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VDAC3 has differing mitochondrial functions in two types of striated muscles

Keltoum Anflous-Pharayra [a,d,*], Nha Lee [a], Dawna L. Armstrong [b], William J. Craigen [a,c]

[a] Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[b] Department of Pathology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[c] Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[d] Department of Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

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ABSTRACT

Voltage-dependent anion channel (VDAC) is an abundant mitochondrial outer membrane protein. In mammals, three VDAC isoforms have been characterized. We have previously reported alterations in the function of mitochondria when assessed *in situ* in different muscle types in VDAC1 deficient mice (Anflous et al., 2001). In the present report we extend the study to VDAC3 deficient muscles and measure the respiratory enzyme activity in both VDAC1 and VDAC3 deficient muscles. While in the heart the absence of VDAC3 causes a decrease in the apparent affinity of *in situ* mitochondria for ADP, in the gastrocnemius, a mixed glycolytic/oxidative muscle, the affinity of *in situ* mitochondria for ADP remains unchanged. The absence of VDAC1 causes multiple defects in respiratory complex activities in both types of muscle. However, in VDAC3 deficient mice the defect is restricted to the heart and only to complex IV. These functional alterations correlate with structural aberrations of mitochondria. These results demonstrate that, unlike VDAC1, there is muscle-type specificity for VDAC3 function and therefore *in vivo* these two isoforms may fulfill different physiologic functions.

1. Introduction

Many biochemical reactions in cells depend on a tightly controlled ratio of ATP to ADP for their execution [1]. In oxidative tissues, this ratio is preserved by regulatory mechanisms that couple the rate of ATP consumption in the cell to the rate of ATP production by oxidative phosphorylation in mitochondria [2]. The mitochondrial outer membrane (MOM), as the boundary structure for mitochondria, plays a regulatory role by integrating sites of ATP consumption and production. Indeed, it has been demonstrated that, *in vivo*, the MOM dynamically separates the intermembrane space from the extramitochondrial space, leading to ADP compartmentation in the mitochondrial intermembrane space [3]. This was further demonstrated by studies on permeabilized heart and skeletal muscle fibers that have shown that the MOM participates in the regulation of mitochondrial respiration *in vivo* [4–7]. By analyzing heart skinned fibers during the first 6 weeks of life, Tiivel et al. [8] reported a gradual decrease in the apparent affinity of mitochondria for ADP ($Km_{(ADP)}$). This suggests a developmental mechanism that increasingly limits the access of cytoplasmic ADP to mitochondria. The communication between the extra-mitochondrial domain and the intermembrane space is conferred by the voltage-dependent anion channels (VDACs). VDACs, also known as mitochondrial porins, are small proteins of the MOM and are the main pathway for the transport of metabolites. Rostovtseva and Colombini [9] were the first to provide evidence that VDACs mediate the flux of ATP. Being at the interface between mitochondria and the cytosol, VDAC regulation potentially defines in which direction mitochondria will go: respiration and viability or apoptosis and cell death (reviewed in [10]). Indeed, it has been reported that a disruption in ATP/ADP exchange across the MOM upon growth factor withdrawal and induction of apoptosis correlates with the changes in conductance properties that accompany closure of VDACs, suggesting that restriction of flux across the channel is an important trigger for the induction of apoptosis. Based upon these observations, it has been proposed that VDACs can control coupled respiration and cell survival [11].

The fact that VDACs interact with kinases and the adenine nucleotide translocase (ANT) in important mitochondrial structures such as special contact sites and megachannels, underlines its regulatory role in mitochondrial energy metabolism [12,13]. A kinase that functionally interacts with VDAC is creatine kinase. The creatine kinase system, along with the glycolytic and adenylate kinase pathways, plays a key role in the energy metabolism of cells with intermittently high and fluctuating energy requirements, such as cardiac and skeletal muscle and neural tissue [14,15]. A primary function of the creatine kinase system in cardiac and oxidative skeletal muscle is the effective channeling of mitochondrial ATP across the intermembrane space to the myofibrils where it is consumed in the contraction cycle. By being functionally coupled to respiration, the mitochondrial isoform of creatine kinase (mi-CK)

Abbreviations: ANT, adenine nucleotide translocase; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; VDAC, voltage-dependent anion channel

* Corresponding author. Baylor College of Medicine, Department of Immunology, One Baylor Plaza, Houston, TX 77030, USA. Tel.: +1 713 798 1560.
E-mail address: kanflous@bcm.tmc.edu (K. Anflous-Pharayra).

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decreases the apparent $Km_{(ADP)}$, and therefore, *in vivo* with its counterpart in the cytosol, plays a key role for the transfer of energy in cardiac and oxidative skeletal muscle [4,5]. Accordingly, a decrease in the apparent $Km_{(ADP)}$ has been reported in mouse mi-CK knock-out muscles [16]. It is believed that this functional coupling increases the ADP concentration close to the ANT and compensates for the barrier to ADP diffusion exerted by the MOM. It has been suggested that creatine diffuses through VDACs to reach mi-CK located in the mitochondrial intermembrane space [17].

In higher eukaryotes, three VDAC isoforms have been characterized: VDAC1, VDAC2 and VDAC3 [18–24]. The three isoforms are encoded by three separate genes [25,26]. Although VDACs are highly conserved across species, the specific function of each isoform remains poorly understood. VDAC1 and VDAC3 deficient mice have been successfully generated [27–29]. Despite a partial *in utero* lethality, VDAC deficient mice are viable and therefore provide an opportunity to study the role(s) of VDACs in cellular metabolism in intact organisms (reviewed in Craigen and Graham, 2008) [30]. We have previously reported an alteration in the MOM permeability for ADP in different muscle types in the absence of VDAC1. However, the functional coupling of mi-CK to respiration remains intact in the absence of VDAC1 [27]. To test the hypothesis that different VDAC isoforms fulfill different roles *in vivo* and function in a tissue-specific fashion, VDAC3 deficient mice were used to measure the functional properties of mitochondria *in situ* in two types of striated muscle, the heart and the gastrocnemius, a mixed glycolytic/oxidative skeletal muscle. We also measured the impact of the absence of VDAC1 and VDAC3 on the activity of the respiratory enzyme complexes. We report alterations of the mitochondrial structure and the permeability of the MOM for ADP in VDAC3 deficient heart but not VDAC3 deficient gastrocnemius. We also report a partial defect of the respiratory enzyme activities in both types of muscle in VDAC1 deficient mice. However in VDAC3 deficient mice, there is a single respiratory chain defect that is restricted to the heart. Together, these data support the hypothesis that, *in vivo*, VDAC1 and VDAC3 fulfill different functions.

