Mitochondrial creatine kinase is critically necessary for normal myocardial high-energy phosphate metabolism

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Spindler, Matthias, Reinhard Niebler, Helga Remkes, Michael Horn, Titus Lanz, and Stefan Neubauer. Mitochondrial creatine kinase is critically necessary for normal myocardial high-energy phosphate metabolism. Am J Physiol Heart Circ Physiol 283: H680–H687, 2002.—First published May 2, 2002; 10.1152/ajpheart.00800.2001.—The individual functional significance of the various creatine kinase (CK) isoenzymes for myocardial energy homeostasis is poorly understood. Whereas transgenic hearts lacking the M subunit of CK (M-CK) show unaltered cardiac energetics and left ventricular (LV) performance, deletion of M-CK in combination with loss of sarcomeric mitochondrial CK (ScCKmit) leads to significant alterations in myocardial high-energy phosphate metabolites. To address the question as to whether this alteration is due to a decrease in total CK activity below a critical threshold or due to the specific loss of ScCKmit, we studied isolated perfused hearts with selective loss of ScCKmit (ScCKmit<sup>−/−</sup>; remaining total CK activity ~70%) using 31P NMR spectroscopy at two different workloads. LV performance in ScCKmit<sup>−/−</sup> hearts (n = 11) was similar compared with wild-type hearts (n = 9). Phosphocreatinine/ATP, however, was significantly reduced in ScCKmit<sup>−/−</sup> compared with wild-type hearts (1.02 ± 0.05 vs. 1.54 ± 0.07, P < 0.05). In parallel, free [ADP] was higher (144 ± 11 vs. 67 ± 7 μM, P < 0.01) and free energy release for ATP hydrolysis (∆G<sub>ATP</sub>) was lower (−55.8 ± 0.5 vs. −58.5 ± 0.5 kJ/mol, P < 0.01) in ScCKmit<sup>−/−</sup> compared with wild-type hearts. These results demonstrate that M- and B-CK containing isoenzymes are unable to fully substitute for the loss of ScCKmit. We conclude that ScCKmit, in contrast to M-CK, is critically necessary to maintain normal high-energy phosphate metabolite levels in the heart.

Creatine kinase; energy metabolism; nuclear magnetic resonance spectroscopy; transgenic mouse

CREATINE KINASE (CK; EC 2.7.2.2) is a key enzyme involved in energy metabolism in tissues with large fluctuations of energetic demand such as the muscle or brain. CK catalyses the reversible transfer of a high-energy phosphoryl group between ATP and phosphocreatine (PCr). Four different isoenzymes of CK are known; three are dimers composed of two subunits (MM-CK, MB-CK, and BB-CK), whereas sarcomeric mitochondrial CK (ScCKmit) can form both dimers and octamers (for a review, see Ref. 28). These isoenzymes are localized in a compartmentalized fashion in the cell. MM-CK, the most abundant muscle isoform, is a structural protein of the myofibrillar M band. ScCKmit, the second most abundant isoform, is found on the outer surface of the inner mitochondrial membrane, forming a functional compartment with porin and adenine nucleotide translocase (29). This characteristic spatial distribution has led to the “CK shuttle” hypothesis, where PCr serves as an energy transfer molecule for fast and efficient transport of phosphoryl moieties from the sites of energy generation (mitochondria) to the sites of energy consumption (myofibrils and ion pumps) (1). On the other hand, the PCr-CK system has been generally regarded as a high-energy buffer system that meets increased energetic requirements during periods of mismatched energy production and consumption. The physiological importance of the CK system in heart muscle is underlined by numerous reports of alterations in a variety of components of the PCr-CK system found in various animal models of heart failure as well as in human heart failure (10, 12–14).

