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Topical Review

The Voltage-dependent Anion Channel in Endoplasmic/Sarcoplasmic Reticulum: Characterization, Modulation and Possible Function

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Abstract. In recent years, it has been recognized that there is a metabolic coupling between the cytosol, ER/SR and mitochondria. In this cross-talk, mitochondrial Ca^{2+} homeostasis and ATP production and supply play a major role. The primary transporter of adenine nucleotides, $Ca^{2+}$and other metabolites into and out of mitochondria is the voltage-dependent anion channel (VDAC) located at the outer mitochondrial membrane, at a crucial position in the cell. VDAC has been established as a key player in mitochondrial metabolite and ion signaling and it has also been proposed that VDAC is present in extramitochondrial membranes. Thus, regulation of VDAC, as the main interface between mitochondrial and cellular metabolism, by other molecules is of utmost importance. This article reviews localization and function of VDAC, and focuses on VDAC as a skeletal muscle sarcoplasmic reticulum channel. The regulation of VDAC activity by associated proteins and by inhibitors is also presented. Several aspects of the physiological relevance of VDAC to Ca^{2+} homeostasis and mitochondria-mediated apoptosis will be discussed.

Key words: VDAC — SR — Ion channel — GAPDH — Mitochondrial porin

Introduction

Sarcoplasmic reticulum (SR) membrane acts as a subcellular Ca^{2+} store, regulating the contraction-relaxation cycle of muscles. The control of myoplasmic Ca^{2+} concentration is mediated by the Ca^{2+}-release channel/ryanodine receptor (RyR) [16, 52, 79] and by an ATP-powered Ca^{2+} pump [24, 41, 49]. SR membranes contain many proteins located in the membrane or in the lumen whose function is associated with the control of intracellular [Ca^{2+}]. The function and regulation of some of these proteins, however, are as yet unknown. Phosphorylation and de-phosphorylation of SR proteins (such as of calsequestrin and phospholamban) are included among the mechanisms for protein activity modulation [13, 48]. SR membranes contain an endogenous phosphorylation system that phosphorylates the luminally located proteins such as histidine-rich Ca^{2+}-binding protein (HCP) and the glycoprotein sarcalumenin [28, 56, 57, 82]. Phosphorylation of luminally located proteins requires the uptake of ATP into the SR lumen. The voltage-dependent anion channel (VDAC) in purified SR has been proposed as the protein that mediates ATP transport into the SR lumen [81].

Characterization of VDAC

VDAC, also referred to as the mitochondrial porin, is a 30–35 kDa protein that has been purified, cloned, sequenced and manipulated utilizing molecular biology techniques [6, 9, 14, 15, 20, 80]. Computer modeling of VDAC's primary amino-acid sequence has led to the development of models showing the transmembrane organization of VDAC, consisting of a single α-helix and 13 or 16 transbilayer β-strands [9, 14, 15]. These β-strands are connected by several peptide loops of different sizes on both sides of the membrane that serve as potential protein-interaction sites. When reconstituted into planar phospholipid membranes, VDAC forms a large (~ 3 nm) voltage-gated pore. VDAC channels are voltage-dependent and can exist in multiple conformational states with different selectivity and permeability [6, 15, 20, 80].

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At zero and low transmembrane potentials, VDAC can exist in a highly conductive open state but is converted into a low-conducting state at high potentials, with reduced conductivity and reversed selectivity [6, 15, 20, 80]. VDAC is not ion-specific, and it transports adenine nucleotides [69], Ca^{2+} [32] and other metabolites [38]. The molecular nature of the VDAC gating mechanism has yet to be resolved.

