Altered Mitochondrial Sensitivity for ADP and Maintenance of Creatine-stimulated Respiration in Oxidative Striated Muscles from VDAC1-deficient Mice*

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Voltage-dependent anion channels (VDACs) form the main pathway for metabolites across the mitochondrial outer membrane. The mouse edac1 gene has been disrupted by gene targeting, and the resulting mutant mice have been examined for defects in muscle physiology. To test the hypothesis that VDAC1 constitutes a pathway for ADP translocation into mitochondria, the apparent mitochondrial sensitivity for ADP (K_m(ADP)) and the calculated rate of respiration in the presence of the maximal ADP concentration (V_max) have been assessed using skinned fibers prepared from two oxidative muscles (ventricle and soleus) and a glycolytic muscle (gastrocnemius) in control and edac1−/− mice. We observed a significant increase in the apparent K_m(ADP) in heart and gastrocnemius, whereas the V_max remained unchanged in both muscles. In contrast, a significant decrease in both the apparent K_m(ADP) and V_max was observed in soleus. To test whether VDAC1 is required for creatine stimulation of mitochondrial respiration in oxidative muscles, the apparent K_m(ADP) and V_max were determined in the presence of 25 mM creatine. The creatine effect on mitochondrial respiration was unchanged in both heart and soleus. These data, together with the significant increase in citrate synthase activity in heart, but not in soleus and gastrocnemius, suggest that distinct metabolic responses to altered mitochondrial outer membrane permeability occur in these different striated muscle types.

The mitochondrial outer membrane (MOM), like the outer membrane of Gram-negative bacteria, contains a major protein that constitutes an aqueous pore, porin, also known as the voltage-dependent anion channel (VDAC). When reconstituted in artificial bilayers, VDAC conductance is high at low voltages, whereas the channel reverts to a low conductance state when the voltage is increased (1). Three VDAC isoforms have been characterized in mammals: VDAC1, VDAC2, and VDAC3 (2–6). These isoforms constitute a gene family that arose by gene duplication and divergence (7). Although the exact in vivo roles of VDACs are not yet known, it has been speculated that they are involved in the coupling of cellular energy demand to mitochondrial energy production since they constitute the main pathway for small metabolites across the MOM (8–10).

Compartmentation of creatine kinase isoforms in the cytosol and mitochondria of tissues with high and fluctuating energy demands has led to the phosphocreatine circuit hypothesis as a mechanism linking sites of ATP consumption and production (11, 12). Bessman and Fonyo (13) were the first to demonstrate that creatine could exert acceptor control of respiration by production of ADP from mitochondrial ATP. The functional coupling of mitochondrial creatine kinase (miCK), located in the intermembrane space, to respiration has been attributed to the direct channeling of ATP and ADP between miCK and the adenine-nucleotide translocase (ANT), located in the inner mitochondrial membrane (14–17). In support of this hypothesis, formation of a complex between the octameric form of miCK and VDAC has been observed in vitro (18), and a complex formed by octameric miCK, VDAC, and ANT has been purified from rat brain mitochondria (19). These complexes may constitute the structural basis for the functional coupling of miCK to respiration. It is believed that the functional coupling of miCK to respiration increases the ADP concentration in the vicinity of the ANT and compensates for the barrier to ADP diffusion exerted by the MOM. Evidence that the MOM may restrict ADP diffusion has been provided by experiments in reconstituted systems showing that mitochondria and pyruvate kinase compete for ADP generated by kinases present at different locations with respect to the MOM (17, 20, 21). The functional coupling of miCK to respiration has more recently been studied in skinned fibers prepared from cardiac ventricle (22, 23). In these preparations, where mitochondria preserve their in vivo structure, miCK coupling to respiration decreases the apparent mitochondrial K_m for ADP (K_m(ADP)). Unlike isolated mitochondria devoid of their intracellular interactions, the in situ apparent K_m(ADP) is relatively high. This high apparent K_m(ADP) is low, similar to that in isolated mitochondria (25, 26).