Despite several decades of research, however, the true nature of the fundamental role of CK, especially in disease states such as myopathies or heart failure, remains ill defined. Transgenic animals with null mutations of one or more of the genes of the CK family may shed new light on the functional significance of the PCr-CK system. For example, skeletal muscle of mice lacking the M subunit of CK, referred to here as M-CK<sup>−/−</sup>, demonstrated a transient impairment in contractile function (burst activity) (25). However, concentrations of high-energy phosphate metabolites were unaltered and PCr was still hydrolyzed and resynthesized during contraction. When ScCKmit is abladed in addition to M-CK (referred to as M/ScCKmit<sup>−/−</sup>), leaving only BB-CK activity, skeletal muscle had a 30% lower PCr-to-ATP ratio (PCr/ATP) and was unable to hydrolyze PCr (22).

In cardiac muscle, contractile performance of isolated perfused hearts was unchanged for low-to-mod-
erate workloads in M-CK−/− and M/ScCKmit−/− mice (20, 26). Whereas hearts of M-CK−/− mice (30% remaining CK activity, consisting of ScCKmit and BB-CK) showed no difference in PCr/ATP compared with wild-type hearts (26), M/ScCKmit−/− hearts (with only 3% remaining CK activity) had a 25% lower PCr/ATP. Thus impaired myocardial energetics have been demonstrated for hearts of M/ScCKmit−/− mice only (19, 20). At present, it is unclear whether these reduced PCR/ATP are predominantly caused by the specific loss of ScCKmit or by the overall decrease of total CK activity below a certain threshold necessary to maintain “normal” energetics. This question can only be directly addressed by studying hearts with an isolated ablation of ScCKmit (referred to as ScCKmit−/−).

The purpose of the present work was, therefore, to define left ventricular (LV) performance, CK activity, isoenzyme distribution, and high-energy phosphate metabolite concentrations under two different workloads in ScCKmit−/− hearts. 31P NMR spectroscopy was used to measure [ATP], [PCr], [Pi], [ADP], free energy for ATP hydrolysis (ΔGATP), and pH in isolated beating hearts of mutant and wild-type mice.

METHODS

Animals. ScCKmit−/− mice were generated in the laboratory of Dr. Bé Wieringa (University of Nijmegen, Nijmegen, The Netherlands) by gene targeting as previously reported (24). Male and female mice of 20–30 wk of age were studied. There was no difference between WT and ScCKmit−/− mice regarding heart weight or heart weight-to-body weight ratios. The genotype of each mouse was confirmed by measuring the isoenzymes of CK present using a Helena Cardio-Rep CK isoenzyme analyzer (Helena Diagnostika). The experimental protocol for the present study followed the American Physiological Society guidelines for the care and use of laboratory animals.

Isolated perfused heart preparation. Hearts of WT and ScCKmit−/− mice were isolated and perfused in the Langendorff preparation in a 10-mm NMR tube as previously described (21). Retrograde perfusion via the aorta was carried out at a constant coronary perfusion pressure of 75 mmHg at 37°C. Coronary flow was measured by collecting coronary sinus effluent through a suction tube. Phosphate-free Krebs-Henseleit buffer containing (in mM) 118 NaCl, 5.3 KCl, 2.0 CaCl2, 1.2 MgSO4, 0.5 EDTA, 25 NaHCO3, 10 glucose, and 0.5 pyruvate as substrates was prepared at the time of the experiment and equilibrated with 95% O2-5% CO2, yielding a pH of 7.4. All hearts were paced at 7 Hz using a pulse width of 1.84 s. The maximum values within a beat of the first derivative of LV pressure (−dP/dt and +dP/dt, respectively), and rate-pressure product (RPP; product of LVDP and heart rate) were calculated off-line.

31P NMR spectroscopy. 31P NMR spectra were obtained at 121.50 MHz using a superwide-bore NMR spectrometer (Bruker; Rheinstetten, Germany) equipped with an Aspect 3000 computer. Hearts were placed in a 10-mm NMR tube and inserted into a custom-made 1H/31P double-tuned probe situated in a 150-mm bore, 7.05-T superconducting magnet. To improve homogeneity of the NMR-sensitive volume, the perfusate level was adjusted so that the heart was submerged in buffer. Spectra were collected at a pulse length of 17.2 μs, pulse angle of 60°, repetition time of 1.84 s, and sweep width of 6,000 Hz. Single spectra were collected for 8-min periods and consisted of 256 consecutive free induction decays.