2. Materials and methods

2.1. Animals

Unless otherwise indicated, wild type control and VDAC3 deficient ($vdac3^{-/-}$) mice studied were on an inbred 129SvEv background. In each set of experiments, at least five littermates wild type control and $vdac3^{-/-}$ mice were used. Mice were ~8 months of age. The genotypes of the mice were determined by PCR, as previously described (27–29). All animal experiments conformed to Baylor College of Medicine IACUC Guidelines for the care of rodents.

2.2. In situ mitochondrial respiratory studies

Mice were anesthetized by intraperitoneal injection of Urethane (0.1 mg/30 g wet weigh). The hearts were quickly removed and placed in a cooled, well oxygenated (95% O_2 + 5% CO_2) 1× PBS solution without calcium (Amersham). Heart and gastrocnemius skinned fibers were prepared as previously described [27,31]. Rates of oxygen consumption were assessed using an oxygraph (Biological Monitor, YSI MODEL 5300) and a Clark electrode (Oxygen probe, YSI 5331) and recorded by a MacLab/200 system (AD Instruments). The ADP-stimulated respiration above basal oxygen consumption were plotted by using a GraphPad PRIZM program, version 2.0C in order to determine the apparent $Km_{(ADP)}$ and the calculated rate of oxygen consumption in the presence of maximal ADP concentration (V_{max}) [27]. The *Km* is termed apparent because the exact concentration of ADP in the mitochondrial intermembrane space is not known. The functional coupling of mi-CK to respiration was assessed in the presence of 25 mM creatine. At the end of the experiments, the fibers were removed from the respiration buffer, put on aluminum paper, dried on a heating plate and weighed. Rate of respiration is given in μmoles of oxygen/min/g dry weigh.

2.3. Electron microscopy

Samples of heart and gastrocnemius muscles from wild type control and $vdac3^{-/-}$ mice were processed and examined by a transmission electron microscopy as described previously [27].

2.4. Western blotting

We used affinity purified rabbit polyclonal antibody specific for VDAC1 (kindly provided by Dr. M. Colombini) and a chicken polyclonal antibody specific for VDAC2 (similarly generated as described in [32]) to quantify the amount of the corresponding VDAC isoforms in $vdac3^{-/-}$ muscles. To detect VDAC3 in wild type samples we used an affinity purified chicken polyclonal antibody [32]. An actin-specific polyclonal antibody (Sigma) was used as a loading control. For COXIV detection we used a monoclonal antibody (Molecular Probes) and cytochrome c as loading control (Santa Cruz Biotechnology). Heart and gastrocnemius muscles from wild type control and $vdac3^{-/-}$ mice were homogenized by a polytron in a buffer containing: Hepes 5 mM (pH 8.0), EGTA 1 mM, DTT 1 mM and triton 0.1%. Antiprotease cocktail (1×) (P8340, Sigma) and phenylmethylsulfonyl (1 mM) were added to the buffer. The homogenates were centrifuged at 550*g*, 5 °C for 5 min. 30 μg of total protein from the supernatant was separated using a 12% Tris/HCl ready polyacrylamide-gel (Bio-Rad) and transferred to polyvinylidene difluoride membrane (Roche Molecular Biochemicals) using a Bio-Rad Trans-Blot system. The membranes were blocked, incubated and developed as described previously [33]. Protein concentrations were determined with the BCA protein assay reagent (PIERCE), using BSA as standard.

2.5. Respiratory chain enzyme activities

Fresh heart and gastrocnemius muscles were homogenized to a final concentration of 10% with a Dounce glass-Teflon homogenizer. The homogenates were prepared in sucrose buffer (Sucrose 150 mM, EDTA 2 mM and Tris–HCl 100 mM, pH 7.45) and centrifuged at 550*g*, 5 °C for 20 min [34]. The assay was carried out in the supernatant at 30 °C using a temperature-controlled spectrophotometer (Pharmacia, Biotech). The activities of complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex I + III (NADH:cytochrome c oxidoreductase), complex II + III (succinate :cytochrome c reductase) and complex IV (cytochrome c oxidase) were assayed using different electron acceptors/donors. The activities of complex I + III and II + III were measured by following the reduction of cytochrome c at 550 nm, of complex I by following the oxidation of NADH at 340 nm, of complex II by following the reduction of 2,6-dichloroindophenol (DCIP) at 600 nm and of complex IV by following the oxidation of reduced cytochrome c at 550 nm [35,36]. Citrate synthase activity was measured to adjust enzymatic activities for mitochondrial content, as previously described [37].

2.6. Statistical analysis

A two-tailed, unpaired t test was used to compare the data. The level of significance was set at $p<0.05$.

3. Results

3.1. In situ mitochondrial respiratory studies

VDAC is a major protein in the MOM. To test the hypothesis that the different VDAC isoforms fulfill different functions within different

muscle types, we used the technique of skinned fibers to study the *in situ* properties of mitochondria in the absence of VDAC3. In the cardiac fibers, we observed that the absence of VDAC3 results in an increase of the apparent $Km_{(ADP)}$, reflecting a decrease in the affinity of mitochondria for ADP (Fig. 1A). In the presence of 25 mM creatine, there is a decrease in the apparent $Km_{(ADP)}$ in both wild type and *vdac3*$^{-/-}$ cardiac fibers, consistent with the maintenance of the creatine effect in the mutant fibers (Fig. 1A). V_{max} (respiration in the presence of maximal ADP concentration) remains statistically unchanged from the control, both in the absence and presence of creatine (Fig. 1B). We used the gastrocnemius to address whether VDAC3 fulfills a different function in a different muscle type. In contrast to the difference in the apparent $Km_{(ADP)}$ observed in VDAC3 deficient cardiac fibers, the absence of VDAC3 has no significant effect on the apparent $Km_{(ADP)}$ in gastrocnemius fibers (Fig. 1C). Likewise, V_{max} remains statistically undistinguishable from the control (Fig. 1D).