VDAC Presence in Extra-mitochondrial Membranes

Although VDAC was originally thought to be located exclusively in the outer mitochondrial membrane (OMM), immunohistochemistry at the light and electron microscopy levels, together with biochemical and electrophysiological methods, have revealed that VDAC is also localized to cell compartments other than mitochondria [12, 21, 34, 42–45, 51, 58, 64, 65, 73, 74, 81, 83, 88]. It has been found in the plasma membrane of transformed human B-lymphocytes [43], rat brain [21], *Torpedo* electric organ [74] and bovine astrocytic plasma membrane [34]. VDAC has been purified from the plasma membrane of intact cells by a procedure utilizing the membrane-impermeable labeling reagent NH-SS-biotin and streptavidin affinity chromatography [58]. Channels recorded from excised patches of plasma membranes of a rat astrocytic cell line exhibited the electrophysiological and pharmacological characteristics of VDAC [34]. A 36 kDa protein was co-purified with the GABA/benzodiazepine receptor and found to be a member of the VDAC family [51]. Endocytotic vesicles isolated from renal cortex endosomes [65] and caveolae and caveolae-like domains [58] were shown to contain VDAC. Additional support for plasmalemmal expression of VDAC came from the identification of a VDAC isoform (pI-VDAC) that contains a leader sequence for its trafficking to the plasma membrane [11], targeting that was prevented by anti-sense oligonucleotide directed against the specific leader sequence [3]. Moreover, electrophysiological studies demonstrated a connection between VDAC and plasma membrane maxi Cl^- channels activated by anti-estrogens [11]. In addition, association between GABAA receptor and VDAC as well as VDAC labeling by a neuroactive steroid analog, 6-azi-peregnanolone, were demonstrated in rat brain [18]. The presence of VDAC in the SR of amphibian [45] and of mammalian skeletal muscles [42, 44, 73, 81] has also been demonstrated. Recently, using immuno-electron microscopy and sub-cellular fractionation, the presence of VDAC in endoplasmic reticulum (ER) of rat cerebellum was demonstrated [83]. Immunogold labeling and EM analysis of the cerebellar molecular layer showed specific VDAC immunostaining of the ER that was highly enhanced in contact sites between mitochondria and associated ER (see Fig. 4). Thus, it is expected that most of the VDAC in the ER is located in ER associated with mitochondria and thus it would be mainly in the mitochondrial fraction and not in free ER. In addition, highly purified ER membranes contain VDAC, but not other mitochondrial proteins [83]. Moreover, quantitative analysis of the estimated amount of VDAC purified from SR is the same (*see* Fig. 1) or higher than that obtained from the same total amount of mitochondrial protein.

In spite of this accumulating evidence, VDAC localization in extra-mitochondrial membranes has been questioned by Yu et al. [94]. The authors expressed epitope-tagged VDAC isoforms in Cos7 cells and rat astrocytes, but found the corresponding gene products exclusively in fractions containing mitochondrial marker proteins and thus, they concluded that if VDACs are present at nonmitochondrial membranes in mammalian cells, these are unlikely to be the known products of HVDAC1 or VDAC2 genes. These results were challenged by biochemical [58] and expression studies showing that VDAC is co-localized with the plasma membrane lipid rafts, and that VDAC follows the secretory pathway on its way to plasmalemma of C1300 mouse neuroblastoma cells [3].

The exact function of extra-mitochondrial VDAC is as yet unknown. Plasmalemmal VDAC has been suggested to make up part of the outwardly rectifying depolarization-induced chloride (ORDIC) channel complex [64], while in astrocytes, the response of VDAC to hypo-osmotic solutions may suggest its involvement in volume regulation in brain tissue [21]. Results demonstrating that VDAC1, directly or indirectly, contributes to ATP release and cell volume regulation in murine cells were also reported [54]. The possible functions of VDAC in SR/ER membranes are presented below (section Possible Function of VDAC in Structural and Functional Coupling between Mitochondria and SR/ER).

Purification and Structural Characterization of VDAC in SR and Mitochondria

We have developed three different methods for purifying VDAC from different membranes, such as liver, brain and retinal mitochondria, skeletal muscle SR, synaptosomes isolated from brain and from *Torpedo* electric organ, and from mitochondria from yeast or from cell lines expressing recombinant or native VDAC. The purification methods include the use of spermine-agarose [73, 81], carboxy methyl cellulose [30, 31, 80] or reactive-red agarose [30, 31, 80] resins and are dependent on the detergent used (β-OG, LDAO, Triton X-100, NP-40) [80]. The purity of the VDAC obtained by these purification methods is over 98%. Regardless of the purification