In the time domain, the amplitudes of the resonances of ATP, PCr, and Pi, which are proportional to the number of phosphorus atoms in the respective compound, were determined using the “AMARES”-routine including prior knowledge. Briefly, J-coupling of 16 Hz, same linewidth, and 1:1 or 1:2:1 ratios for the amplitudes within each duplet or triplet of ATP was used as prior knowledge for the ATP signals. The calculation of PCR/ATP was based on the fit integrals of PCR and γ-ATP. By comparing the peak amplitude of fully relaxed (recycle time 15 s) and those of partially saturated (recycle time 1.84 s) spectra, we calculated the correction factors for saturation for ATP (1.0), PCr (1.3), and Pi (1.05).

To independently confirm that the ATP concentrations were not different among the two groups, separate wild-type and ScCKmit−/− hearts were analyzed for ATP content via HPLC as described below (for details, see Biochemical assays). The concentrations of ATP were 10.6 ± 0.4 mM for wild-type hearts and 9.6 ± 0.4 mM for ScCKmit−/− hearts, respectively (not significantly different). Thus the ATP resonance area in the first spectrum of each heart was set to the average concentration of ATP measured biochemically for that group. The γ-ATP resonance area of each heart was used as an internal standard to correct resonance areas of PCr and Pi to their respective concentrations.

Intracellular pH (pHi) was determined by comparing the chemical shift of the P, Pi, and PCr peaks in each spectrum to values from a standard curve.

Cytosolic free [ADP] was calculated using the equilibrium constant of the CK reaction and from values obtained by NMR spectroscopy and biochemical assays

\[
[ADP] = ([ATP][free Cr]/[PCr][H+])K_{eq}
\]

where the equilibrium constant (K_{eq}) is 1.66 × 10^8 M^-1 for a [Mg^{2+}] of 1.0 mM (9).

The free energy stored in the high-energy phosphate bonds of ATP and released by ΔG_{ATP} is a negative number. For purposes of clarity, all values of ΔG_{ATP} are expressed as their absolute values (|ΔG_{ATP}|). Thus increases in |ΔG_{ATP}| (in kcal/mol) indicate an increase in free energy release

\[
|ΔG_{ATP}| = |ΔG^*| + RT \ln ([ADP][Pi]/[ATP])
\]

where ΔG^* (−30.5 kcal/mol) is the value of ΔG_{ATP} under standard conditions of molarity, temperature, pH, and [Mg^{2+}]; R is the gas constant (8.3 J/mol⋅K), and T is the temperature (in K) (4).
Biochemical assays. In one group of hearts (6 wild type and 7 ScCKmit−/−), concentrations of the primary nucleotides and nucleosides were measured using HPLC (Pharmacia). After 16 min of baseline perfusion, these hearts were freeze clamped, the tissue was homogenized in 0.4 N perchloric acid at 0°C, and aliquots of the homogenate were removed for protein determination. The homogenates were neutralized with saturated KOH and centrifugated for 5 min. Aliquots of the supernatant were applied to a HPLC column (Supelcosil LC-18, 4.6 × 250 mm, Supelco). Nucleosides and nucleotides were eluted at 30°C isocratically using 0.2 M phosphate buffer (pH 6.0) at a flow rate of 0.8 ml/min. The column effluent was analyzed at 205 nm for ATP, and amounts were calculated using external standards. In these hearts, the measured [ATP] were 22.4 ± 1.7 and 24.3 ± 1.1 nmol ATP/mg protein in wild-type and ScCKmit−/− hearts, respectively. With the use of measured values for protein concentration and the literature value for the ratio of intracellular volume to total cell volume of 0.48 (16), these values were converted to [ATP] of 10.6 ± 0.4 and 9.6 ± 0.4 mM for wild-type and ScCKmit−/− hearts, respectively. With the use of other aliquots, total creatine content was measured using the method of Kammermeier (8). Noncollagen protein was measured by the method of Lowry with bovine serum albumin as the standard (11). Total lactate dehydrogenase (LDH) activity, LDH isozymes, and citrate synthase activity were measured as previously described (14). Creatine transporter protein was quantified by Western blot as previously published by our group (15).