3.2. Electron microscopy

To determine if the alterations in mitochondrial function are associated with mitochondrial structural alterations, electron microscopy was used to examine the morphology of mitochondria in both muscle types from wild type control and *vdac3*$^{-/-}$ mice. The intermyofibrillar mitochondria from *vdac3*$^{-/-}$ heart appear enlarged with irregular cristae, with an appearance reminiscent of fingerprints (Fig. 2A and B). While in wild type heart, an average of two to three mitochondria span two sarcomere units (indicated by asterisk in Fig. 2A), in the VDAC3 deficient heart, on average one mitochondrion spans one sarcomere unit and even may span two sarcomere units (indicated by head arrow in Fig. 2B). This enlargement of mitochondria in the absence of VDAC3 is similar to that seen with *vdac1*$^{-/-}$ mice, suggestive of a similar underlying mechanism. In the *vdac3*$^{-/-}$ gastrocnemius, the mitochondria appear indistinguishable from the wild type control sample in both intermyofibrillar and subsarcolemmal populations (Fig. 2C, D, E and F).

3.3. VDAC protein quantitation

We used western blot analysis to determine whether the absence of VDAC3 has an impact on the amount of the remaining isoforms. The amounts of VDAC1 and VDAC2 in the mutant heart and gastrocnemius remain indistinguishable from the control muscles (Fig. 3). Therefore, the effects described reflect the absence of VDAC3 and are not due to an alteration in the amount of the remaining VDAC isoforms.

3.4. Respiratory chain complex activities

As a component of the MOM, the absence of VDAC may potentially influence the respiratory chain activity in two ways: by restricting the access of substrates to the respiratory chain or by disturbing the assembly or structure of the complexes, which in turn may affect their functions. We determined spectrophotometrically the activity of different respiratory complex activities in the heart and the gastrocnemius from *vdac1*$^{-/-}$ and *vdac3*$^{-/-}$ mice. We observed statistically significant reductions in complex I + III, II + III and IV activities in *vdac1*$^{-/-}$ muscles (Fig. 4A and B). Whereas *vdac1*$^{-/-}$ heart does not exhibit any defect in complex I and II activities, there is a defect in complex II activity in the gastrocnemius of *vdac1*$^{-/-}$ mice. Complex I activity was not determined for *vdac1*$^{-/-}$ gastrocnemius. For *vdac3*$^{-/-}$ mice, the respiratory chain defect is restricted to complex IV and is only observed in the heart (Fig. 4A and B).

4. Discussion

In this report we have shown that while the absence of VDAC3 in mice results in altered functional and structural properties of *in situ* mitochondria in cardiac fibers, mitochondria in the mixed muscle gastrocnemius remain indistinguishable from control mitochondria. We have previously reported that the absence of VDAC1 causes an increase in the apparent $Km_{(ADP)}$ as well as an alteration in the structure of mitochondria in both cardiac and gastrocnemius fibers;

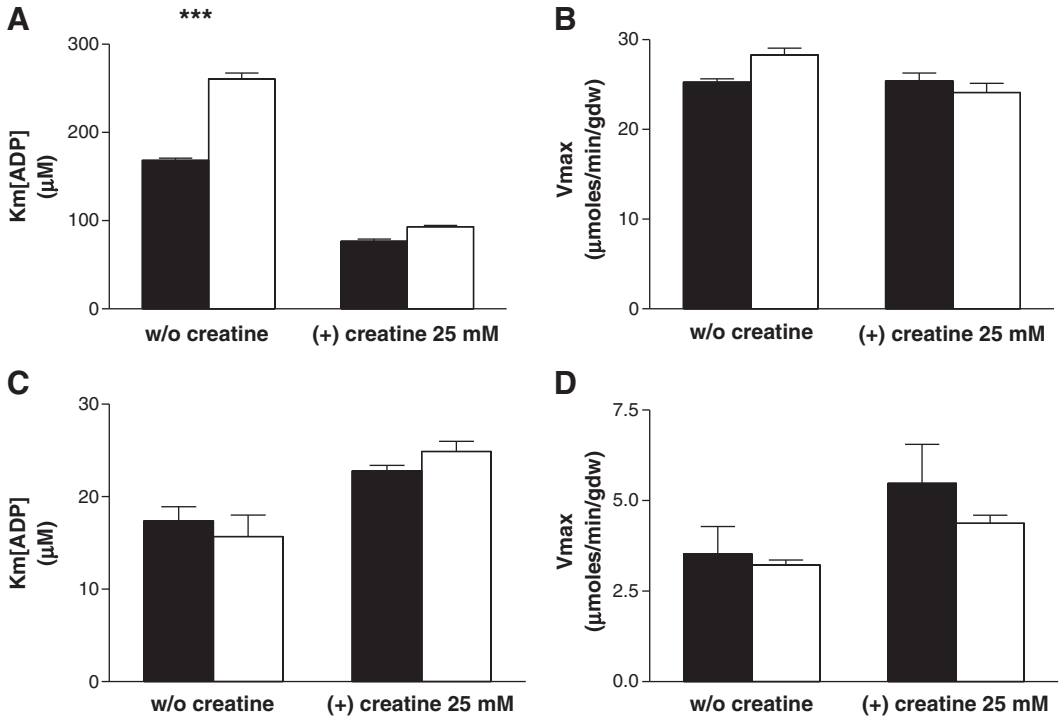

Fig. 1. Functional properties of mitochondria. The *in situ* affinity of mitochondria for ADP (Km[ADP] in µM) was determined in saponin-skinned fibers prepared from wild type control (black bar) and *vdac3*$^{-/-}$ (white bar) heart (A) and gastrocnemius (C) in the absence (w/o) and presence of 25 mM creatine. Calculated rate of respiration in the presence of the maximal ADP concentration (V_{max}) was determined in fibers prepared from wild type control (black bar) and *vdac3*$^{-/-}$ (white bar) heart (B) and gastrocnemius (D) in the absence (w/o) and presence of 25 mM creatine. Values are expressed in µmoles of oxygen/min/g dry weigh (gdw). The error bar represents the SEM. ***$p<0.001$ vs. wild type.

