Mitochondrial creatine kinases form octameric structures composed of four active and stable dimers. Octamer formation has been postulated to occur via interaction of the charged amino acids in the N-terminal peptide of the mature enzyme. We altered codons for charged amino acids in the N-terminal region of mature sarcomeric mitochondrial creatine kinase (sMtCK) to those encoding neutral amino acids. Transfection of normal sMtCK cDNA or those with the mutations R42G, E43G/H45G, and K46G into rat neonatal cardiomyocytes resulted in enzymatically active sMtCK expression in all. After hypoosmotic treatment of isolated mitochondria, mitochondrial inner membrane-associated and soluble sMtCK from the intermembranous space were measured. The R42G and E43G/H45G double mutation caused destabilization of the octameric structure of sMtCK and a profound reduction in binding of sMtCK to the inner mitochondrial membrane. The other mutant sMtCK proteins had modest reductions in binding. Creatine-stimulated respiration was markedly reduced in mitochondria isolated from cells transfected with the R42G and E43G/H45G mutant cDNA as compared with those transfected with normal sMtCK cDNA. We conclude that neutralization of charges in N-terminal peptide resulted in destabilization of octamer structure of sMtCK. Thus, charged amino acids at the N-terminal moiety of mature sMtCK are essential for octamer formation, binding of sMtCK with inner mitochondrial membrane, and coupling of sMtCK to oxidative phosphorylation.

Sarcomeric mitochondrial creatine kinase (sMtCK) is a member of a gene family of four homologous CK genes (1–4). The M-CK and B-CK genes encode soluble, cytoplasmic homodimeric enzymes necessary for ATP production from creatine phosphate at sites of high energy utilization, such as the contractile apparatus. These cytosolic CKs share about 85% similarity to each other in a particular species, and each has 77–91% homology among vertebrate species. Two additional genes encode different mitochondrial CKs, which are localized on the outer surface of the inner mitochondrial membrane (IMM) (2, 3). sMtCK expression is restricted to heart and skeletal muscle, but ubiquitous MtCK is present in many tissues, especially brain and smooth muscle. Mammalian MtCKs are only 60–65% similar to cytosolic CKs and about 80% homologous to each other, but MtCKs share 95% identity across species. This suggests that, in addition to enzymatic functions, ubiquitous MtCK and sMtCK may have slightly different structural roles requiring sequence conservation across species.

We previously isolated and characterized cDNA clones encoding human (2, 3) and rat (7) MtCKs. The rat MtCK cDNA coding region consists of 1260 nucleotides, and the first 117 bp encode the transit peptide, responsible for transport of de novo synthesized precursor sMtCK from cytosol into mitochondria. During this translocation of pre-sMtCK to the intermembranous space, the transit peptide is proteolytically removed, allowing formation of the active, mature subunit (2, 4).

Both MtCK proteins can form either homo-octamers or homodimeric structures (5). sMtCK is functionally coupled with the mitochondrial oxidative phosphorylation system via the ADP-ATP translocase (adenine nucleotide translocase; ANT) and directly converts ATP to phosphocreatine, which is exported as an easily diffusible energy carrier to the cytosolic sites of energy utilization, such as the contractile apparatus or ion channels and pumps. Published results suggest that only membrane-associated sMtCK is effectively coupled with the mitochondrial oxidative phosphorylation system (6, 7). Displacement of the enzyme from the IMM by high salt concentration causes a decrease of the efficiency of this coupling (6, 7). sMtCK binding to the outer side of the IMM is thus thought to be mediated by electrostatic interactions (6, 8).

Functionally active mitochondrial creatine kinase in vivo exists as a homooctamer, which can be reversibly dissociated into dimers in the presence of ADP, creatine, and NO3 (9). The physiologic significance of dissociation in vivo remains unclear. Experiments in vitro showed that the N-terminal peptide of mature sMtCK is crucial for stable octamer formation in vitro, and an important role for the glutamic acid at residue 43 was suggested (15). However, no experiments in intact cells or in vivo to examine the hypothesis that glutamic acid 43 and other N-terminal residues are essential in sMtCK function have been done.