Since VDACs constitute a multigenic family with multiple isoforms, it is reasonable to think that the different functions assigned to VDACs may be carried out by distinct isoforms. The fact that expression of each mouse VDAC isoform in yeast leads to different permeability characteristics (27), as well as the differing ability of each isoform to complement yvdac1-deficient yeast (7), supports this hypothesis. In an attempt to further
elucidate the roles of VDAC1 in cell metabolism, VDAC1-deficient mice have been generated. The mice are viable and thus provide a means for addressing VDAC1 function in vivo. As VDACs are believed to be responsible for MOM permeability for small metabolites, including ADP, we studied the apparent $K_{m,ADP}$ in skinned fiber preparations from two oxidative striated muscle types (ventricle and soleus) and from a glycolytic skeletal muscle (gastrocnemius). To examine the role of VDAC1 in creatine coupling, we also studied the effect of creatine on mitochondrial respiration.

**Experimental Procedures**

**Chemicals—**Chemical products were purchased from Sigma.

**Animals—**The control and VDAC1-deficient (vdac1-/-) mice used in this study were of a mixed genetic background (C57BL6/129SvEv). Unless otherwise noted, in each set of experiments, at least five littermate control and five vdac1-/- mice were used. All mice were ~3 months of age.

**Preparation of Skinned Fibers—**Control and vdac1-/- mice were anesthetized with Metofo, and the hearts were quickly removed and placed in a cooled, well oxygenated (95% O$_2$ + 5% CO$_2$), modified Krebs solution containing 118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO$_3$, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, (25). To study the in situ properties of mitochondria, we used the method of skinned fiber preparation described by Veksel et al. (29). For heart skinned fibers, the left ventricle was opened, and muscle strips from the endocardium were cut lengthwise to avoid mechanical damage of the cells. Muscle fiber bundles from the left ventricle, soleus (slow-twitch skeletal muscle), and gastrocnemius (fast-twitch skeletal muscle) were placed in a cooled solution containing 2.77 mM CaK$_2$EGTA, 7.23 mM K$_2$EGTA, 6.56 mM MgCl$_2$, 5.7 mM Na$_2$ATP, 15 mM phosphocreatine, 0.5 mM dithiothreitol, 50 mM potassium methanesulfonate, 20 mM imidazole, and 20 mM taunine (pH 7.1) containing 50 μg/ml saponin. The fibers were incubated with gentle stirring at 4 °C for 30 min to solubilize the sarcolemma. Permeabilized (skinned) fibers were then placed in buffers A (2.77 mM CaK$_2$EGTA, 7.23 mM K$_2$EGTA, 1.38 mM MgCl$_2$, 0.5 mM dithiothreitol, 3 mM K$_2$HPO$_4$, 2 mM malic acid, 5 mM pyruvic acid, 100 mM potassium methanesulfonate, 20 mM imidazole, and 20 mM taunine (pH 7.1)) for 10 min with gentle stirring at 4 °C to wash out soluble metabolites, in particular ADP (30). The wash was repeated twice.

**In Situ Mitochondrial Respiratory Studies—**Respiratory rates in the presence of increasing concentrations of ADP were assessed using an oxygraph (biological oxygen monitor, YSI Model 5300) and a Clark-type electrode (oxygen probe, YSI Model 5331). Skinned fibers prepared from different striated muscles were incubated at 22 °C in 4 ml of buffer A containing 2 mg/ml bovine serum albumin. The rate of oxygen consumption was recorded by a MacLab/200 system (AD Instruments). The solubility of oxygen was taken as 230 nmol of oxygen/ml. The ADP concentrations used were 0.05–2 mM for ventricle and soleus fibers and 0.1–2 mM for gastrocnemius fibers. The functional coupling of miCK to respiration was assessed in the presence of 25 mM creatine, and the ADP concentration used was 0.025–2 mM. At the end of the experiments, fibers were removed, dried, and weighed. Rates of respiration are given in μmol of oxygen/min/g (dry weight) (Fig. 1). The integrity of the MOM was assessed in the presence of 8 μM cytochrome c (31, 32).