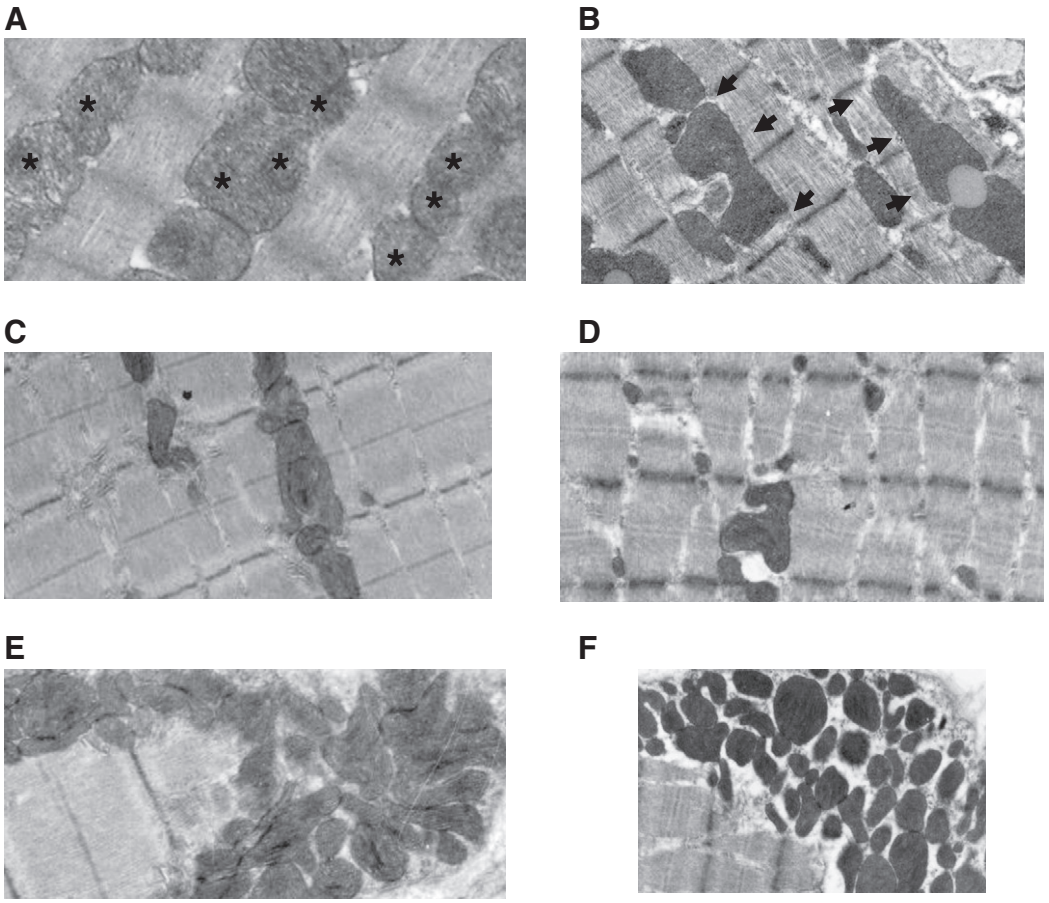

Fig. 2. Electron microscopy images of muscles. Heart and gastrocnemius muscles were dissected from wild type and *vdac3*$^{-/-}$ mice and mounted for electron microscopy analysis. A and B show intermyofibrillar mitochondria from wild type and *vdac3*$^{-/-}$ heart respectively. The head arrows indicate one mitochondrion that spans two sarcomere units. The asterisks indicate an average of mitochondria within two sarcomere units. C and D show intermyofibrillar mitochondria from wild type and *vdac3*$^{-/-}$ gastrocnemius respectively. E and F show subsarcolemmal mitochondria from wild type and *vdac3*$^{-/-}$ gastrocnemius respectively. Magnification ×2000 (A) and (B), ×6000 (C), (D), (E) and (F).

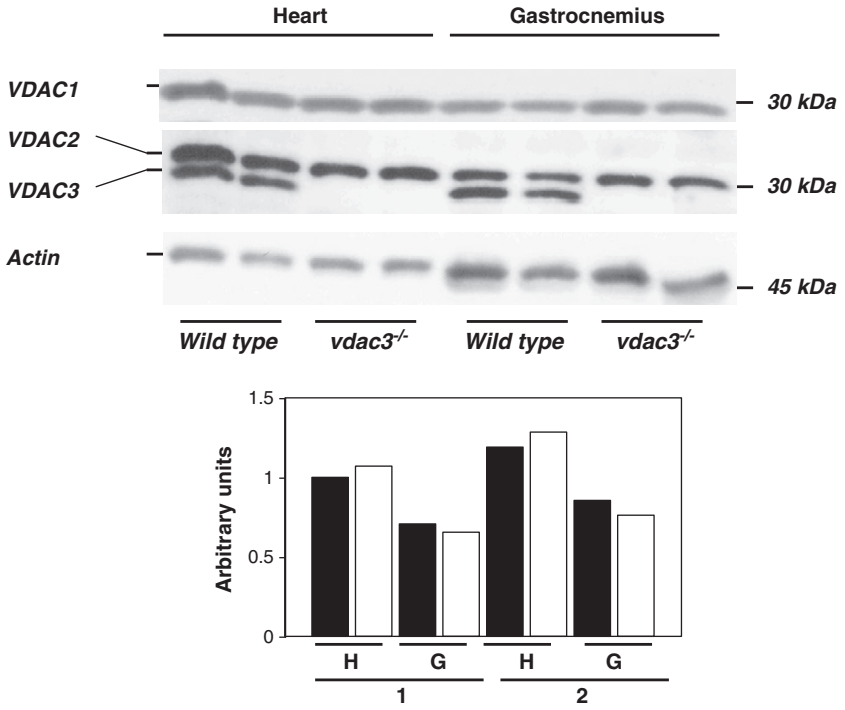

Fig. 3. Expression analysis of different VDAC isoforms. Western blots corresponding to the sample homogenates from wild type and *vdac3*$^{-/-}$ muscles were probed with antibodies specifics for different VDAC isoforms. Actin antibody was used for loading control. The panel indicates the ratio of VDAC1 (1) and VDAC2 (2) band signal to actin band signal in wild type (black bar) and *vdac3*$^{-/-}$ (white bar) heart (H) and gastrocnemius (G). The bands were quantified by a Densitometer. Each bar represents the mean of two values.

