[Frontiers in Bioscience E5, 1011-1016, June 1, 2013]

Evidence for a relation between plasma membrane coenzyme Q and autism

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1. ABSTRACT

Voltage Dependent Anion Channel (VDAC) in the cell membrane transports important molecules and ions across the cell membrane. It was recently shown that VDAC also acts as a trans membrane NADH dehydrogenase. A recent study showed that autistic children have increased antibodies to VDAC proteins and such a binding inhibits both the transport and dehydrogenase activities of VDAC. The derived function of VDAC, therefore, might underlie the development of autism. It has also recently been shown that the dehydrogenase in erythrocyte membranes requires coenzyme Q. Since the plasma membrane oxidase is not in erythrocyte membranes, the coenzyme Q requirement must be for VDAC. This is consistent with sensitivity of the dehydrogenase to SH inhibitors. This is a novel site for coenzyme Q function but it has an analogy with the Q requirement for the mitochondrial uncoupler protein and the permeability transition pore.

2. INTRODUCTION

Voltage Dependent Anion Channel (VDAC) is one of two NADH oxidoreductases found in the plasma membrane of most cells. It only is known to use ferricyanide or coenzyme Q as electron acceptors. The natural acceptor may be ascorbate free radical (AFR). It only has two cysteine SH as known electron carriers (1, 2). The SH groups are on the inside of the channel (2). The choice of ferricyanide as electron acceptor was fortunate and was based on earlier studies of NADH dehydrogenase in mitochondria (3) and the impermeability of ferricyanide (4). The other enzyme system is a multicomponent NADH oxidase (5, 6). The oxidase consists of three components: A NADH coenzyme Q reductase on the inside of the plasma membrane, coenzyme Q in the membrane, (7) and a copper containing coenzyme Q oxidase on the outer surface of the cell. The oxidase is found in all cells tested except erythrocytes. Most cells tested have both oxidase and the VDAC dehydrogenase. Both the oxidase and