**Citrate Synthase Activity—**Fresh heart, soleus, and gastrocnemius were homogenized in 5 mM Hepes buffer (pH 8.0) containing 1 mM EDTA and 1 mM dithiothreitol. Protein concentrations were determined using the BCA protein assay (Pierce). Citrate synthase activity was measured in the presence of 0.1 mM 5,5′dithio-bis-(2-nitrobenzoic acid), 0.3 mM acetyl-CoA, and 0.5 mM oxalacetate using 30 μg of protein from heart and soleus homogenates and 100 μg of protein from gastrocnemius homogenate. The initial rate of reaction of liberated CoA-SH was followed at 412 nm for 3 min.

**Western Blotting—**To quantify the amount of each VDAC isoform in different striated muscles, a commercial monoclonal antibody (mAb1, Calbiochem) specific for VDAC1 (33) and two polyclonal antibodies specific for VDAC2 (similarly generated as described (34)) and VDAC3 (34) were used. To control for variation in protein content, a cytochrome c-specific polyclonal antibody (H-104, Santa Cruz Biotechnology) was used. Different tissues from control and vdac1-/- mice were homogenized in a Polytron in 5 mM Hepes buffer (pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride and anti-proteases (2 μg/ml aprotonin, 2 μg/ml leupentin, 1 μg/ml pepstatin A, and 2 μg/ml antipain). 20–30 μg of total protein were separated on duplicate 4–12% gradient SDS-polyacrylamide gels. The separated proteins were transferred to polyvinylidene difluoride membranes (Roche Molecular Biochemicals) using a Bio-Rad Trans-Blot system at 380 mA for 1 h in buffer containing 50 mM Tris, 40 mM glycerine, 0.05% SDS, and 20% ethanol. Membranes were blocked with 10% skimmed milk in buffer containing 25 mM Tris, 3 mM potassium chloride, 140 mM sodium chloride, and 0.05% Tween 20 (pH 7.4) overnight at 4 °C and then incubated with mAb1 (5 μg/ml), anti-VDAC2 antibody (1:50 dilution), anti-VDAC3 antibody (1:500 dilution), or anti-cytochrome c antibody (1:500 dilution) for 1 h at room temperature. The secondary antibody used was peroxidase-conjugated antibody IgG (1:10,000 dilution) for mAb1, anti-chicken IgY (1:10,000 dilution) for the two polyclonal antibodies specific for VDAC2 and VDAC3, and anti-rabbit IgG (1:5000 dilution) for the polyclonal antibody specific for cytochrome c. The membranes were developed using an ECL reaction and exposed to Kodak X-Omat films for 1–10 min.

**Electron Microscopy—**Following dissection, small pieces of the left ventricle, soleus muscle, and gastrocnemius muscle from control and vdac1-/- mice were fixed immediately in 3% phosphate-buffered glutaraldehyde. Samples were post-fixed in 1% buffered osmium tetroxide and then dehydrated through graded concentrations of alcohol, followed by embedding in Araldite. Thin and thick sections were made, and the tissue was examined on thin sections using a Joel 1210 transmission electron microscopy. Photographs were made of selected regions.

**Statistical Analysis—**A two-tailed, unpaired t test (GraphPAD PRISM Version 2.0C) was employed.

**Results**

**In Situ Mitochondrial Sensitivity for ADP—**In skinned muscle fibers, the apparent $K_{m,ADP}$ reflects the in situ mitochondrial sensitivity for ADP. It is “apparent” because the exact concentration of ADP in the mitochondrial intermembrane space is not determined. This value varies significantly between muscle types (25, 26). To determine whether the absence of VDAC1 increases the barrier to ADP diffusion across the MOM, we determined the apparent $K_{m,ADP}$ in skinned fibers prepared from different striated muscle types in control and vdac1-/- mice.