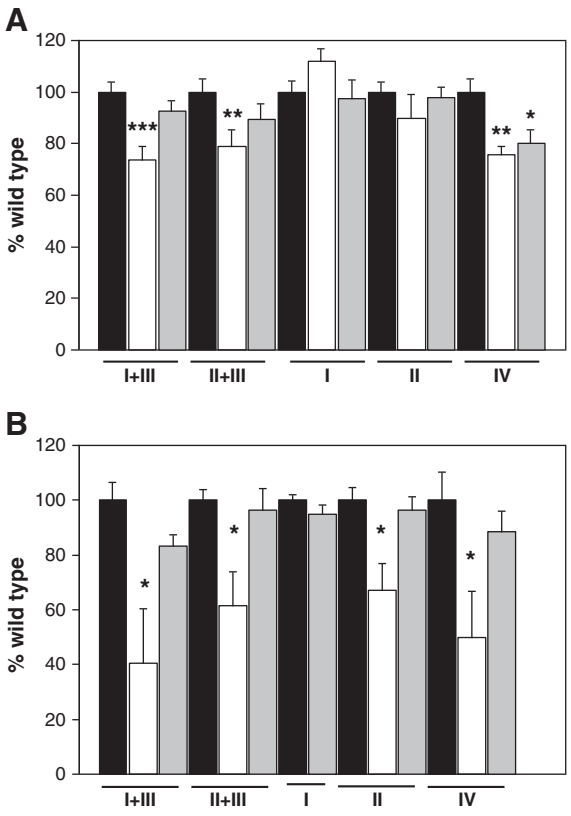

Fig. 4. Respiratory enzyme activities. The activities of complex I, II, I + III, II + III and IV were determined and normalized to citrate synthase activity for mitochondrial content. The normalized activity in wild type control was taken as 100% and the normalized activity in the mutant muscles was calculated as a percent of the wild type control. (A) Normalized respiratory enzyme activity in the heart. (B) Normalized respiratory enzyme activity in the gastrocnemius. The black bar represents wild type muscle, the white bar represents *vdac1*$^{-/-}$ muscle and the gray bar represents *vdac3*$^{-/-}$ muscle. The error bars refer to SEM. *$p<0.05$ vs. wild type, **$p<0.01$ vs. wild type, ***$p<0.001$ vs. wild type.

therefore, unlike VDAC1, there is a muscle type specificity for VDAC3 function. Recently, at a more integrated level, we reported impaired glucose tolerance with reduced mitochondria-bound hexokinase activity in VDAC1 deficient mice but not in VDAC3 deficient mice [33], further supporting the hypothesis that, *in vivo*, VDAC1 and VDAC3 fulfill different physiologic functions.

4.1. Muscle type specificity for VDAC3 function

Fundamental differences between the oxidative and glycolytic striated muscles have previously been reported [38]. These differences also include the organization of the CK system as well as the permeability of the MOM for ADP [16,17,27,39]. Being the main pathway in the MOM for small metabolites, it is believed that VDACs regulate the permeability of the MOM and the functional coupling of mi-CK to respiration. Similar to VDAC1, while VDAC3 is involved in the exchange of ADP in cardiac muscle, its involvement in the creatine effect on respiration remains questionable. This raises the possibility that VDAC2 may be the isoform involved in the exchange of creatine across the MOM. Since VDAC2 deficiency appears to be lethal to the mouse [40], addressing this question depends upon the generation of a conditional deletion of VDAC2 in muscles. In the gastrocnemius, VDAC3 may fulfill a different function since we did not detect any alteration in the apparent $Km_{(ADP)}$ between control and mutant fibers. The results of western blotting favor this hypothesis since there is no variation in the amount of VDAC1 or VDAC2 in the absence of VDAC3 that would reflect a specific compensatory upregulation of these isoforms. On the other hand, it has been reported that tubulin binds to VDAC and regulates mitochondrial respiration [41]. More recently, Saks et al. suggested that VDAC and tubulin are part of a supercomplex that includes mi-CK and the ATP synthasome [42]. This supercomplex assures continuous recycling of adenine nucleotides between mitochondria and cytoplasm. Although it is not known if VDAC3 interacts with tubulin, one can speculate that the difference in the mitochondrial affinity for ADP between cardiac muscle and the gastrocnemius may be due to a muscle type specificity in the interaction of VDAC3 with tubulin.

4.2. The organization of the mitochondrial network is intact in the absence of VDAC3

The functional alterations of mitochondria in *vdac3*$^{-/-}$ heart are consistent with altered ultrastructure observed by electron microscopy. Their relatively increased size suggests alterations in mitochondrial fusion/fission processes. Since it has been reported that VDAC is the binding site for microtubules [41,43], one would expect alterations in the organization of the mitochondrial network in VDAC deficient muscles. Indeed, it was recently shown that the cytoskeleton may play a role in the organization of mitochondrial networks and their incorporation into functional complexes with the sarcomere and sarcoplasmic reticulum [44]. Upon brief proteolytic treatment with trypsin, the authors demonstrated that disorganization of microtubular and plectin networks together with the mitochondrial network occur. Interestingly, mitochondria in *vdac3*$^{-/-}$ heart preserve their organization within the myofibrils suggesting that the structural interaction between the cytoskeletal elements and mitochondria is preserved in the absence of VDAC3. The same observation has previously been made in VDAC1 deficient muscles [27]. These results suggest a redundancy among VDAC isoforms in their structural interaction with the cytoskeleton and/or that VDAC2 is the main isoform interacting with the microtubular network.