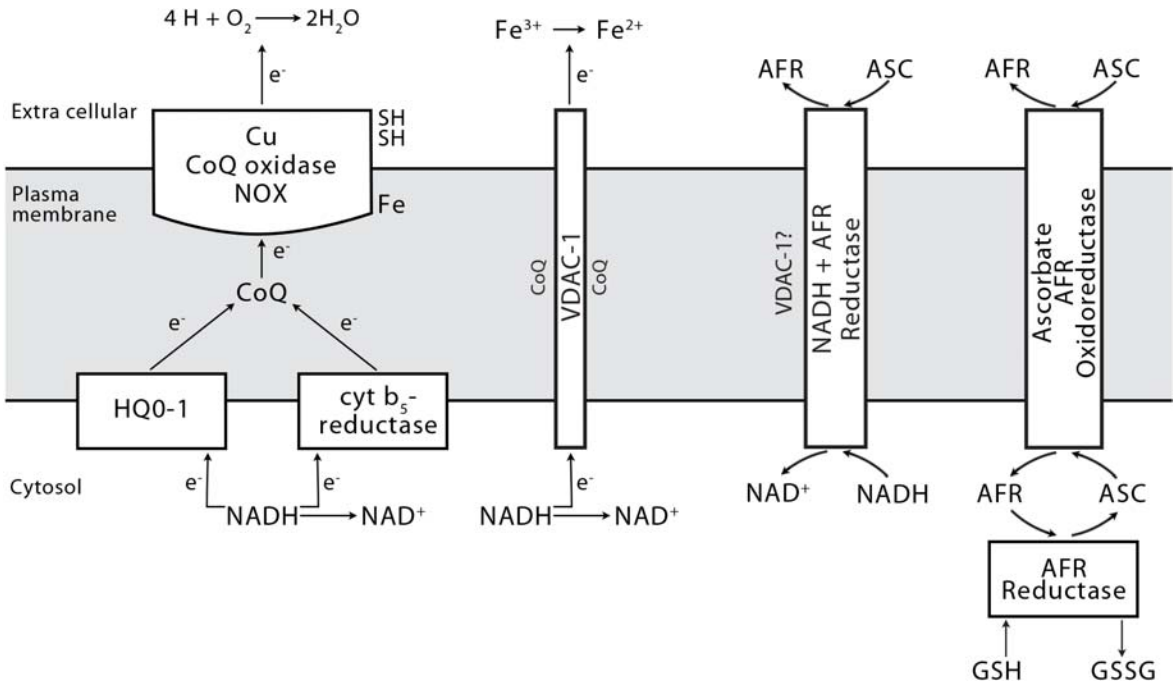

Figure 1. Diagram of different systems for trans plasma membrane electron transport. Left to right: NADH -Oxygen oxidoreductase (NADH Oxidase.) 2.NADH Ferricyanide reductase,,(Voltage Dependent Anion Channel ,VDAC) 3. NADH Ascorbiate free radical reductase.(31) 4. Ascorbate Ascorbic free radical oxidoreductase. Other possibilities are shown in Ref (31). The parts of the oxidase Ref (7, 32)

dehydrogenase move electrons from cytosolic NADH to acceptors outside the cell to increase cytosolic NAD. Increased NAD can have physiological consequences, such as stimulating glycoliosis and activation of sirtuin to activate transcription factors (Figure 1).

Another group of trans plasma membrane electron carriers are based on ascorbate cycling. A possible interaction between the ascorbate oxidation and the ferricyanide reductase could be semidehydroascorbate generated by free radicle oxidation acting as an electron acceptor for VDAC on the outside of the cell (Figure 1).

3. EXPERIMENTAL BACKGROUND

Because of the exposed SH groups, the NADH ferricyanide reductase of VDAC is extremely sensitive to the SH reagent parachloro mercury benzoate (PCMB) with 100% inhibition of the reductase at 1 μM (8, 9). The erythrocyte plasma membrane is the only one with no oxidase so the NADH dehydrogenase is exclusively based on VDAC. This conclusion is supported by100% inhibition at 1 μM PCMB in the erythrocyte membrane. With membranes from other cells such as rat liver, Ehrlich ascites, or HeLa there is only partial inhibition at 100 μM indicating the presence of the less sensitive oxidase as a contributor to NADH ferricyanide reduction (Table 1).

Extraction with hexane to remove coenzyme Q and adding back Q is another test for a requirement for coenzyme Q. As shown in Figure 2, extraction of isolated erythrocyte membrane decreases NADH ferricyanide reductase 80%. Addition of coenzyme Q restored 75% of the lost activity supporting the requirement for coenzyme Q.

Further evidence for coenzyme Q function in VDAC is inhibition of ferricyanide reduction by coenzyme Q analogs such as piericidin, ethoxy coenzyme Q, capsaicin, or chloroquine (6, 10) (Table 2). The inhibition by these analogs is reversed by coenzyme Q. This analog inhibition reversed by coenzyme Q indicates specific binding sites for Q action.

Further evidence for selective coenzyme Q reduction by VDAC is indicated by demonstration of NADH coenzyme Q reductase. Coenzyme Q reduction by NADH has been demonstrated with intact membrane acting as an NADH coenzyme Q reductase. This activity would be part of the overall NADH ferricyanide reductase with coenzyme Q acting as an intermediate carrier between NADH and ferricyanide. This is easy to demonstrate, because erythrocyte membranes do not have a functional oxidase so electrons stop at coenzyme Q. This reaction is easy to show in mitochondria because there are good inhibitors for cytochrome oxidase, but difficult with most plasma membranes because there is no good inhibitor for ENOX, so the QH2 is rapidly reduced. The assay is similar to one used in mitochondria (3, 11).