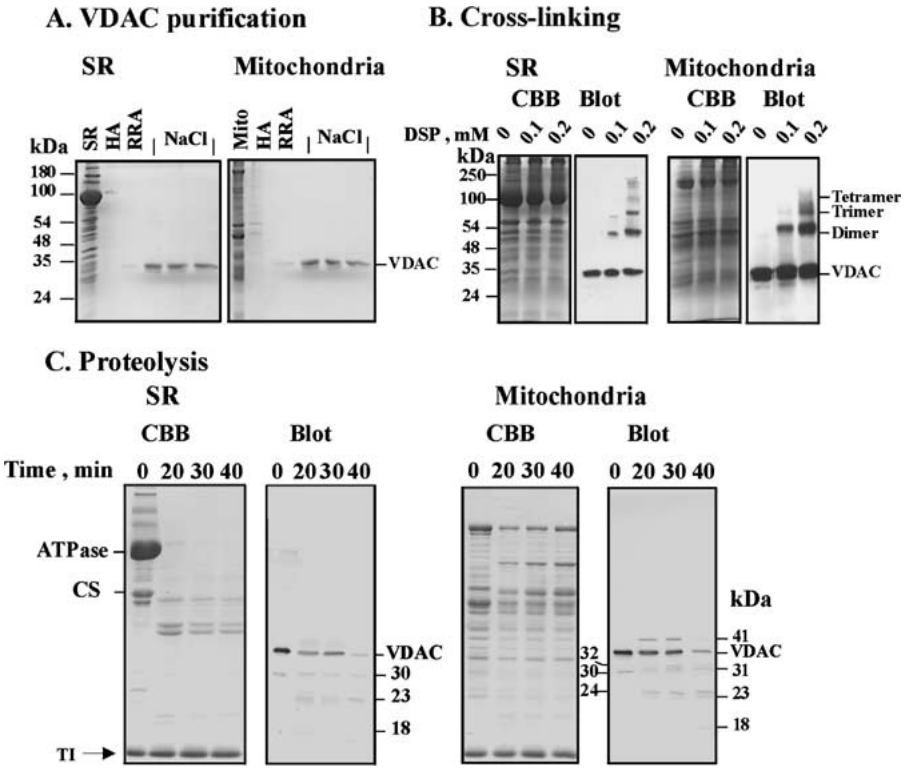

Fig. 1. (*A*) Purification of VDAC from SR membranes. SR (50 mg) was solubilized with Triton X-100 (3.0%, 2 mg protein/ml). Following centrifugation (44,000 × *g* for 30 min), the supernatant (Triton extract, Ext.) was applied to a hydroxyapatite column, from which VDAC was eluted with 10 mM Tris/HCl, pH 7.2 containing 3% Triton X-100. VDAC-containing fractions were collected, diluted 3-fold with 10 mM Tricine, pH 7.2 and applied to a reactive red-agarose column from which VDAC was eluted with 10 mM Tris/HCl, pH 7.2 containing 0.3% NP-40 and 0.3 M NaCl. Note that a similar VDAC purification profile was obtained for the two membranal preparations. (*B*) Cross-linking of VDAC in SR and mitochondria. Mitochondria and SR (1mg/ml) were incubated for 10 min at 30°C in 10 mM Tricine, pH 8.3, with the indicated concentration of the cross-linking reagent DSP. Sample buffer for electrophoresis without β-mercaptoethanol was added and the samples were subjected to SDS-PAGE (10% acrylamide), stained with Coomassie blue (CBB) or subjected to Western blot analysis using anti-VDAC antibodies (blot). (*C*) Chymotrypsin digestion of SR and mitochondrial VDAC. SR and mitochondria (5 mg/ml) were incubated with chymotrypsin (1:20, w/w) for the indicated time in a solution containing 0.5 M sucrose and 10 mM Tricine, pH 8.0. Proteolysis was stopped by addition of trypsin inhibitor (5-fold excess over chymotrypsin) and the samples were subjected to SDS-PAGE (10% acrylamide), stained with Coomassie blue (CBB) or subjected to Western blot analysis using anti-VDAC antibodies and alkaline phosphatase-conjugated secondary antibodies. *ATPase*, (Ca + Mg)ATPase; *CS*, calsequestrin; *TI*, trypsin inhibitor.

method, VDAC reconstituted into a planar lipid bilayer (PLB) shows the fingerprints of VDAC channel activity [30, 31, 80]. SR and mitochondrial VDAC show high similarity with respect to purification procedures, tryptic map, and oligomeric structure (Fig. 1). The similar yield of purified VDAC obtained from isolated skeletal muscle SR and from mitochondria argues against the VDAC being from a contamination of the SR with mitochondria. We have shown recently that in brain ER [83] or liver mitochondria [95], VDAC exists in a dynamic equilibrium between dimers and tetramers, as is also shown here (Fig. 1*B*) for VDAC in skeletal muscle SR.