In the ventricle, there was a 30% increase in the apparent $K_{m,ADP}$, in vdac1-/- mice (309.4 ± 21.9 μM in vdac1-/- versus 220.6 ± 22.2 μM in control mice; $p = 0.020$) (Fig. 2A). In the presence of 25 mM creatine, the apparent $K_{m,ADP}$ decreased in both control (74.40 ± 6.9 μM) and vdac1-/- (78.86 ± 4.25 μM) mice, these two values being comparable to each other (Fig. 2A). The $V_{max}$ (calculated rate of oxygen consumption in the
presence of the maximal ADP concentration) was not significantly different between control and \textit{vdac1}\textsuperscript{−/−} mice in the absence or presence of 25 mM creatine (Fig. 2B and Table I). In comparison with wild-type mice, basal respiration (no added ADP) was unchanged in \textit{vdac1}\textsuperscript{−/−} mice in the absence or presence of 25 mM creatine (Table I).

In soleus fibers, surprisingly, the apparent \(K_{m,(ADP)}\) decreased significantly in \textit{vdac1}\textsuperscript{−/−} mice (212.0 \pm 11.3 \mu M) in comparison with control mice (304.7 \pm 2.7 \mu M; \(p < 0.0001\)) (Fig. 3A). In the presence of 25 mM creatine, the apparent \(K_{m,(ADP)}\) decreased in both control (106.0 \pm 2.4 \mu M) and \textit{vdac1}\textsuperscript{−/−} (71.40 \pm 8.90 \mu M) mice, these two values being significantly different (\(p = 0.0074\)) (Fig. 3A). In comparison with wild-type fibers, there was a significant decrease in the \(V_{\text{max}}\) in \textit{vdac1}\textsuperscript{−/−} mice, and this was unchanged in the presence of 25 mM creatine (Fig. 3B and Table I). Basal respiration remained unchanged between control and \textit{vdac1}\textsuperscript{−/−} mice in the absence or presence of 25 mM creatine (Table I). This reduction in the apparent \(K_{m,(ADP)}\) led us to consider the possibility that the VDAC1-deficient MOM of soleus muscle is rendered more fragile and therefore is damaged by saponin treatment. However, the percentage of stimulation of respiration by cytochrome c was not significantly different between control (7.27 \pm 4.94\%, \(n = 3\)) and \textit{vdac1}\textsuperscript{−/−} (5.85 \pm 1.86\%, \(n = 5\)) soleus muscles. This result suggests that the integrity of the MOM in \textit{vdac1}\textsuperscript{−/−} soleus fibers is similar to that in control soleus fibers.

In gastrocnemius fibers, the apparent \(K_{m,(ADP)}\) increased significantly in \textit{vdac1}\textsuperscript{−/−} mice (16.13 \pm 2.37 \mu M) in comparison with control mice (7.77 \pm 0.42 \mu M; \(p = 0.0085\)) (Fig. 4A). In the presence of 25 mM creatine, the apparent \(K_{m,(ADP)}\) increased in both control (13.52 \pm 2.36 \mu M) and \textit{vdac1}\textsuperscript{−/−} (25.37 \pm 3.88 \mu M) mice, these two values being significantly different (\(p = 0.0395\)) (Fig. 4A). In gastrocnemius muscle, miCK is not functionally coupled to respiration; the apparent \(K_{m,(ADP)}\) is relatively low and is unchanged by the addition of creatine (25). The increased apparent \(K_{m,(ADP)}\) in the gastrocnemius in the presence of creatine in this study is similar to what has previously been

### Table I

| Muscle   | \(V_{\text{max}}\) (\mu mol/min/g dw) | \(V_{\text{max}} + \text{ADP}\) (\mu mol/min/g dw) | RCR (ADP) | Respiration control ratio (ADP) |
|----------|----------------------------------|----------------------------------|-----------|---------------------------------|
| Ventricle | -                               | -                               | -         | -                               |
| Soleus   | 3.92 \pm 0.57                    | 24.3 \pm 2.0                    | 2.77      | 8.16                            |
| Gastroc. | 13.5 \pm 1.7                     | 26.3 \pm 2.4                    | 1.45      | 5.79                            |

Values (means \pm S.D.) are expressed in \(\mu\) mol of oxygen/min/g (dry weight).