Total CK activity and the proportion of this activity attributable to each isoenzyme of CK was measured using methods previously described. CK activities were measured in international units per milligram of protein and converted to micromolars per second using the measured concentrations of cardiac protein. All values are expressed as micromolars per second at 37°C. The percentage of total CK activity attributable to each isoenzyme was measured using a Helena Cardio-Rep CK isoenzyme analyzer (17).

Statistical analysis. All data are expressed as means ± SE. Paired and unpaired Student’s t-tests as appropriate were used to compare ScCKmit−/− and wild-type hearts at baseline and high workload. Statistical analyses were performed with the use of Statview (Brainpower; Calabasas, CA), and values of P < 0.05 were considered statistically significant.

RESULTS

General characteristics and CK system of wild-type and ScCKmit−/− mice. Body weight, LV weight, and LV weight-to-body weight ratios were similar in both groups, ruling out gross cardiac hypertrophy (Table 1). Coronary flow was also unchanged in ScCKmit−/− compared with wild-type hearts (data not shown).

Total CK activity in hearts of ScCKmit−/− mice was 30% less than that of wild-type tissue (39.8 ± 3.1 mM/s for ScCKmit−/− vs. 58.2 ± 1.4 mM/s for wild type). Maximal activities of CK isoenzymes and isoenzyme distribution relative to total CK activity are summarized in Table 2. In contrast to what has been described for skeletal muscle of ScCKmit−/− mice, there was no compensatory increase in absolute MM-CK activity in heart tissue.

Furthermore, there was no difference in citrate synthase, a marker of mitochondrial density, and in total LDH activities (see Table 1) or LDH isoenzyme distribution, an index of the ratio of oxidative to glycolytic capacity (data not shown).

Intracellular noncollagen protein concentration, measured as Lowry protein, was 0.147 ± 0.005 and 0.141 ± 0.006 mg protein/mg wet wt for wild-type and ScCKmit−/− hearts, respectively.

Cardiac function and high-energy phosphate metabolism under baseline conditions. When EDP was set to ~8 mmHg and isolated hearts were paced at 420 beats/min, LVDP was not different between wild-type and ScCKmit−/− hearts (see Table 3). Likewise, no difference was observable with regard to RPP, +dP/dt, −dP/dt, and coronary flow/hem weight. As described previously for isolated hearts with isolated knockout of M-C or combined knockout of M-C and ScCKmit (20), contractile performance was stable with variations, i.e., in RPP of <5% during a 30-min baseline perfusion period.

Representative 31P NMR spectra from a wild-type and a ScCKmit−/− heart during baseline perfusion are shown in Fig. 1. As visible in these spectra, [PCr] was ~35% lower in the ScCKmit−/− heart (10.2 ± 0.5 mM) compared with the wild-type heart (15.5 ± 0.7 mM). [ATP], [Pi], and pHi were not different between both groups. Accordingly, PCr/ATP were lower in ScCKmit−/− hearts compared with wild-type ones.

Table 1. Heart weight, body weight, and biochemical data of wild-type and ScCKmit−/− mice

<table>
<thead>
<tr>
<th></th>
<th>Wild type (n = 10)</th>
<th>ScCKmit−/− (n = 10)</th>
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<tbody>
<tr>
<td>Body Weight, g</td>
<td>29.2 ± 0.8</td>
<td>28.4 ± 1.1</td>
</tr>
<tr>
<td>LV Weight, mg</td>
<td>111 ± 3</td>
<td>101 ± 6</td>
</tr>
<tr>
<td>Protein, mg</td>
<td>4.9 ± 0.1</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>mm protein/mg wet wt</td>
<td>1.147 ± 0.005</td>
<td>1.141 ± 0.006</td>
</tr>
<tr>
<td>Creatine, mM</td>
<td>18.3 ± 1.2</td>
<td>18.0 ± 1.2</td>
</tr>
<tr>
<td>Citrate Synthase, IU/mg protein</td>
<td>1.13 ± 0.11</td>
<td>1.16 ± 0.06</td>
</tr>
<tr>
<td>LDH, IU/mg protein</td>
<td>0.48 ± 0.01</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>Na+/Creatine Transporter, AU of OD</td>
<td>27.4 ± 3.3</td>
<td>24.3 ± 3.9</td>
</tr>
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</table>