Modulation of VDAC Channel Activity

Detergent-extracted VDAC reconstituted into a planar lipid bilayer (PLB), in its native form, never completely closes [6, 15, 20]. If this is an intrinsic property of VDAC, then the location of VDAC in the plasma membrane or SR is difficult to reconcile. However, the complete closure of VDAC, even at moderate applied voltages, has been demonstrated [5]. Moreover, VDAC closure can be induced by various inhibitors, such as ruthenium red (RuR), dicyclohexylcarbodiimide (DCCD) and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), regardless of its source—mitochondria or SR [31, 32, 73, 80, 81, Fig. 2 and Table 1]. In addition, it has been shown that the closure of mitochondrial VDAC can be enhanced by a soluble protein [39, 47], induced by hexokinase [1], and modulated by NADH [96] and glutamate [29, 30, 80]. Furthermore, many exogenous effectors, including polyamines [40] and polyanions [15] also modify channel activity. Thus, under physiological conditions, permeability of VDAC may be

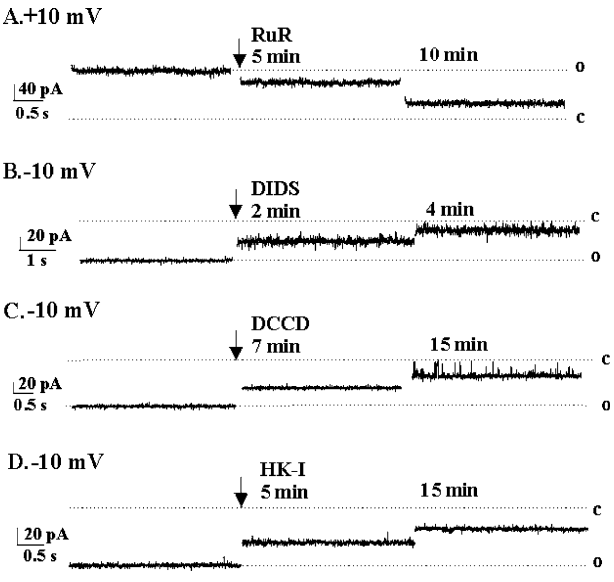

Fig. 2. Bilayer-reconstituted VDAC channel can be closed by different inhibitors. Purified SR VDAC was reconstituted into PLB. The effects of RuR (*A*), DIDS (*B*), DCCD (*C*) and HK-I (*D*) on the channel activity are shown. Currents through VDAC before and after the addition of the indicated inhibitor in response to a voltage step from 0 to +10 mV or 0 to −10 mV were recorded. The dashed lines indicate current levels at open (*o*) or closed (*c*) states. The experiments are representative of 3–4 similar experiments.

controlled by associated proteins as well as by endogenous effectors such as NADH and ATP.

VDAC Interacts with Cytosolic and Mitochondrial Proteins

Accumulating evidence points to a complex regulation of VDAC permeability involving regulation by associated proteins. Several proteins have been suggested to interact with VDAC; most of the proteins are localized to the mitochondria, or occur in association with the mitochondria. VDAC has been shown to have binding sites for several cytosolic enzymes, including hexokinase [8, 27, 53, 90], and for the mitochondrial intermembranal-space creatine kinase [8, 10, 90]. In addition, complexes between VDAC and other proteins, such as the microtubule-associated protein 2 (MAP2) [46], benzodiazepine receptor [51], adenine nucleotide translocator [90], the ORDIC channel [64], dynein light chain and the heat shock protein mtHSP70 [72] have been proposed.

VDAC has also been shown to be regulated by the Bcl-2 family of proteins via direct interaction, where Bax/Bak and Bcl-x_L, but not Bik or Bid, can bind directly to VDAC and modulate its activity [75–78]. However, controversial results have been reported. While some reports show that the anti-apoptotic Bcl-x(L) completely closes VDAC, in contrast to Bax [75–78], other reports suggest that Bcl-x_L promotes VDAC in the open configuration

Table 1. Modulation of VDAC channel activity by various reagents

Compound	SRVDAC	Mitochondrial VDAC
RuR	This work	Refs. 32, 80
Ru360	This work	Refs. 31,80
DIDS	Ref. 81	This work
DCCD	Refs. 73, 81	Ref. 73
HK-I	This work	Ref. 1
LaCl3	This work	Refs. 31, 32
Glutamate	This work	Refs. 29, 30, 80

The experiments were carried out as described in the indicated references

[89]. Furthermore, it has been reported recently that Bid, but not Bax, regulates VDAC channels [70]. Recently, modulation of VDAC channel activity by HK-I was demonstrated [1].