4.3. Complex IV deficiency in VDAC3 deficient heart

Structural alteration of mitochondria is a hallmark of patients with a variety of mitochondrial diseases [45,46]. Despite extensive studies of the function and structure of mitochondria, much of the underlying pathogenic processes of mitochondrial diseases remain poorly understood (reviewed in [47]). A large number of mitochondrial diseases originate from respiratory chain dysfunction. The demonstration that loss of a MOM channel leads to a variety of respiratory chain perturbations reflects the complex interactions between the inner and outer membranes. The core of the respiratory chain and oxidative phosphorylation pathway is composed of five multisubunit complexes (I-V) located within the mitochondrial inner membrane (MIM). In addition to mutations in mitochondrial DNA-encoded proteins, tRNAs and ribosomal RNAs, mutations in nuclear genes not directly related to oxidative phosphorylation, such as assembly factors, chaperones, and enzymes of phospholipid metabolism, have emerged as potential causes of mitochondrial respiratory chain disorders. These defects leading to secondary loss in oxidative function have been referred to as class II respiratory chain defects (reviewed in [48–51]). While the absence of VDAC1 leads to a reduction in multiple respiratory chain enzymatic activities in both the heart and the gastrocnemius, the absence of VDAC3 is only associated with complex IV deficiency in the heart and no detectable deficiency in the gastrocnemius. Since these deficiencies are observed in both intact and frozen mitochondrial samples, a failure to conduct metabolites across the MOM cannot explain these observations. Therefore, there is a structural dependence between VDAC and different respiratory enzyme complexes. Complex IV (COX), the terminal enzyme in the mitochondrial respiratory chain, is composed of 13 subunits, 10 of which are encoded by nuclear genes. The three

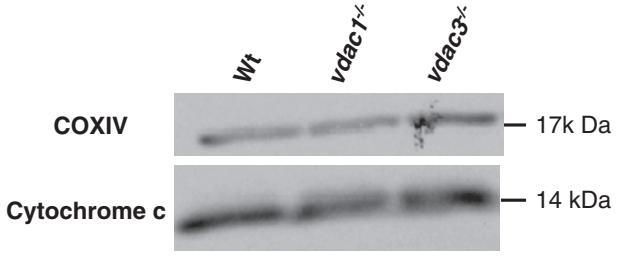

Fig. 5. Expression analysis of COXIV subunit. Western blot corresponding to the sample homogenates from wild type (*Wt*), *vdac1*$^{-/-}$ and *vdac3*$^{-/-}$ heart was probed with an antibody specific for COXIV subunit. Cytochrome c was used for loading control.

mitochondrial DNA-encoded subunits (COX I-III) form the catalytic core of the enzyme. The details of the assembly of complex IV are still poorly understood. In a subset of patients with Leigh Syndrome, an early onset, fatal neurodegenerative disorder, COX activity is reduced to 10–25% of control levels. Mutations in SURF1, a nuclear gene involved in COX assembly, have been reported in some Leigh Syndrome patients [52,53]. Whether the same mechanism is at play in VDAC deficient muscles, or whether there is a defect in subunit import into mitochondria is the subject of ongoing studies. Preliminary data with a western blot analysis using COXIV specific antibody does not show any alteration in the amount of this subunit in VDAC deficient hearts (Fig. 5).

5. Conclusions

In conclusion, we present evidence that unlike VDAC1, VDAC3 fulfills different functions in two types of striated muscles. We also show that VDACs functionally interact with the MIM as evidenced by a reduction in respiratory chain activities. Although the nature of this interaction is at present unknown, VDAC deficient mice provide a model for class II respiratory chain defects and therefore can be used to dissect some of the molecular mechanisms underlying respiratory chain defects observed in mitochondrial diseases.