Values are means ± SE; n = 9 wild-type and 11 scarkotic mitochondrial creatinine kinase (CK)-deficient (ScCKmit−/−) mice. LV, left ventricular; LDH, lactate dehydrogenase; AU, arbitrary units; OD, optical density.

Table 2. CK isoenzyme distribution and isoenzyme activities in hearts of wild-type and ScCKmit−/− mice

<table>
<thead>
<tr>
<th></th>
<th>Wild Type (n = 10)</th>
<th>ScCKmit−/− (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CK, mM/s</td>
<td>58.2 ± 1.4</td>
<td>39.8 ± 3.1*</td>
</tr>
<tr>
<td>MM-CK, mM/s</td>
<td>33.6 ± 0.8</td>
<td>37.1 ± 2.9</td>
</tr>
<tr>
<td>BB-CK, mM/s</td>
<td>1.6 ± 0.1</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>ScCKmit, mM/s</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>MM-CK, %total</td>
<td>57.8 ± 1.2</td>
<td>ND</td>
</tr>
<tr>
<td>MB-CK, %total</td>
<td>2.8 ± 0.2</td>
<td>5.3 ± 0.3*</td>
</tr>
<tr>
<td>BB-CK, %total</td>
<td>0.8 ± 0.2</td>
<td>1.5 ± 0.2*</td>
</tr>
<tr>
<td>ScCKmit, %total</td>
<td>38.6 ± 1.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of hearts. ND, not detectable. *P < 0.05, ScCKmit−/− vs. wild-type hearts.
ScCKmit−/− (1.02 ± 0.05) versus wild-type hearts (1.54 ± 0.07) (Fig. 2). Total creatine concentration, measured biochemically with HPLC in the same hearts, revealed similar values for ScCKmit−/− and wild-type hearts (Table 1). Furthermore, myocardial Na+–creatine cotransporter, the protein responsible for regulating the myocardial creatine concentration, was also similar in both groups.

When intracellular free [ADP] was calculated from the equilibrium equation for the CK reaction, significantly higher [ADP] were found for ScCKmit−/− (144 ± 11 μM) compared with wild-type hearts (67 ± 7 μM) (Fig. 2). Because of this doubling of free [ADP], calculated ΔGATP was significantly lower in ScCKmit−/− compared with wild-type hearts (−55.8 ± 0.5 vs. −58.5 ± 0.5 kJ/mol, respectively) (Fig. 2).

Cardiac high-energy phosphate metabolism and cardiac function during increased work and during recovery. When workload was increased by doubling of the extracellular [Ca2+] and increasing pacing frequency from 420 to 600 beats/min, EDP increased slightly and LVDP essentially remained constant, leading to an increase in RPP on average of 40–55% (see Table 3). There were no differences in relative and absolute increases in RPP between both groups during increased work.

When perfusate [Ca2+] and pacing frequency was returned to baseline levels, LVDP and RPP fell below values observed during baseline perfusion. On average, RPP returned to 75 ± 4% of baseline for wild-type hearts and 79 ± 3% for ScCKmit−/− hearts after 16 min of recovery (see Table 3). There were no significant differences between isolated wild-type and ScCKmit−/− hearts with regard to EDP, LVDP, +dP/dt, and −dP/dt during high workload and during recovery from increased cardiac work.

Increasing workload by 40–55% lead to the expected changes of high- and low-energy phosphate concentrations. [Pi] more than doubled, whereas [PCr] decreased 5.7 mM (by 37%) in wild-type hearts and 2.4 mM (by 24%) in ScCKmit−/− hearts. During recovery, a similar amount of PCr was resynthetized in both groups of hearts, and [PCr] after 10 min of recovery reached almost the baseline level. In parallel to this resynthesis of PCr, [Pi] decreased in both groups to baseline levels.