\(V_{\text{max}}\), calculated rate of respiration in the presence of the maximal ADP concentration; RCR, respiratory control ratio (\(V_{\text{max}} + \text{ADP}/V_{\text{max}}\)) ventricles in the absence and presence of 25 mM creatine (Cr).
measured in rat fibers prepared from the gastrocnemius (26) and atria (35). The V_{max} remained unchanged between control and VDAC1-deficient fibers and was unaffected by the addition of 25 mM creatine (Fig. 4B and Table I). Basal respiration also remained unchanged between control and VDAC1-deficient fibers and was unaffected by the addition of 25 mM creatine (Table I).

Citrate Synthase Activity—Ventricle homogenates from vdac1^{-/-} mice had a higher citrate synthase activity (348 ± 63 mIU/mg of protein) in comparison with control mice (174 ± 14 mIU/mg of protein; p = 0.0030) (Fig. 5). There was no significant change in citrate synthase activity between control and vdac1^{-/-} mice in both the soleus (193 ± 8 mIU/mg of protein in control versus 220 ± 46 mIU/mg of protein in vdac1^{-/-} mice) and gastrocnemius (64 ± 9 mIU/mg of protein in control versus 72 ± 13 mIU/mg of protein in vdac1^{-/-} mice) (Fig. 5).

Western Blot Analysis—It is possible that loss of VDAC1 function leads to an alteration in the expression level of the remaining VDACs. Using antibodies specific for each VDAC isoform, we determined that the striated muscles surveyed in this study express all three VDAC isoforms (Fig. 6). After normalizing the expression level to the mitochondrial protein cytochrome c, we did not detect any significant alteration in the expression of VDAC2 or VDAC3 in any of the striated muscles surveyed in vdac1^{-/-} mice (Fig. 6).

Ultrastructural Examination—To examine whether the loss of VDAC1 results in structural alterations of mitochondria, the different striated muscles surveyed in control and vdac1^{-/-} mice were studied by electron microscopy. Although the three striated muscles showed alterations in mitochondrial structure, the extent of these changes was qualitatively different among the three muscles. In the control soleus sample, the subsarcolemmal population of mitochondria were considerably smaller than adjacent nuclei and had regular cristae (Fig. 7A). The same population of mitochondria in the vdac1^{-/-} soleus approached the size of the adjacent nuclei, and the cristae were more compacted (Figs. 7B and 8). In the vdac1^{-/-} gastrocnemius, the subsarcolemmal population of mitochondria were also increased in size in comparison with the same population in the control mice (Fig. 9). The structural changes in heart mitochondria were less pronounced than in the two skeletal muscles. Although mitochondria from the vdac1^{-/-} ventricle were increased in size relative to control mitochondria, they did not reach the size of the nucleus (Fig. 10). Furthermore, the cristae were still visible and appeared less compacted in the mutant mice (Fig. 10B).
DISCUSSION

For adequate mitochondrial energy production, a variety of small metabolites such as pyruvate, ADP, and ATP must be transported across the two mitochondrial membranes. VDACs form the main pathway for small metabolites across the MOM and potentially offer another mechanism for integrating cytosolic energy demand with mitochondrial energy production. In this report, we describe the characteristics of in situ mitochondria from different striated muscles lacking VDAC1.