4. DISCUSSON

Gvozdjakova *et al* have shown that with long-term supplementation at high levels of coenzyme Q, the symptoms of autism decrease (12). Their study supports a

Table 1. Inhibition of plasma membrane NADH ferricyanide reductase in isolated plasma membrane by PCMB

Membrane	p-dichloromercui benzoate (PCMB) concentration	% Inhibition
Human erythrocyte	1 µM	100%
Pig erythrocyte	1 µM	95%
HeLa	100 µM	55%
Ehrlich ascites	100 µM	89%
Rat liver	5 µM	60%

Table 2. Analog inhibition of erythrocyte NADH ferricyanide reductase

Addition	NADH Ferricyanide reductase (nmol/min/mg protein
None	308 ± 43
EthoxycoQ 30 µg/ml	30
EthoxycoQ + CoQ 10 µM	232 ± 19
DichloroQ 25 µg/ml	113 ± 37
DClcoQ + CoQ 10 µM	215 ± 26

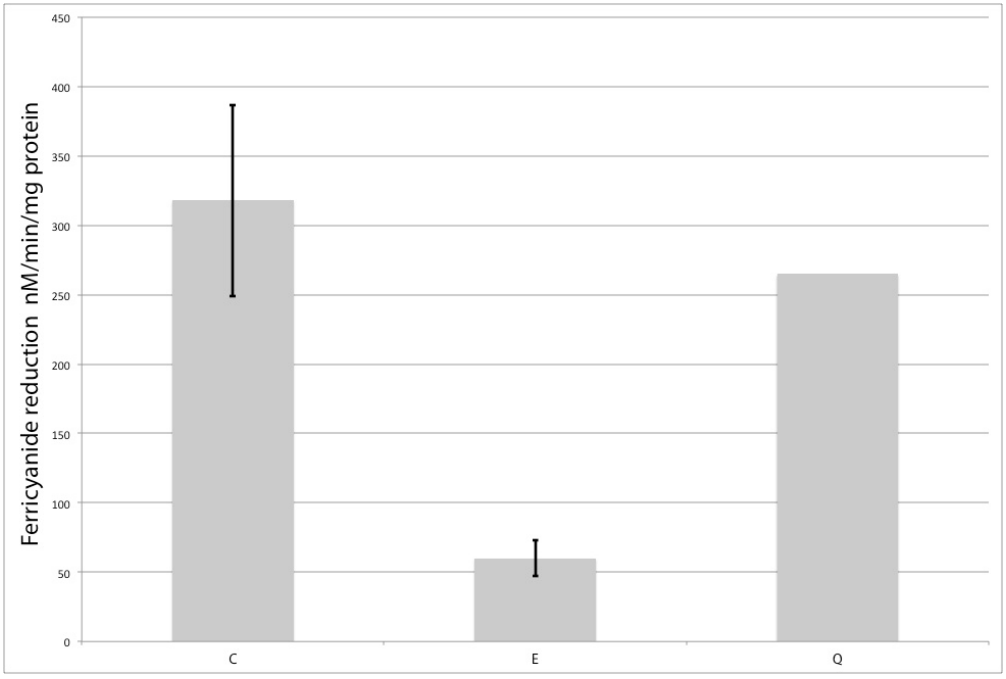

Figure 2. Hexane extraction of erythrocyte membrane. Ferricyanide reduction reported as nM/min/mg protein. C = untreated control (318 ± 69), E = extracted (60 ± 13), Q = extracted + CoQ 10 µM (265). Data with permission from Ref (7)

role for coenzyme Q as a controlling factor and indicates channel control in VDAC as a contributing factor in autism. Channel control is necessary to prevent loss of cell function and induction of apoptosis. Hexokinase and NADH are known controls. There is precedence for coenzyme Q binding to individual channel proteins. An example is the uncoupler UCP, the mitochondrial uncoupler protein (13). The inhibition by coenzyme Q analogs such as piericidin with aromatic 2,3 dimethoxy groups calls for precaution since many aromatic 2,3 dimethoxy lipophylic compounds tend to inhibit coenzyme Q function. Dissociation of Coenzyme Q from its binding site may also increase with low levels of coenzyme Q in blood. Low levels of Q may also decrease uptake in the brain has been questioned before, but with high levels Gvozdjakova *et al* have shown uptake in mouse brain (14).

