast to the normal mitochondrial response time of hearts where the cytosolic isoform of AK has been ablated. These results support the role of creatine kinase as a buffer for rapid oscillations in local ATP concentrations near sites of ATP usage, which delays the transfer of the metabolic stimulus to oxidative phosphorylation.
REFERENCES


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