The octamerization of sMtCK has also been proposed as necessary for formation of a multienzyme complex with porin, an anionic channel in the outer mitochondrial membrane, and...
mitochondrial ANT (10–13). Formation of this multienzyme complex may facilitate metabolic channeling between mitochondrial matrix and cytoplasm by forming contact sites (13, 14). Determination of the molecular structure (16) of sMtCK revealed surprising features consistent with this structural role. sMtCK forms a highly symmetrical, cube-like octameric structure with a channel extending along the four-fold axis. Previous work suggested that sMtCK specifically interacts with cardiolipin molecules in the inner mitochondrial membrane (8, 17). The structural analysis suggested the possibility that charged N-terminal residues might be required for or facilitate binding to ANT, cardiolipin, or porin to form the contact sites, allowing metabolic channeling.

Our published sMtCK cDNA sequence data (1–4), which allow some cross-species comparisons, reveal that the N-terminal region is highly positively charged and contains several conserved residues (Fig. 1). To test the hypothesis that octamer formation is essential for coupling of sMtCK with the mitochondrial oxidative phosphorylation system directly in vivo in intact cells, we created mutations in the mouse sMtCK cDNA. Following transfection and overexpression of normal and mutant sMtCK mRNAs in primary cultures of neonatal rat cardiomyocytes, we characterized octamer formation and binding to the IMM of the expressed sMtCKs and quantified creatine-stimulated respiration to assess functional and physiologic coupling to oxidative phosphorylation.

EXPERIMENTAL PROCEDURES

Isolation and Cloning of Mouse sMtCK and Plasmid Construction—An sMtCK cDNA was isolated from an adult mouse cardiac cDNA library in Lambda ZAP II vector (Stratagene) by standard plaque screening techniques (18) using a 550-bp EcoRI–DraIII coding region fragment from rat sMtCK cDNA (4). The purified clone containing mouse sMtCK was isolated and subcloned using ApoI and XhoI sites in the pcDNA3 vector (Invitrogen) designed for eukaryotic expression. DNA sequence analysis (19) revealed that the insert contained 1394 bp and encompassed the entire sMtCK coding region, the 3′-untranslated region, and the poly(A) tail.

Introducing Mutations in the N-terminal Domain of sMtCK cDNA—Mutations were created with the primer-directed mutagenesis technique (20) with overlapping “inside” primers in both orientations containing the desired codon alterations (Table 1). Flanking, “outside,” normal sense and antisense primers were 125–190 bp upstream or 690–640 bp downstream, respectively, of the mutation sites. Two sets of primary polymerase chain reactions were performed to amplify the two overlapping fragments carrying the desired mutations using the Pfu1-linearized sMtCK cDNA in pcDNA3 vector (100 ng) as template DNA. Each cycle of amplification consisted of denaturation at 96 °C for 30 s, annealing at 50 °C for 30 s, and primer extension at 72 °C for 60 s (30 cycles). After amplification, approximately 10 ng of these overlapping primary products were mixed, denatured, and allowed to reanneal. Subsequent reamplification with the outside primers gave 860-bp, full-length secondary products, containing the desired mutations. These products were digested by XhoI and DraIII, restriction sites that flank the mutated region, and the purified 319-bp fragment containing mutations was inserted into the sMtCK cDNA in pcDNA3. All mutations were confirmed by sequence determinations of the entire fragment, which also ruled out the presence of other, undesired polymerase chain reaction-generated abnormalities.

Cell Cultures—Primary cultures of cardiomyocytes were prepared from 1–2-day-old newborn rat hearts using repeated collagenase digestion (130 units/ml) (21). Cardiomyocytes were separated from non-muscle cells by centrifugation through two steps of a Percoll density gradient (1.059 and 1.082 g/ml) for 30 min at 2000 × g. Muscle cells from the middle band were collected by centrifugation and suspended in the PC-1 media (Hycor catalog no. 77232). Approximately 3 × 10⁵ cells were transferred to collagen-coated culture dishes. After 24 h, PC-1 medium was changed to PC-1/F-12 minimal essential medium (1:2 v/v) mixture. Expression of the克隆的cDNA在新生鼠心脏细胞中—LipofectAMINE® reagent (Life Technologies, Inc.) was employed for transfection of normal and mutant sMtCK cDNA into primary cultures of cardiomyocytes. Approximately 24 μg of plasmid DNA and 60 μl of the LipofectAMINE® reagent were used for each 60-mm plate. Cells were exposed to the transfection mixture for 4 h. After this, cells were grown in PC-1/F-12 minimal essential medium (1:2 v/v) mixture for an additional 48 h.