HK-I Interaction with VDAC

Cancer cells are characterized by a high rate of glycolysis, which serves as their primary energy-generating pathway [60]. The molecular basis of this high rate of glycolysis involves a number of genetic and biochemical events, including over-expression of mitochondrial-bound isoforms of hexokinase (HK-I and HK-II) [60]. HK-I and HK-II interact with mitochondrial VDAC via their hydrophobic 15-amino-acid sequence at the N-terminal region [92]. Recently [1], we demonstrated that HK-I binds directly to a bilayer-reconstituted VDAC and induces closure of its channel (*see* also Fig. 2) that was reversed by glucose-6-phosphate. Furthermore, HK-I prevents the opening of the mitochondrial permeability transition (PTP) and cytochrome c release, thereby blocking the gateway to apoptosis. Indeed, overexpression of HK-I in the tumor-derived cell line U-973 or vascular smooth muscle cells suppresses staurosporine-induced apoptotic cell death [1]. A decrease in apoptotic cell death and increase in cell proliferation due to HK-II expression in NIH-373 [26] and Rat 1a cell lines have also been reported [33]. Moreover, HK-II has been shown to interfere with the ability of the pro-apoptotic Bax protein to bind to mitochondria and induce release of cytochrome c [59]. These results suggest that HK-I, by interacting with VDAC, prevents key events in mitochondria-mediated apoptosis.

Protein(s) Specifically Associated with the SR VDAC

Specific interactions between VDAC and associated protein(s) could be of essential importance in the biological function of VDAC in SR. To identify VDAC-associated proteins, affinity chromatography,