Increasing RPP caused [ATP] to decrease similarly in both groups, by 1.5 mM in wild-type hearts and by 0.9 mM in ScCKmit−/− hearts. The total cardiac phos-

Table 3. Isovolumic LV contractile performance at baseline, during increased work, and during recovery of wild-type and ScCKmit−/− hearts

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Increased Work</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>EDP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>7 ± 1</td>
<td>12 ± 2</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>ScCKmit−/−</td>
<td>8 ± 1</td>
<td>11 ± 1</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>58.5 ± 0.5</td>
<td>55.8 ± 0.5</td>
<td>55.8 ± 0.5</td>
</tr>
<tr>
<td>ScCKmit−/−</td>
<td>58.5 ± 0.5</td>
<td>55.8 ± 0.5</td>
<td>55.8 ± 0.5</td>
</tr>
<tr>
<td>RPP, mmHg·beats·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>34,800 ± 1,900</td>
<td>47,000 ± 3,000</td>
<td>25,900 ± 1,400</td>
</tr>
<tr>
<td>ScCKmit−/−</td>
<td>34,400 ± 1,200</td>
<td>53,200 ± 2,300</td>
<td>28,100 ± 1,300</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>3,100 ± 100</td>
<td>3,400 ± 200</td>
<td>2,300 ± 100</td>
</tr>
<tr>
<td>ScCKmit−/−</td>
<td>3,100 ± 100</td>
<td>3,600 ± 200</td>
<td>2,400 ± 200</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>2,400 ± 200</td>
<td>3,200 ± 300</td>
<td>1,600 ± 100</td>
</tr>
<tr>
<td>ScCKmit−/−</td>
<td>2,500 ± 100</td>
<td>3,400 ± 300</td>
<td>1,700 ± 200</td>
</tr>
</tbody>
</table>

Values are means ± SE. EDP, end-diastolic pressure; LVDP, LV developed pressure; RPP, rate-pressure product; +dP/dt and −dP/dt, maximum and minimum values within a beat of the first derivative of LV pressure. There were no significant differences between the two groups of hearts at baseline (heart rate = 420 beats/min, [Ca2+] = 2.0 mM), during increased work (heart rate = 600 beats/min, [Ca2+] = 4.0 mM), or during recovery (heart rate = 420 beats/min, [Ca2+] = 2.0 mM) for any measure of cardiac function.
phosphate pool ([P_i] + [PCr] + 3 × [ATP]) decreased by ∼15% from baseline to recovery in the two groups of hearts (data not shown).

Free [ADP] increased by 82 µM in wild-type hearts and by 42 µM in ScCKmit−/− hearts in response to increased workload (P < 0.05). In absolute terms, free [ADP] at high workload showed a trend for higher concentrations in ScCKmit−/− compared with wild-type hearts. The ΔG_ATP decreased significantly as workload was increased in both groups, 4.9 kJ/mol for wild-type hearts and 2.9 kJ/mol for ScCKmit−/− hearts (Fig. 2). However, in contrast to baseline conditions, no differences were detectable in ΔG_ATP during increased workload and after recovery between wild-type and ScCKmit−/− hearts.

**DISCUSSION**

The CK system constitutes a highly organized isoenzyme system of central importance for energy maintenance, transfer, and buffering in excitable tissue, evidenced by a large body of work examining the kinetic, thermodynamic, and functional properties of this enzyme under various conditions and disease states (6, 18, 28). However, the functional coupling and complex interactions of the CK system with other components of the energy generation, transport, and utilization machinery of the cell have challenged the classical view of an enzyme system only necessary to catalyze the reversible transfer of a phosphoryl group between PCr and ATP via the following reaction: PCr + ADP + H⁺ ↔ ATP + creatine. Because specific and selective inhibitors of CK are not available, gene knockout technology offers a unique opportunity to dissect the functional significance of the different CK isoenzymes in muscle.