The different types of mammalian muscle fibers differ in such aspects as myosin heavy chain composition and mitochondrial content, conferring relatively distinct physiological and biochemical properties (36). Skeletal muscle fibers have generally been categorized into three types, identified as slow oxidative, fast oxidative glycolytic, and fast glycolytic (37). Cardiac muscle is composed of slow oxidative fibers. In this study, we have shown that the different striated muscles surveyed express all three VDAC isoforms in wild-type mice. The absence of VDAC1 did not affect the expression of the other VDAC isoforms, so we can assume that the results described in this report are related directly to the absence of VDAC1. The different striated muscles from mice lacking VDAC1 exhibited different types of in situ mitochondrial adaptation. In ventricle and gastrocnemius fibers, we measured a 30% increase in the apparent $K_m(ADP)$ in the absence of VDAC1, which indicates that the barrier to ADP diffusion across the MOM has increased. This result supports the hypothesis that VDAC1 constitutes a pathway for ADP across the MOM and is consistent with recent results of Vander Heiden et al. (38), who showed a disruption of ATP/ADP exchange across the MOM upon growth.

**FIG. 6.** Western blot showing the distribution of VDAC isoforms in different striated muscle types from wild-type (+) and vdac1−/− (−) mice. Cytochrome c was used as a control signal to normalize the protein load. As determined by densitometry, the relative values corresponding to this representative figure (VDAC signal/cytochrome c signal) are as follows. Anti-VDAC2 antibody: wild-type and vdac1−/− ventricles, 0.566 and 0.579, respectively; wild-type and vdac1−/− soleus muscles, 2.034 and 1.691, respectively; and wild-type and vdac1−/− gastrocnemius muscles, 0.454 and 0.285, respectively. Anti-VDAC3 antibody: wild-type and vdac1−/− ventricles, 0.507 and 0.475, respectively; wild-type and vdac1−/− soleus muscles, 4.684 and 2.831, respectively; and wild-type and vdac1−/− gastrocnemius muscles, 0.608 and 0.305, respectively.

**FIG. 7.** Electron microscopic study showing subsarcolemmal population of mitochondria from wild-type (A) and vdac1−/− (B) soleus muscles. Magnification × 8000.

**FIG. 8.** Electron microscopic study showing subsarcolemmal population of mitochondria in the vdac1−/− soleus. Magnification × 3200 (A) and 9600 (B).

**FIG. 9.** Electron microscopic study showing mitochondria from wild-type (A) and vdac1−/− (B) gastrocnemius muscles. Magnification × 2500 (A) and 4000 (B).

**FIG. 10.** Electron microscopic study showing intermyofibrillar population of mitochondria from wild-type (A) and vdac1−/− (B) ventricles. Magnification × 10,000.
factor withdrawal and induction of apoptosis. This defect results from a loss of MOM permeability to metabolic anions and correlates with the changes in conductance properties that accompany closure ofVDACs. The observation that the \( V_{\text{max}} \) did not change in the absence ofVDAC1 may mean that, in these two striated muscles, the absence ofVDAC1 alters the properties of only the MOM, without affecting the mitochondrial inner membrane.

In contrast to the ventricle and gastrocnemius, the soleus exhibited a decrease in both the apparent \( K_{\text{MADP}} \) and \( V_{\text{max}} \) which suggests that, in this oxidative skeletal muscle, the absence ofVDAC1 affects the properties of both the MOM and the mitochondrial inner membrane. In mammals, three ANT isoforms have been characterized, and their tissue specificity determined (40–42). In ANT1-deficient mice, a severe reduction in the state III respiration rate has been reported in skeletal muscle (39). It is possible that, in the \( v \text{d}ac1^{-/–} \) soleus, there is a decrease in the expression ofANT1 and/or ANT3, thus accounting for the reduction in the \( V_{\text{max}} \). However, a child with deficiency ofVDAC1 in skeletal muscle was reported to have only a slight decrease in ANT at the protein level (43). Alternatively, a defect in the respiratory chain may be present.

The decreased apparent \( K_{\text{MADP}} \) in the \( v \text{d}ac1^{-/–} \) soleus could correspond to a change in the biophysical properties of the MOM, e.g., the MOM may become nonspecifically permeable. Cytochrome c effect on respiration is typically used to determine the integrity of the MOM (31, 32). Since we did not find a difference in the stimulation of respiration by cytochrome c between control and \( v \text{d}ac1^{-/–} \) mice, we conclude that the decreased apparent \( K_{\text{MADP}} \) in the \( v \text{d}ac1^{-/–} \) soleus is not due to the disruption of the MOM by saponin treatment.