Acknowledgments

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References

[1] F. Buttgereit, M.D. Brand, A hierarchy of ATP-consuming processes in mammalian cells, Biochem. J. 312 (1995) 163–167.
[2] G.C. Brown, P.L. Lakin-Thomas, M.D. Brand, Control of respiration and oxidative phosphorylation in isolated rat liver cells, Eur. J. Biochem. 192 (1990) 355–362.
[3] F.N. Gellerich, R. Bohnensack, W. Kunz, Role of the mitochondrial outer membrane in dynamic compartmentation of adenine nucleotides, in: A. Azzi, K.A. Nalecz, M.J. Nalecz, L. Wojtczak (Eds.), The Anion Carriers of the Mitochondrial Membranes, Springer Verlag, Berlin Heidelberg, 1989, pp. 349–359.
[4] V.A. Saks, Y.O. Belikova, A.V. Kuznetsov, In vivo regulation of mitochondrial respiration in cardiomyocytes: specific restriction for intracellular diffusion for ADP, Biochim. Biophys. Acta 1074 (1991) 302–311.
[5] V.A. Saks, E. Vasil'eva, Y.O. Belikova, A.V. Kuznetsov, S. Lyapina, L. Petrova, N.A. Perov, Retarded diffusion of ADP in cardiomyocytes: possible role of mitochondrial outer membrane and creatine kinase in cellular regulation of oxidative phosphorylation, Biochim. Biophys. Acta 1144 (1993) 134–148.
[6] V.A. Saks, A.V. Kuznetsov, Z.A. Khuchua, E.V. Vasilyeva, J.O. Belikova, T. Kesvatera, T. Tiivel, Control of cellular respiration by mitochondrial outer membrane and by creatine kinase in normal muscle and in pathology, J. Mol. Cell. Cardiol. 27 (1995) 625–645.
[7] V. Saks, A. Kuznetsov, T. Andrienko, Y. Usson, F. Appaix, K. Guerrero, T. Kaambre, P. Sikk, M. Lemba, M. Vendelin, Heterogeneity of ADP diffusion and regulation of respiration in cardiac cells, Biophys. J. 84 (2003) 3436–3456.
[8] T. Tiivel, L. Kadaya, A. Kuznetsov, T. Kaambre, N. Peet, P. Sikk, U. Braun, R. Ventura-Clapier, V. Saks, E.K. Seppet, Developmental changes in regulation of mitochondrial respiration by ADP and creatine in rat heart in vivo, Mol. Cell. Biochem. 208 (2000) 119–128.
[9] T. Rostovtseva, M. Colombini, VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function, Biophys J. 72 (1997) 1954–1962.
[10] T.K. Rostovtseva, S.M. Bezrukov, VDAC regulation: role of cytosolic proteins and mitochondrial lipids, J. Bioenerg. Biomembr. 40 (2008) 163–170.
[11] M. Vander Heiden, N.S. Chandel, X.X. Li, P.T. Schumacker, M. Colombini, C.B. Thompson, Outer mitochondrial membrane permeability can regulate coupled respiration and cell survival, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4666–4671.
[12] D. Brdiczka, T. Wallimann, The importance of the outer mitochondrial compartment in regulation of energy metabolism, Mol. Cell. Biochem. 133/134 (1994) 69–83.
[13] F.N. Gellerich, M. Kapischke, W. Kunz, W. Neumann, A. Kuznetsov, D. Brdiczka, K. Nicolay, The influence of the cytosolic oncotic pressure on the permeability of the mitochondrial outer membrane for ADP: implications for the kinetic properties of mitochondrial creatine kinase and for ADP channeling into the intermembrane space, Mol. Cell. Biochem. 133/134 (1994) 85–104.
[14] P.P. Dzeja, A. Terzic, B. Wieringa, Phosphotransfer dynamics in skeletal muscle from creatine kinase gene-deleted mice, Mol. Cell. Biochem. 256/257 (2004) 13–27.
[15] T. Wallimann, M. Dolder, U. Schlattner, M. Eder, T. Hornemann, E. O'Gorman, A. Ruck, D. Brdiczka, Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology, Biofactors 8 (1998) 229–234.
[16] V.I. Veksler, A.V. Kuznetsov, K. Anflous, P. Mateo, J. van Deursen, B. Wieringa, R. Ventura-Clapier, Muscle creatine kinase-deficient mice. II. Cardiac and skeletal muscles exhibit tissue-specific adaptation of the mitochondrial function, J. Biol. Chem. 270 (1995) 19921–19929.
[17] T. Wallimann, M. Wyss, D. Brdiczka, K. Nicolay, H.M. Eppenberger, Intracellular compartmentalization, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the "phosphocreatine circuit" for cellular energy homeostasis, Biochem. J. 281 (1992) 21–40.
[18] K. Anflous, O. Blondel, A. Bernard, M. Khrestchatiski, R. Ventura-Clapier, Characterization of rat porin isoforms: cloning of a cardiac type-3 variant encoding an additional methionine at its putative N-terminal region, Biochim. Biophys. Acta. 1399 (1998) 47–50.
[19] E. Blachly-Dyson, E.B. Zambronicz, W.H. Yu, V. Adams, E.R. MacCabe, J. Adelman, M. Colombini, M. Forte, Cloning and functional expression in yeast of two human isoforms of the outer mitochondrial membrane channel, the voltage-dependent anion channel, J. Biol. Chem. 268 (1993) 1835–1841.
[20] M.H. Bureau, M. Khrestchatiski, M.A. Heeren, E.B. Zambrowicz, H. Kim, T.M. Grizar, M. Colombini, A.J. Tobin, R.W. Olsen, Isolation and cloning of a voltage-dependent anion channel-like Mr 36, 000 polypeptide from mammalian brain, J. Biol. Chem. 267 (1992) 8679–8684.
[21] H. Ha, P. Hajek, D.M. Bedwell, P.D. Burrows, A mitochondrial porin cDNA predicts the existence of multiple human porins, J. Biol. Chem. 268 (1993) 12143–12149.
[22] M.J. Sampson, R.S. Lovell, W.J. Craigen, Isolation, characterization, and mapping of two mouse mitochondrial voltage-dependent anion channel isoforms, Genomics 33 (1996) 283–288.
[23] M.J. Sampson, R.S. Lovell, D.B. Davison, W.J. Craigen, A novel mouse mitochondrial voltage-dependent anion channel gene localizes to chromosome 8, Genomics 36 (1996) 192–196.
[24] M.J. Sampson, L. Ross, W.K. Decker, W.J. Craigen, A novel isoform of the mitochondrial outer membrane protein VDAC3 via alternative splicing of a 3-base exon, J. Biol. Chem. 273 (1998) 30482–30486.
[25] W.K. Decker, K.R. Boules, E.C. Schatte, J.A. Towbin, W.J. Craigen, Revised fine mapping of the human voltage-dependent anion channel loci by radiation hybrid analysis, Mamm. Genome 10 (1999) 1041–1042.
[26] M.J. Sampson, R.S. Lovell, W.J. Craigen, The murine voltage-dependent anion channel gene family, conserved structure and function, J. Biol. Chem. 272 (1997) 18966–18973.
[27] K. Anflous, D. Armstrong, W.J. Craigen, Altered mitochondrial sensitivity for ADP and maintenance of creatine stimulated respiration in oxidative striated muscles from VDAC1 deficient mice, J. Biol. Chem. 276 (2001) 1954–1960.
[28] M.J. Sampson, W.K. Decker, A.L. Beaudet, W. Ruitenbeek, D. Armstrong, M.J. Hicks, W.J. Craigen, Immotile sperm and infertility in mice lacking mitochondrial voltage-dependent anion channel type 3, J. Biol. Chem. 276 (2001) 39206–39212.
[29] E.J. Weeber, M. Levy, M. Sampson, K. Anflous, D.L. Armstrong, S.E. Brown, J.D. Sweatt, W.J. Craigen, The role of mitochondrial porins and the permeability transition pore in learning and synaptic plasticity, J. Biol. Chem. 277 (2002) 18891–18897.
[30] W.J. Craigen, B.H. Graham, Genetic strategies for dissecting mammalian and Drosophila voltage-dependent anion channel functions, J. Bioenerg. Biomembr. 40 (2008) 207–212.
[31] V.I. Veksler, A.V. Kuznetsov, V.G. Sharov, V.I. Kapelko, V.A. Saks, Mitochondrial respiratory parameters in cardiac tissue: a novel method of assessment by using saponin-skinned fibers, Biochim. Biophys. Acta 892 (1987) 191–196.
[32] W.K. Decker, W.J. Craigen, The tissue-specific, alternatively spliced single ATG exon of the type 3 voltage-dependent anion channel gene does not create a truncated protein isoform in vivo, Mol. Genet. Metab. 70 (2000) 69–74.
[33] K. Anflous-Pharayra, Z.J. Cai, W.J. Craigen, VDAC1 serves as a mitochondrial binding site for hexokinase in oxidative muscles, Biochim. Biophys. Acta 1767 (2007) 136–142.