The present study thus sought to determine LV function, high-energy phosphate metabolism, and isoenzyme distribution in hearts exclusively lacking the ScCKmit isoenzyme (30% decrease in total CK activity). Specifically, we asked whether lowering CK activity below a certain critical threshold was responsible for the inability to maintain normal energetics or, alternatively, whether the specific loss of ScCKmit lead to the “energetic phenotype” of M/ScCKmit−/− hearts.

Our results demonstrate that, first, no compensatory increase in other CK isoforms or in a variety of other enzymes involved in cellular energy homeostasis was observed in ScCKmit−/− hearts. Second, deletion of ScCKmit is not associated with any functional alteration in isolated perfused hearts during different workloads. Third, and most importantly, hearts from ScCKmit−/− mice demonstrate significant changes in...
high-energy phosphate metabolism similar to the changes in M/ScCKmit−/− hearts, such as an increase in free [ADP] and a decrease in ΔGATP.

Biochemical effects of deletion of ScCKmit. Deletion of ScCKmit produced the expected 30% decrease in total CK activity. We did not observe any compensatory increase in the specific activity of the remaining CK isoenzymes. This is in line with the results of Saupe et al. (19) demonstrating complete independence of CK isoenzyme regulation in developing and mature hearts from M-CK−/− and M/ScCKmit−/− compared with wild-type mice. In contrast, Boehm et al. (3) found an ~30% decrease in MM-CK activity in ScCKmit−/− hearts, leading to an overall 47% decrease in total CK activity in these hearts. This decrease in MM-CK activity was only observed in ventricular muscle; in skeletal muscle, a significant increase in MM-CK activity of ScCKmit−/− mice was reported. The reasons for the discrepancy between these two investigations cannot be elucidated from the currently available data and warrant further study.

In agreement with others (23, 24), we did not detect changes in total LDH activity and LDH isoenzyme distribution in hearts from ScCKmit−/−. Boehm et al. (3) showed that this distribution, reflecting the oxidative or glycolytic preference of carbohydrate metabolism, was altered in slow twitch skeletal muscle of ScCKmit−/− mice toward a more glycolytic pattern. This shift towards a more glycolytic energy production in skeletal muscle was not observed in heart muscle. Similarly, Steeghs et al. (23) did not find a difference in the ability of mitochondria of ScCKmit−/− hearts to oxidize pyruvate. Although changes in substrate utilization cannot completely be ruled out by these measurements, it is apparent that more adaptational changes take place in skeletal muscle than in heart muscle from ScCKmit−/− mice. This is further confirmed by our finding that citrate synthase activity, a marker of mitochondrial mass, is unaffected in ScCKmit−/− hearts.

Myocardial function and energy metabolism with varying workload demand. During baseline perfusion conditions, producing a moderate degree of workload in wild-type mice, contractile performance was similar in ScCKmit−/− and wild-type hearts. Despite comparable isovolumic contractile work, hearts from ScCKmit−/− mice had a significantly lower (~34%) concentration of PCr. In parallel, P showed a trend for higher values in ScCKmit−/− mice; statistical significance, however, was not reached. When the free cytosolic [ADP] was calculated, a significant increase was observed in ScCKmit−/− hearts. These results are remarkable for several reasons: It has previously been shown that hearts with deletion of M-CK−/− (remaining CK activity 28%) were able to maintain a completely normal high-energy phosphate profile during baseline and increased workload, whereas a combined loss of M-CK and ScCKmit (leaving only 3% CK activity) led to reduced PCr, increased ADP, and decreased ΔGATP values (20). The assumption that a total CK activity below a critical threshold is necessary to maintain “normal” energetics has led to the prediction that ScCKmit−/− hearts, with a remaining total CK activity of 70%, will have unchanged high-energy phosphates. The view was supported by the results of skeletal muscle of ScCKmit−/− mice, showing no impairment in baseline energetics and a normal capacity to hydrolyze PCr during ischemia (5). For the other CK knockout strains such as M-CK−/− and M/ScCKmit−/− mice, the changes in high-energy phosphate profile in the heart were correctly “predicted” by the results from skeletal muscle. This, however, is not the case for ScCKmit−/− hearts. This report is therefore the first to describe significant discrepancies in steady-state high-energy phosphate concentrations between the heart and skeletal muscle in CK knockout mice. These differences highlight the unique role of ScCKmit in cardiac compared with skeletal muscle. This may be related to the fact that cardiac muscle with its higher mitochondrial density and larger proportion of ScCKmit but lower total CK activity relies predominantly on aerobic ATP generation (2, 27).