Expression of the Clone dDNA in Escherichia coli—We employed E. coli strain BL21(DE3)pLyS and pET21a vector (Novagen) for expression of normal and mutant sMtCK in bacteria. The coding sequence of mature mouse sMtCK was generated by primer-directed polymerase chain reaction with sMtCK containing pcDNA3 plasmid as template to create an Ndel site and new initiator methionine codon (Table 1) at the 5′-end and an XhoI site immediately after the translation stop codon (Table 1). The Ndel–XhoI fragment, containing the complete sequence of mature sMtCK, was inserted into the expression vector, pET21a. Mutations in the N terminus were created using the strategy outlined above.

Freshly transformed single colonies of the expression host, BL21(DE3)pLyS, were inoculated in 150 ml of CIRCLEGROW® (Bio 101) medium, and bacteria were grown at 37 °C to an A₆₀₀ of about 0.4 and induced by the addition of 0.4 mM isopropyl β-D-thiogalactopyranoside. After 4 h, cells were harvested by centrifugation for 10 min at 5000 × g; resuspended in 15 ml of 50 mM Tris–HCl, pH 8.0, 1 mM EDTA; and sonicated for 5 min (10-s pulse, 15-s pause) at 0 °C. The cell debris was removed by centrifugation at 15,000 × g for 30 min. Clear supernatants were used for further CK assay and gel permeation chromatography.

Gel Permeation Chromatography—The proportion of sMtCK as oc- tamer or dimers was estimated by measuring the CK activities after
isolated, membrane-associated CK activity was extracted from the mitochondrial pellet with 100 µl of buffer containing 100 mM K2HPO4 (pH 8.5), 15 mM MgCl2, 5 mM K-Hepes, 4 mM glutamate, 2 mM malate, and 0.5 mM dithiothreitol. For permeabilization of the sarcolemma, cells were incubated with saponin (20 µg/ml) for 5 min. Basal respiration (Vb) was recorded during this time. Respiration was stimulated by 60 µM ADP to submaximal level (Vmax). Respiration rate was enhanced further by the addition of 25 mM creatine (Vcreat). Near-maximal respiration rates were observed at 1 mM ADP (Vcreat), which was inhibited by atractyloside, an inhibitor of the ATP-ADP translocator.

For determination of total CK activity, CK isoenzymes, and protein concentration, cells were scraped on ice in 2 ml of F-12 medium, collected by centrifugation at 2,000 g for 5 min, and resuspended in the 0.2 ml of extraction buffer, containing 100 mM K2HPO4 (pH 8.5), 15 mM N-acetyl-l-cysteine, 1 mM EGTA, and 0.1% Nonidet P-40. The entire volume was taken for determination of CK activity.

Biochemical and Physiologic Determinations—Cardiomyocyte respiration rates were determined at 22 °C using an oxygraph (Yellow Spring Instruments) and Clark electrode in a special attachment, as described by Seraydarian and Yamada (23). Oxygen consumption by mitochondria in a special attachment, as described by Seraydarian and Yamada (23). Oxygen consumption by mitochondria was measured in air-saturated medium containing 120 mM K-MES, 10 mM NaCl, 20 mM imidazole (pH 7.2), 20 mM taurine, 8 mM MgCl2, 5 mM K-Hepes, 4 mM glutamate, 2 mM malate, and 0.5 mM dithiothreitol. For permeabilization of the sarcolemma, samples were pooled from both steps. The remaining, membrane-associated CK activity was extracted from the mitochondrial pellet with 100 µl of buffer containing 100 mM K2HPO4 (pH 8.9), 15 mM N-acetyl-l-cysteine, 1 mM EGTA, and 0.1% Nonidet P-40. The entire volume was taken for determination of CK activity.

Expression of the Endogenous Cardiac CKs—We chose the neonatal rat cardiomyocyte primary culture system to test sMtCK overexpression and binding with inner mitochondrial membrane because of the low level of the endogenous sMtCK expression, the high number of mitochondria, and sufficient organ size to isolate enough cardiomyocytes for transfection and biochemical analyses. We first characterized this system by analyzing expression of endogenous CK isoenzymes. Total CK activity in nontransfected, cultured cells was 0.96 ± 0.15 IU/mg of protein. In mitochondria isolated from these cells, endogenous sMtCK activity was 0.18 ± 0.07 IU/mg of mitochondrial protein. For comparison, sMtCK activity from freshly isolated adult rat heart mitochondria is 10-fold greater (2.0–2.9 IU/mg of mitochondrial protein) (Table II). To confirm that these measured activities represent the different CK isoenzymes, we performed non-denaturing gel electrophoresis with subsequent in situ activity measurements (Fig. 2) (25). Total CK activity (Fig. 2) from adult heart (lane 1) and neonatal cardiomyocytes (lane 2) could easily be separated into the cytosolic isoenzymes, MM, MB, and BB CK, and the octameric and dimeric of the mitochondrial isoenzyme, sMtCK. These results demonstrate that the cardiomyocyte preparation and CK assay procedures work well in separating endogenous sMtCK from cytosolic CKs and provide a basis for comparison of sMtCK expression in transfected cardiomyocytes.