The apparent \( K_{\text{MADP}} \) represents an adaptable mechanism for mitochondrial regulation. Indeed, a 3-fold decrease in the apparent \( K_{\text{MADP}} \) in skinned cardiac fibers in rats fed a creatine analog that competitively inhibits creatine transport across the plasmalemma has been reported (44). This decreased apparent \( K_{\text{MADP}} \) is thought to compensate for the reduced cellular level of creatine and phosphocreatine that would presumably affect the phosphocreatine/creatine shuttle system. It has also been reported that a decrease in the apparent \( K_{\text{MADP}} \) in skinned cardiac and oxidative skeletal muscle fibers occurs in mice deficient in microtubule-associated protein (25). As an adaptation to the absence ofVDAC1, soleus fibers may take on the characteristics of glycolytic muscle, accounting for the decrease in the apparent \( K_{\text{MADP}} \). Xu et al. (27) have reported that, when reconstituted into liposomes, VDAC2 appears to exist in two forms differing with respect to conductance and selectivity. It is plausible that, in the soleus of \( v \text{d}ac1^{-/–} \) mice, VDAC2 exists predominantly in its high conductance state, thus accounting for their relatively low apparent \( K_{\text{MADP}} \).

There is a tissue specificity of mitochondria with respect to morphology, structural organization, and oxidative capacity (45–47). In striated muscles, there are subsarcomemal and intermyofibrillar mitochondria. Abnormal subsarcomemal and intermyofibrillar mitochondria associated with defects of components of the mitochondrial electron transport chain have also been described (48–50). By analyzing muscle sections by electron microscopy, we observed an abnormal configuration of the cristae of subsarcomemal mitochondria in the skeletal muscles of \( v \text{d}ac1^{-/–} \) mice. The morphologically abnormal mitochondria in the \( v \text{d}ac1^{-/–} \) skeletal muscles may result from an alteration in the interaction of cytoskeletal elements with the MOM. In support of this notion, it has been reported thatVDACs bind the cytoskeletal element MAP2 in brain mitochondria (51).

The functional coupling ofmiCK to mitochondrial respiration decreases the apparent \( K_{\text{MADP}} \), a finding that supports the hypothesis that, in vivo, the stimulation of oxidative phosphorylation depends on the activity of peripheral kinases (52). Creatine-stimulated respiration occurs when miCK is functionally coupled to oxidative phosphorylation, and it has been suggested that the octameric form of miCK located in the intermembrane space connects the MOM via VDACs to ANT. It has been theorized that creatine diffuses through VDACs and is converted by miCK in the presence of ATP to phosphocreatine and ADP. Phosphocreatine then leaves the mitochondria and is used at ATP-consuming sites, whereas ADP returns to the matrix via ANT to generate ATP (11, 12). In this study, we did not observe an alteration of the effect of creatine on mitochondrial respiration, which could mean either that VDAC1 is not required for the diffusion of creatine through the MOM or that VDAC2 and VDAC3 (or some alternate means) are sufficient for diffusion. From this study, we can also conclude either that the structural basis for the functional coupling of miCK to respiration does not require VDAC1 or that VDAC2 and VDAC3 are sufficient to account for the connection between the MOM and ANT via miCK.

In conclusion, our results suggest thatVDAC1 is involved in the transport of ADP across the MOM. We cannot draw the same conclusion for creatine. The absence ofVDAC1 has differing effects on the properties of in situ mitochondria from different striated muscles. Similar studies on mice lacking VDAC2, VDAC3, or some combination ofVDAC isoforms will be necessary to completely elucidate the roles ofVDAC isoforms in cellular metabolism and their possible involvement in mitochondrial diseases.

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