In agreement with the results from skeletal muscle showing preserved ability to hydrolyze PCr during ischemia (23), ScCKmit−/− hearts were able to hydrolyze PCr during increased work and, more importantly, resynthesize PCr during recovery. Because changes in PCr content reflect the balance between ATP synthesis and hydrolysis, this result demonstrates that during recovery the rate of ATP synthesis and therefore PCr generation exceeds the rate of ATP hydrolysis. Thus we conclude that not only is oxidative energy production still workload dependent in ScCKmit−/− hearts, but also that effective high-energy phosphate transport across the mitochondrial membrane is maintained without ScCKmit. Whether the remaining CK isoenzymes directly substitute for ScCKmit, relocating into intermembrane space, or whether other enzyme systems are upregulated cannot directly be answered from our data.

It is, however, important to point out the possibility that complex adaptational processes and subcellular rearrangements already during fetal development might have taken place in the CK-deficient hearts, ensuring an alternative energy transfer and signal transduction system. This hypothesis has been recently confirmed in M/ScCKmit−/− hearts showing significant cytoarchitectural rearrangements and biochemical adaptations (7). It is concluded that the genetically induced loss of an important component of the CK system is obviously partially compensated by an effective rearrangement of subcellular organelles, explaining the preserved cardiac function of CK-deficient mice under moderate workload. Whether these compensatory changes and mechanisms enable the ScCKmit−/− hearts to withstand acute or chronic stress conditions is completely unknown at present and warrant further examinations.

When cardiac energetics of ScCKmit−/− hearts from this study are compared with M/ScCKmit−/− hearts from previous studies, two differences are apparent: First, whereas we found ΔGATP to be significantly re-
duced in ScCKmit−/− compared with wild-type hearts, Saupe et al. (20) found ΔG_{ATP} to be comparable between M/ScCKmit−/− and wild-type hearts. Second, ScCKmit−/− and wild-type hearts showed significant changes in free ADP and ΔG_{ATP} during increased work in the present study, whereas similar workload-dependent changes were seen previously only in M/ScCKmit−/− but not in wild-type and M-CK−/− hearts. The major difference between both studies is that glucose was the sole substrate for M/ScCKmit−/− hearts, whereas ScCKmit−/− hearts in the present study received pyruvate as an additional substrate for oxidative phosphorylation. It is reasonable to assume that M/ScCKmit−/− hearts perfused with glucose as the sole exogenous substrate are partially substrate limited so that the near complete loss of CK cannot be fully compensated leading to an increased [ADP].

Substrate-dependent changes in myocardial high-energy phosphate metabolites have been recently described for CK-deficient hearts (19). When pyruvate was included in the perfusate, free ADP and ΔG_{ATP} significantly changed only in wild-type and M-CK−/− but not in M/ScCKmit−/− hearts. This effect of the different metabolic substrates on ΔG_{ATP} in the CK knockout mice is in complete agreement with our observation that hearts with loss of ScCKmit only have lower ΔG_{ATP} and higher free ADP compared with wild-type hearts. This further underscores the unique role of ScCKmit for myocardial energy homeostasis during different workloads and substrate conditions.

Taken together, our results demonstrate that ScCKmit is critically necessary to maintain normal high-energy phosphate metabolites in the heart. The previously reported changes in myocardial energetics of mice with combined loss of M-CK and ScCKmit are due to the deletion of ScCKmit rather than to lowering CK activity below a certain threshold level. We conclude that ScCKmit is the isoenzyme primarily responsible for myocardial energy homeostasis during different workloads.

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