Neonatal Cardiomyocyte Overexpression of Transfected Normal and Mutant sMtCKs—We used the cardiomyocyte culture system to analyze expression of transfected sMtCK cDNAs. Forty-eight hours following transfection with expression plasmids containing normal and mutant sMtCK cDNA, cardiomyo-
pressed in bacteria and subjected to gel permeation chromatography, retained by the column. 

Lane 6 contains-transfection with cDNAs encoding normal sMtCK (lanes 3–6). Transfection with normal sMtCK (lane 1) and cultured cardiomyocytes before transfection, and UV light. (arrows) are normal sMtCK expression 48 h after induction with isopropyl β-D-thiogalactopyranoside, 70–80 IU/ml of CK activity was present in each supernatants representing the sMtCK bound to the IMM. This result confirms that neonatal cardiomyocytes contain sufficient binding sites for the high levels of overexpressed normal sMtCK, as expected.

Cytochrome c oxidase activity was unchanged and that sMtCK activity was dramatically increased, both in cells transfected with normal sMtCK cDNA (lane 3) and any of three mutant plasmids (lanes 4–6), as compared with nontransfected cells (lane 2). After isolation of the mitochondria, we determined that the overexpressed sMtCK was present in mitochondria and active (Table II). Again, cells transfected with the normal or any of the three mutated cDNAs expressed similar levels of active sMtCK localized to mitochondria. By comparison, nontransfected neonatal cells have substantially lower sMtCK activity in this assay. We conclude that all of the increase in total CK activity in transfected cells is attributable to increased sMtCK enzyme (Table II and Fig. 2).

Nondenaturing gel electrophoresis of CK isoenzymes allowed us to distinguish the sMtCK fast moving octamer from slow moving dimer (Fig. 2). In adult rat heart and cardiomyocytes transfected with normal sMtCK, octamer is the predominant form of the enzyme (Fig. 2, lanes 1 and 3). However, substitution of Arg42 and both Glu43 and His45 with glycine resulted in partial dissociation of sMtCK octamer into dimers (Fig. 2, lanes 4 and 5). The K46G substitution has a minimal effect on stability of sMtCK octamer (Fig. 2, lane 6). These data strongly suggest that the first 6 amino acid residues at the N terminus are crucial for sMtCK octamer stability.

Suborganellar Localization of Expressed Normal and Mutant sMtCKs—Previous studies document that sMtCK in heart is located in the intermembranous space of mitochondria bound to the IMM (27). We developed an assay to localize sMtCK and test for binding to the IMM, both as normally expressed in heart, and after transfection of isolated cardiomyocytes (Table III). Incubation of purified adult rat heart mitochondria in hypotonic solution released only 15% of sMtCK, leaving 85% firmly bound with the IMM, confirming the reliability of the assay. In mitochondria isolated from neonatal rat cardiomyocytes transfected with normal sMtCK cDNA, enzymatic activity was distributed between supernatant and membrane pellet in the same proportion as in mitochondria from adult rat heart: 19% in supernatant, representing the free or soluble fraction from the intermembranous space, and 81% in the pellet, representing the sMtCK bound to the IMM. This result confirms that neonatal cardiomyocytes contain sufficient binding sites for the high levels of overexpressed normal sMtCK, as expected.

Assays for membrane-bound sMtCK were done in cardiomyocytes transfected with sMtCK cDNAs containing each of the three mutations in charged amino acids (Table III). Substitution of glycine for lysine at position 46 caused a significant but modest reduction in bound sMtCK, with 36% released to soluble form. This is 2-fold more soluble, unbound sMtCK than in cells transfected with normal sMtCK cDNA. In contrast, the R42G and double mutant, E43G/H45G, demonstrated more obvious reduction of binding with mitochondrial membrane, with 65–70% of mutant sMtCK soluble. This is 4-fold more than overexpressed, normal sMtCK. Electrophoresis of the hypotonic supernatant and pellet of mitochondria isolated from R42G mutant-transfected cardiomyocytes demonstrates that the membrane-associated fraction contains only sMtCK octamer. The soluble fraction has only dimer (Fig. 3). Thus, neutralization of charges in Arg42 alone or Glu43 and His45 destabilizes sMtCK octamer and reduces membrane binding.

