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 (MV̇O₂). 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 mmHg) and lower in AK⁻⁻ hearts (46.7 ± 9.4 mmHg) compared with wild-type hearts (60.7 ± 10.1 mmHg) at the basal pacing rate. Developed pressure fell slightly when heart rate was increased in all three groups. Basal MV̇O₂ 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 and 8.0 ± 0.4 s for M/MtCK⁻⁻, AK⁻⁻, and wild-type hearts, respectively. Our results demonstrate that MV̇O₂ 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. Changes in basal MV̇O₂ and tmito reflect the changes in ATP hydrolysis and mitochondrial oxidative phosphorylation.

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AMP and can transfer ~10% of high-energy phospho-
rlys 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 work-
loads and high-energy phosphate levels can be main-
tained without active CK. However, contractile reserve
is compromised in the hearts, with the evidence point-
ing to elevated ADP levels causing myofibrillar dys-
function (14, 26). Furthermore, energy transfer is de-
layed 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 func-
tion 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 regu-
lation 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 mito-
chondrial 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 mito-
chondrial 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 ac-
tivation 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 back-
ground (C57/BL6 and 129/Sv), whereas wild-type mice were
of the C57/BL6 strain. Male and female mice weighing be-
 tween 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 trache-
otomy was performed and the mouse was artificially venti-
lated with 100% O2. After the thorax was opened, heparin
(200 IU) was injected intravenously. The aorta was cannu-
lated 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 solu-
tion 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 pre-
paration. 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 ventricu-
lar (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 venous oxygen tensions. The heart
was submerged and electrically stimulated via an elec-

drode 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 (tv) is determined from the
time course of the venous oxygen tension to a step in heart
rate. To derive the true tmito, i.e., the response time of oxygen
consumption, the mean time necessary for oxygen diffusion
and vascular transport, ttransp, is subtracted from tv (tv =
tmito − 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 tmito = tv − ttransp −
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 calcu-
lated as the product of the flow collected from the pulmonary
artery and the arteriovenous oxygen concentration differ-
ence.

Experimental protocol. The response time of oxygen con-
sumption 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 signifi-
cantly 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

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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.

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 the wild-type hearts, which fell from 60.7 ± 10 to 55.8 ± 2.4 and 50.0 ± 2.1 mmHg when pacing was set to 360 and 400 beats/min, respectively. A similar fall in LVDP was observed in the AK−/− hearts exhibited. At basal pacing rate of 300 beats/min, RPP values were 18,200 beats/min, respectively. At the basal pacing rate (300 beats/min) resulted in a spike in LVDP and the pacing rate, did not increase significantly compared with the wild-type, M/MtCK−/−, and AK−/− mice, respectively. The signiﬁcantly higher RPPs found in the M/MtCK−/− hearts, however, did not result in higher LVDP compared with the wild-type, M/MtCK−/−, and AK−/− mice, respectively. In contrast, there was no difference between the three groups of the ratio of dP/dtmin to dP/dtmax 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 MVO2 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 (MVO2), 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 MVO2 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 fmito in...
The major finding of the present study is that the mitochondrial response time to a step in \( \dot{V}_O_2 \) 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 \( \approx 8 \) s without inhibition to 3 s with inhibition. Previous biochemical assays performed on M/MtCK\(^{-/-}\) hearts indicated.

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.

Fig. 3. Rate pressure products (RPP; mmHg/min) (A) and myocardial oxygen consumption (MV\(_O_2\)) (\( \mu\)mol·min\(^{-1}\)·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.

Fig. 4. Relationship between rate-pressure product (RPP) and MV\(_O_2\) (means ± SE) for wild-type (●), AK\(^{-/-}\) (■), or M/MtCK\(^{-/-}\) (▲) hearts.
cate residual myocardial CK activity to be \( \sim 4\% \) (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 \(^{13}\)O phosphorl-labeling techniques (6). Furthermore, Pucar et al. (18) have shown that AK2-catalyzed phosphotransfer 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 \( \frac{\partial P}{\partial \text{d}_{\text{min}}} \) to \( \frac{\partial P}{\partial \text{d}_{\text{max}}} \) 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 (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 MV\(_2\) 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 MV\(_2\). 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 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 glyceraldehyde 3-phosphate dehydrogenase, two key glycolytic enzymes (21). In vitro measurements of glycolytic enzyme activity do not, however, exclude the possibility of an enhancement of 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 isozyme 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.
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REFERENCES