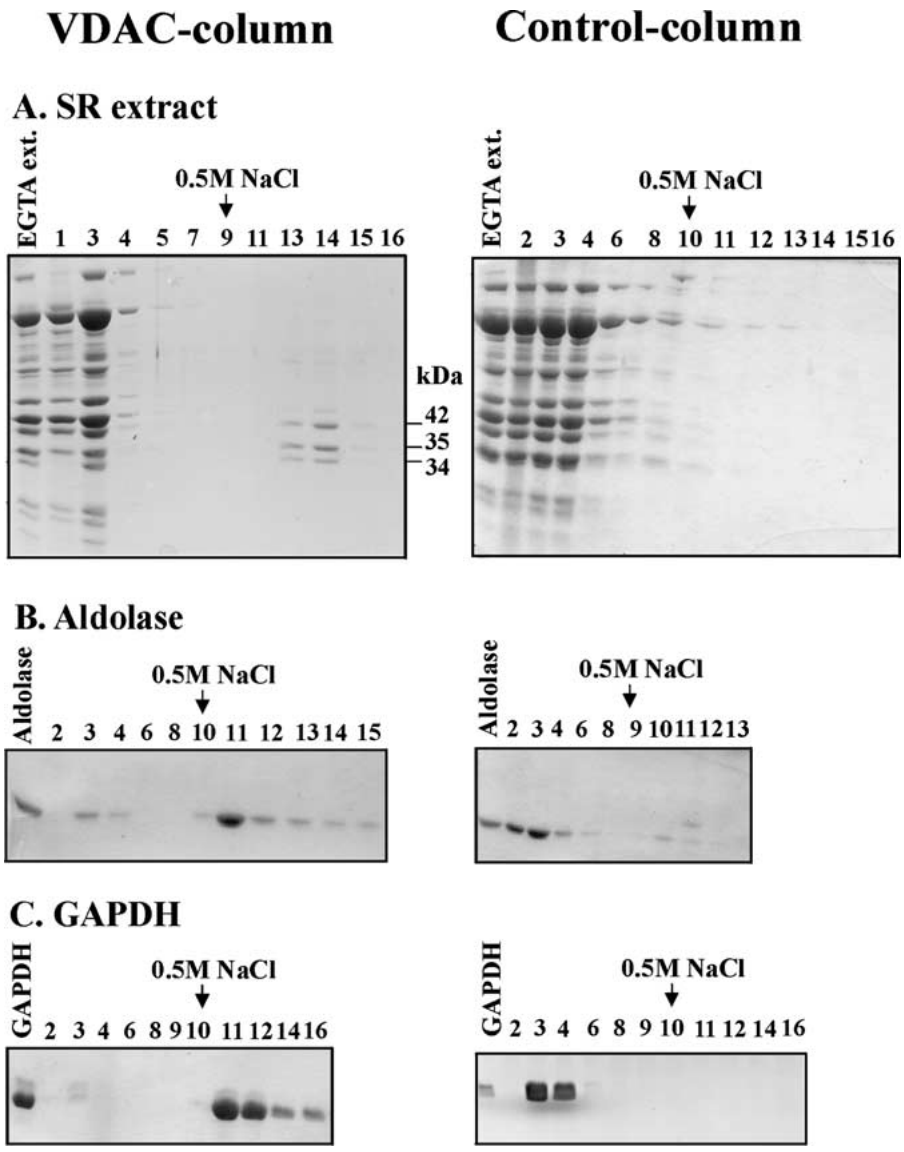

Fig. 3. Purification of VDAC-associated proteins on VDAC-Sepharose column. A VDAC-Sepharose column was coupled to CNBr-activated Sepharose 4B as suggested by Pharmacia. Briefly, purified VDAC (5 mg) was first dialyzed against 0.1 M $NaHCO_3$, pH 8.5 containing 0.5 M NaCl and 0.3% NP-40 and then added to 1 ml of CNBr-activated Sepharose 4B (pre-washed according to Pharmacia). The mixture was incubated overnight at 4°C with gentle stirring. The beads were washed twice with 10 ml of distilled water and the remaining active CNBr groups on the beads were blocked by incubation with 0.1 M ethanolamine, pH 8.5 for 2 h. The beads were then washed with and stored in 0.5 M NaCl, 0.1% NaN_3 and 20 mM Tris pH 7.2 at 4 °C until use. Control columns were prepared by using either BSA instead of VDAC or no protein was added. (*A*) EGTA extract of SR at pH 8.5 was obtained by incubation of SR (5 mg/ml) for 30 min at 4 °C in a solution of 10 mM Tris/HCl, pH 8.5, and 1 mM EGTA. After centrifugation, the pH of the extract was changed to 7.2 and applied to the VDAC-column or control column (1 ml syringe). The columns were washed with 10 mM Tris/HCl, pH 7.2, followed by washing with the same buffer containing 0.5 M NaCl. Aldolase (0.4 mg) (*B*) and GAPDH (0.1 mg/ml) (*C*) were applied to VDAC- or control-column and the columns were washed with 10 mM Tris/HCl, pH 7.2 followed by washing with the same buffer containing 0.5 M NaCl. Selected fractions were subjected to SDS-PAGE.

using VDAC-Sepharose column, of SR membranes extracted with EGTA at alkaline pH or 1 M NaCl was used. Among the extracted SR proteins, three proteins with apparent molecular masses of 40, 37 and 34 kDa were adsorbed to the VDAC-Sepharose column, but not to control Sepharose column, and were eluted from it by 0.5 M NaCl (Fig. 3*A*). By partial amino-acid sequencing and enzymatic activity assays, these proteins were identified as aldolase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The binding of purified GAPDH and aldolase to VDAC-Sepharose column, but not to the control column, and their elution by 0.5 M NaCl are shown in Fig. 3*B* and 3*C*.