Expression of sMtCK in E. coli—The coding sequence of mature normal or the three mutant sMtCKs was fused to the T7 promoter of the bacterial expression vector, pET21a. Four hours after induction with isopropyl β-D-thiogalactopyranoside, 70–80 IU/ml of CK activity was present in each supernatants of four E. coli lysates. Electrophoresis under denaturing conditions and subsequent Western blot of the bacterially expressed,
recombinant sMtCKs showed a subunit of $M_r$ 42,000 (results not shown). Moreover, equal expression of all four soluble sMtCKs, without detectable formation of aggregates in lysate pellets, was noted.

The ability of recombinant sMtCK expressed in E. coli to form octamers was reported by Furter and co-workers (28). In our studies, gel permeation chromatography of recombinant normal sMtCK showed that more than 80% of applied CK activity was octamer (in the void volume). Dimer (20% of total applied CK activity) was retained (Fig. 4).

Substitution of charged amino acid residues Arg$^{42}$ or both Glu$^{43}$ and His$^{45}$ by the neutral amino acid, glycine, shifted the octamer/dimer equilibrium toward dimer, with only one-third of CK activity eluted as octamer. The octamer/dimer ratio in K46G mutant sMtCK is the same as in normal sMtCK (Fig. 4, Table IV). Thus, gel permeation analysis of recombinant normal and mutant sMtCKs confirms that charged amino acid residues Arg$^{42}$ and Glu$^{43}$ or His$^{45}$ at the N terminus of mature sMtCK are essential for stable sMtCK octamer formation.

The Dependence of Creatine-stimulated Respiration on Oligomeric Structure and Binding of sMtCK to the Inner Mitochondrial Membrane—To study the changes in functional activity of sMtCK associated with changes of oligomeric structure and in membrane association, mitochondrial function was investigated in saponin-treated neonatal rat cardiomyocytes (29). Treatment of cells with this detergent permeabilizes the sarcolemma, allowing entry of small molecules into the mitochondrial intermembranous space, but does not alter sMtCK localization or function (10, 29, 30). In adult rat cardiomyocytes, the addition of 25 mM creatine in the presence of 60 $\mu$M ADP, unlimited substrates, and high oxygen concentration allows mitochondria to develop near-maximal rates of oxygen consumption. This high level of mitochondrial respiration requires close functional interactions between mitochondrial CK and the ATP/ADP translocase, the phenomenon known as coupling (31). We examined the coupling phenomenon in rat neonatal cardiomyocytes (Fig. 5, trace A) by measuring oxygen consumption after various experimental manipulations. In saponin-treated, nontransfected cells, the addition of 60 $\mu$M ADP accelerated oxygen consumption, but further addition of creatine did not affect the rate, giving a respiratory ratio of 1.2 (Table V). We believe this is because very little endogenous sMtCK was present, and ATP/ADP translocase activity was not affected.

Recent functional, morphologic, and structural studies with sMtCK have led to the hypothesis that the sMtCK plays a structural role in forming contact sites linking the inner and outer mitochondrial membranes through interactions with ANT and porin, thus forming a pore or channel that is essential in coupling oxidative phosphorylation to cytosolic energy consumption by facilitating metabolite channeling (10–13, 16).

**DISCUSSION**

Recent functional, morphologic, and structural studies with sMtCK have led to the hypothesis that the sMtCK plays a structural role in forming contact sites linking the inner and outer mitochondrial membranes through interactions with ANT and porin, thus forming a pore or channel that is essential in coupling oxidative phosphorylation to cytosolic energy consumption by facilitating metabolite channeling (10–13, 16).
The elucidation of the crystal structure of sMtCK strongly supports the concept that octameric sMtCK is essential for this structural role as the bridge connecting inner and outer membranes (16). Moreover, charged N-terminal residues were postulated as critical in interactions with ANT, cardiolipin, or porin. Although data from in vitro experiments supported this hypothesis (15, 31), both the physiological significance of sMtCK octamer formation in vivo and the residues essential for membrane interactions in vivo in intact cardiac mitochondria have not been evaluated.