[34] F.R. Wiedemann, G. Manfredi, C. Mawrin, M. Flint Beal, E.A. Schon, Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients, J. Neurochem. 80 (2002) 616–625.
[35] G.L. Sottocasa, B. Kuylenstierna, L. Ernster, A. Bergstrand, An electron-transport system associated with the outer membrane of liver mitochondria, A biochemical and morphological study, J. Cell Biol. 32 (1967) 415–438.
[36] T.H. Vu, M. Sciacco, K. Tanji, C. Nichter, E. Bonilla, S. Chatkupt, P. Maertens, S. Shanske, J. Mendell, M.R. Koenigsberger, L. Sharer, E.A. Schon, S. DiMauro, D.C. DeVivo, Clinical manifestations of mitochondrial DNA depletion, Neurology 50 (1998) 1783–1790.
[37] H.S.A. Sherratt, N.J. Watmough, M.A. Johnson, D.M. Turnbull, Methods for study of normal and abnormal skeletal muscle mitochondria, Methods Biochem. Anal. 33 (1988) 243–335.
[38] S. Schiaffino, C. Reggiani, Molecular diversity of myofibrillar proteins: gene regulation and functional significance, Physiol. Rev. 76 (1996) 371–423 (Review).
[39] A.V. Kuznetsov, T. Tiivel, P. Sikk, T. Kaambre, L. Kay, Z. Daneshrad, A. Rossi, L. Kadaja, N. Peet, E. Seppet, V.A. Saks, Striking differences between the kinetics of regulation of respiration by ADP in slow-twitch and fast-twitch muscles in vivo, Eur. J. Biochem. 241 (1996) 909–915.
[40] E.H. Heng, T. Sheiko, J.K. Fisher, W.J. Craigen, S.J. Korsmeyer, VDAC2 inhibits BAK activation and mitochondrial apoptosis, Science 301 (2003) 513–517.
[41] T.K. Rostovtseva, K.L. Sheldon, E. Hassanzadeh, C. Monge, V. Saks, S.M. Bezrukov, D.L. Sacket, Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 18746–18751.
[42] V. Saks, R. Guzun, N. Timohhina, K. Tepp, M. Varikmaa, C. Monge, N. Beraud, T. Kaambre, A. Kuznetsov, L. Kadaja, M. Eimre, E. Seppet, Structure-function relationships in feedback regulation of energy fluxes in vivo in health and disease: mitochondrial interactosome, Biochim. Biophys. Acta 1797 (2010) 678–697.
[43] M. Linden, G. Karlsson, Identification of porin as a binding site for MAP2, Biochem. Biophys. Res. Commun. 218 (1996) 833–836.
[44] F. Appaix, A.V. Kuznetsov, Y. Usson, L. Kay, T. Andrienko, J. Olivares, T. Kaambre, P. Sikk, R. Margreiter, V. Saks, Possible role of cytoskeleton in intracellular arrangement and regulation of mitochondria, Exp. Physiol. 88.1 (2003) 175–190.
[45] S. DiMauro, F. Bonilla, M. Zeviani, M. Nakagawa, D.C. DeVivo, Mitochondrial myopathies, Ann. Neurol. 17 (1985) 521–538.
[46] E. Bonilla, K. Tanji, Ultrastructural alterations in encephalomyopathies of mitochondrial origin, Biofactors 7 (1998) 231–236.
[47] J.J. Briere, D. Chretien, P. Benit, P. Rustin, Respiratory chain defects: what do we know for sure about their consequences in vivo? Biochim. Biophys. Acta 1659 (2004) 172–177.
[48] J.V. Leonard, A.H.V. Schapira, Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects, Lancet 355 (2000) 389–394.
[49] A. Oldfors, M. Tulinius, Mitochondrial Encephalomyopathies regulation and functional significance, J. Neurop. Exp. Neurol. Physiol. Rev. 76 (2003) 371–423 (Review).
[50] D.R. Thorburn, Mitochondrial disorders: prevalence, myths and advances, J. Inherit. Metab. Dis. 27 (2004) 349–362.
[51] D.C. Wallace, Animal models for mitochondrial diseases, Meth. Mol. Biol. 197 (2002) 3–54.
[52] C.M. Sue, C. Karadimas, N. Checcarelli, K. Tanji, L.C. Papadopoulou, F. Pallotti, F.L. Guo, S. Shanske, M. Hirano, D.C. De Vivo, R. Van Coster, P. Kaplan, E. Bonilla, S. DiMauro, Differential features of patients with mutations in two COX assembly genes, SURF-1 and SCO2, Ann. Neurol. 47 (2000) 589–595.
[53] V. Tiranti, M. Jaksch, S. Hofmann, C. Galimberti, K. Hoertnagel, L. Lulli, P. Freisinger, L. Bindoff, K.D. Gerbitz, G.P. Comi, G. Uziel, M. Zeviani, T. Meitinger, Loss of function mutations of SURF-1 are specifically associated with Leigh syndrome with cytochrome c oxidase deficiency, Ann. Neurol. 46 (1999) 161–166.