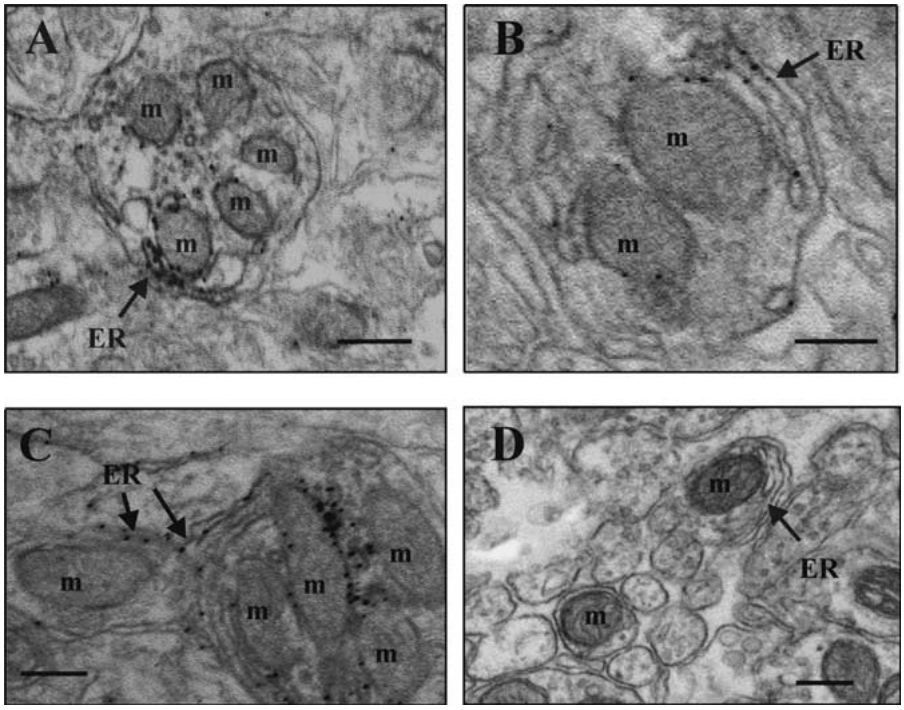

Fig. 4. VDAC in mitochondria and associated ER. Immunostaining was carried out as described previously [77]. (*A*) to (*C*) Micrographs showing that immunostaining is localized not only to outer mitochondrial membrane, but also to ER membrane (arrows), especially to portions that contact mitochondria. (*D*) Low background staining was observed when the primary antibody was omitted. Scale bars are 250 nm.

The glycolytic enzymes have a net positive charge [55]. Furthermore, aldolase and GAPDH are known to carry at least two clusters of net positive charge known as hot spots [91]. VDAC is a basic protein; therefore, its interaction with other proteins is not due to the net charge, but rather to more specific properties.

Glycogenolytic enzymes, known to be associated with the SR, and enzymes of the glycolytic pathway have also been found in skeletal muscle SR [37] and cardiac SR [62]. The association of the entire chain of glycolytic enzymes with both cardiac and skeletal muscle SR has been demonstrated [93].

Under physiological conditions, the interaction of the associated protein with VDAC could be regulated by VDAC binding of metabolites (NADPH, ATP) or inorganic ions such as Ca^{2+} or Mg^{2+}, by changes in local pH, or by phosphorylation of the associated protein. Phosphorylation of GAPDH by CaM Kinase $II\beta_M$ has recently been demonstrated [85]. CaM Kinase $II\beta_M$ directly phosphorylates GAPDH and this leads to a 3-to 4-fold increase in its activity in a Ca^{2+}/calmodulin-dependent manner. This may suggest that GAPDH activity is modulated in response to calcium signaling.

Recent evidence indicates new, intriguing roles for the glycolytic protein GAPDH [7, 25, 86, 87]. These include membranal, cytoplasmic and nuclear functions in endocytosis, mRNA regulation and tRNA export, DNA replication and DNA repair [86, 87]. Moreover, it has been considered as a pro-apoptotic protein [7, 25]. In this respect, the interaction of GAPDH with VDAC, a protein controlling mitochondria-mediated apoptosis, is of utmost importance.

Possible Function of VDAC in Structural and Functional Coupling between Mitochondria and SR/ER

Mitochondria have been emerging as key players in cellular signaling, particularly in intracellular calcium signaling [reviewed in 2, 36, 61, 66], where interplay between mitochondrial function and cellular Ca^{2+} signaling, including SR/ER, exists [4, 23, 68, 84]. Indeed, subdomains of the ER have been visualized in close association with mitochondria [50, 83]. VDAC immunostaining was detected in ER membranes associated with mitochondria (Fig. 4), but not in ER membranes far from mitochondria. Analysis of an area containing 19 ER cisternae associated with mitochondria were less than 20 nm apart (average distance was 13.3 ± 1.0 nm, $n = 15$) and 13 of those junctions were strongly stained for VDAC. This labeling suggests that VDAC is preferentially distributed adjacent to the mitochondria-associated domains of the ER.