Our results demonstrate that neonatal rat cardiomyocytes can be used to examine sMtCK octamer formation, sMtCK interactions with mitochondrial membranes, and coupling to oxidative phosphorylation. This cardiomyocyte assay system benefits from low endogenous sMtCK expression and sufficient transfection efficiencies to compare normal sMtCK function to mutant sMtCK in a physiologic environment. Sufficient cardiomyocytes can be isolated and transfected to allow measurements of respiration, complex formation of sMtCK with membranes, and sMtCK octamer/dimer ratios. Our results suggest the possibility that transfection of cardiomyocytes with other components of the metabolite channeling complex, such as porin or ANT, to analyze residues required for both structural interactions and function may be feasible.

Using this assay system, we tested the hypothesis that only sMtCK octamers can form a functionally active unit, capable of effectively phosphorylating cytosolic creatine using the ATP synthesized in mitochondrial oxidative phosphorylation and forming metabolite channels at contact sites. We changed the equilibrium between sMtCK octamer and dimer toward dimer by altering charged amino acids at the N terminus of the mature sMtCK. Our results showed that substitution of charged amino acids with the neutral amino acid, glycine, at the N terminus did not alter the enzymatic activity of sMtCK, as expected. These mutations did significantly reduce octamer formation, consistent with the hypothesis that these N-terminal charged residues are critical for octamer stability. However, these mutations did not completely abolish octamer formation (Table IV), suggesting that octamer formation is mediated by multiple contact sites between sMtCK dimers. This is in agreement with predictions from the crystal structure analysis of chicken sMtCK (16). Deletion of the entire N-terminal pentapeptide or substitution of tryptophan residues at positions 245, 252, and 262 of mouse sMtCK might generate complete dissociation of sMtCK octamer, as suggested by the work of Gross et al. (32). However, reduction of enzymatic activity makes these tryptophan mutants useless for functional analysis of creatine-stimulated respiration in neonatal cardiomyocytes and suggests that the mutants cannot fold sufficiently properly to generate active dimers.

Our data also show that neutralization of charges at the N terminus of sMtCK has a strong effect on binding of sMtCK with the inner mitochondrial membrane. Both octamer and dimer are able to associate with the inner mitochondrial membrane but with different pH optima (9). In our studies, we chose hypoosmotic buffer (pH 8.1), which allowed selective release of dimeric sMtCK from mitochondria during the hypoosmotic treatment (Fig. 5). Dislodging of sMtCK from the IMM resulted in a reduced rate of mitochondrial creatine-stimulated respiration in permeabilized cardiomyocytes. Based on these results, we conclude that only membrane-bound sMtCK is capable of effectively phosphorylating cytosolic creatine using ATP delivered from mitochondrial oxidative phosphorylation and efficiently conducting metabolite channeling between mitochondrial and a cytosolic compartments in cardiac cells.

Because sMtCK has preferential access to intramitochondrial ATP and because ADP produced in the mitochondrial CK reaction is more potent in stimulation of oxidative phosphorylation than extramitochondrial ADP, localization of sMtCK in contact sites is probably crucial for regulation of oxidative phosphorylation in cardiac mitochondria. The involvement of sMtCK in the architectural assembly of contact sites between inner and outer mitochondrial membranes indicates a metabolic advantage of this location. Based on this concept, we propose that the complex of octameric sMtCK, porin, and the ANT prevents rapid equilibration of substrate concentrations between extra- and intramitochondrial compartments and allows the maintenance of a high cytosolic ATP/ADP ratio. Due to this microcompartmentalization and preferential access to intramitochondrial ATP, phosphocreatine production by sMtCK is velocity of oxygen consumption after the addition of 60 mM ADP, V_{max} is the velocity of oxygen consumption after the addition of 25 mM creatine, V_{max} indicates the maximal rate of oxygen consumption as reached following the addition of 1 mM ADP, and Atr indicates the time of the addition of atractyloside, an inhibitor of ANT that blocks oxygen consumption in mitochondria.

Table V

| Plasmid transfected | (V_{Cr} - V_{ADP}) / V_{ADP} | (V_{Cr} - V_{ADP}) / V_{ADP} |
|---------------------|-------------------------------|-------------------------------|
| None                | 1.20 ± 0.17                   | 2.34 ± 0.64                   |
| Normal sMtCK       |                               |                               |
| Arg42 → Gly mutant  | 1.46 ± 0.16                   |                               |
the regulation of contact site formation. Because sMtCK activity is localized all along the cristae in the coupled state with ANT, our data also show that it is probably the octameric form of sMtCK that participates in metabolite channeling across the mitochondrial membranes in all of these sites of functional coupling.

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Sarcomeric Mitochondrial Creatine Kinase Coupling