Structural interactions between mitochondria and between ER and mitochondria have been demonstrated with electron microscopic tomographic reconstruction of mitochondria in situ. Using this technique, it was shown that mitochondria may occur in clusters with stacks of endoplasmic reticulum [50, 83]. Functional interaction between mitochondria and ER was demonstrated by the close coupling between the intracellular Ca^{2+}-release channels ryanodine receptor and IP_3 receptor in the ER or SR and subsequent mitochondrial Ca^{2+} uptake [4, 23, 67, 68, 84]. Such cross-talk is important since, in addition to ATP synthesis, mitochondria are critical for modu-

lation of the cell's redox status, osmotic regulation, pH control, cytosolic calcium homeostasis and signaling [71]. However, the structures that ensure efficient transmission of the Ca^{2+} signal at the ER/SR-mitochondria interface have not been identified. IP3R and RyR are highly concentrated at the ER/SR, and form clusters in the membranes aligned with the mitochondria [36]. Ca^{2+} permeability of the outer mitochondrial membrane (OMM) is mediated by VDAC, suggesting that VDAC plays a role in the rapid transfer of the Ca^{2+} released from ER through RyR and IP_3R to the OMM, allowing its uptake by the uniporter [17, 36, 63].

Recently, we presented evidence that in the central nervous system, the junctions between mitochondria and between mitochondria and ER are enriched with VDAC. VDAC is also present in ER membranes that come into physical contact with neighboring mitochondria [83 and Fig. 4]. Using cross-linking experiments, we showed that VDAC exists as dimers to tetramers [83, 95 and Fig. 1*B*]. Accordingly, we suggest that VDAC localized at the junction between mitochondria and between ER and mitochondria, transporting Ca^{2+}, ATP and other metabolites, in a monomeric or oligomeric (VDAC-VDAC) form, may play an important role in the structural and functional coupling between mitochondria and between ER and mitochondria. As such, VDAC acts as a multifunctional channel controlling the mitochondrially-mediated energy and Ca^{2+} homeostasis of the cell, as well as the communication between mitochondria and associated organelles.

Possible Functions of VDAC in SR/ER and Mitochondria

The presence of VDAC in the ER/SR membranes [42–45, 73, 83] suggests a role for the protein in these organelles. This role is most likely related to the properties of VDAC as a channel with its large pore size, high conductance, and permeability to ions, nucleotides and Ca^{2+}. Mitochondrial VDAC has been shown to mediate the transport of nucleotides across the mitochondrial outer membrane [69] and similar activity has been demonstrated for the SR-VDAC [81]. We have shown that SR vesicles catalyze the transport of ATP, which is used to phosphorylate the luminal proteins sarcalumenin and HCP, and furthermore, this transport is inhibited by DCCD and DIDS, which inhibit the phosphorylation of the luminal SR proteins as well as VDAC channel activity [73, 81].

In the SR, the Ca^{2+}-storage compartment, the suggested functions for VDAC are: ATP transport to allow phosphorylation of luminal SR proteins, exchange of metabolites between the cytoplasm and the SR lumen, and minimization of osmotic and potential changes during uptake and release of Ca^{2+} by exerting high permeability of the SR to monovalent cations and anions [81]. If, as has been suggested [4, 23, 61, 67, 68, 83, 84], Ca^{2+} transport between SR/ER and mitochondria exists, then Ca^{2+} binding and translocation by VDAC provide a possible regulatory mechanism for the interplay between mitochondrial and ER Ca^{2+} signaling.

The presence of divalent metal binding sites in VDAC is supported by different experiments. As in mitochondrial VDAC [32, 80], in SR, the organometallic hexavalent dye RuR and ruthenium amine binuclear complex, Ru360, known to specifically interact with Ca^{2+}-binding proteins [80], induce VDAC channel closure in a time-dependent manner, and stabilize the channel in a completely closed state (Fig. 2). Thus, VDAC, as Ca^{2+} transporter at the outer mitochondrial membrane, may act as the "gate keeper" for Ca^{2+} entry into and release from mitochondria [32]. Hence, VDAC would control the production of energy, including the control of Ca^{2+}-dependent pyruvate dehydrogenase and iso-citrate dehydrogenase activities in the mitochondria [19, 22], and also various Ca^{2+}-dependent reactions in the cytosol via mediation of Ca^{2+} efflux from the mitochondria to the cytosol [35]. Based on these findings, VDAC has a very important role in Ca^{2+} homeostasis and cell survival.

To conclude, VDAC, in the SR as in the mitochondria, provides a pathway for transport of Ca^{2+}, nucleotides and other metabolites across the membrane. VDAC, participating in supramolecular complexes, is involved in apoptosis and intracellular communication, including calcium signal delivery between the ER and mitochondria.

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