Other factors that either increase or decrease autism also have parallel effects on VDAC. Compounds that stimulate plasma membrane oxidase activity also decrease autistic symptoms. Ascorbate has been used to treat autism increases plasma membrane oxidase activity and the ascorbate free radical can act as a natural electron acceptor for VDAC. The positive effects of hyperbaric oxygen can also be based on oxidase stimulation. Slowing the electron transport can be associated with increasing autism. The extreme sensitivity to mercurials in inhibition of VDAC is the basis of the wide concern that mercury can cause autism. Similar concerns about lead poisoning have a basis in inhibition of VDAC. Coenzym Q in human erythrocyte membranes ranges from 6 to 13 pmol/mg protein (15). The inhibition of NADH ferricyanide reductase in plasma membrane by low levels of zinc may provide another specific inhibitor tor VDAC dehydrogenase activity. Zinc inhibits plasma membrane NADH ferricyanide reductase 67% at 2 µM, whereas 1 mM Mg++ stimulates 14 % and 1 mM Na+ has no effect (16). Since VDAC is inhibited at low concentration of zinc, there may a connection to excess environmental zinc. Triiodothyronine activates the plasma membrane oxidase

and is low in autism, which brings up the question of whether increased activity of oxidase contributes to the effect of the dehydrogenase on autism. Combined action may be necessary for maximum improvement (17). Glutathione is decreased in autism so the effect of GSH or GSSG on VDAC activity should be tested for stimulation of the dehydrogenase (18). On the negative side is inhibition of plasma membrane ferricyanide reductase by the cytokines TNFalpha and IL 2 which are increased in autism (19). The net effect of agents that inhibit VDAC electron transport is to increase the chances of developing symptoms of autism. What is not clear is the relation of the plasma membrane NADH oxidase, if any, to the effect of NADH oxidation by VDAC. If oxidation of cytosolic NADH is the controlling factor then the enzymes oxidizing NADH from the same source should have additive effects. An indication that the oxidase can be involved in control of autism may be seen in the lower level of transferrin in serum of autistic children (20) since diferrictransferrin stimulates the oxidase. VDAC can also relate to apoptosis but the relation varies. For example in neurons ferricyanide reduction is increased during apoptosis whereas neural stem cells have no VDAC ferricyanide reductase. If trans membrane redox function is necessary for growth the stem cells may use the oxidase or they require ascorbate supplement (21). Transferrin is one of the best stimulators of the plasma membrane oxidase so a lower level of transferrin could lead to less oxidation of NADH (20, 22).

4.1 Ascorbic acid

A trans plasma membrane electron transport based on ascorbic has been developed by May (23) and by Lawens group (24, 25). They have proposed different mechanisms for the transfer. One proposal is transfer of electrons from cytosolic NADH to external ascorbate free radical as electron accepter. An alternative to that would be to have ascorbate inside the cell act as electron donor instead of NADH. Entry of ascorbate or AFR would use the glucose transporter to get into the cell. Inside the cell AFR would be reduced by glutathione. This would allow glutathione to keep the ascorbate reduced inside the cell. This would be a membrane system producing AFR instead of NAD+. We do not know if coenzyme Q is involved in the ascorbate systems.

Lane *et al.* found in preloaded cells that oxidation of ascorbate could account for 90% of external ferricyanide reduction (25). The oxidation of a pool of substrate could account for the initial rapid rate of ferricyanide reduction observed when cells reduce ferricyanide. Without preloading with ascorbate the initial rate is usually only two times the slow rate, which would be the metabolic renewal for NADH.

The question is why would the plasma membrane oxidase contribute to control of autism. Since VDAC, which constitutes a significant part of the trans membrane electron transport, is a channel for transport of needed components, the electron flow may be a part of channel transport control and failure of transport could affect mental function. The high level of ascorbate in brain would support the described redox systems. As discussed by Chernyak in his review of the Mitochondrial Permeability Transition Pore (MTP), which is a porin similar to VDAC, the electron transport would control the redox state of the thiols in the channel, which would control transport (26). This control could be coordinated with the control of VDAC by blocking with hexokinase or separate from the blocking agent.

In other words does production of NAD+ serve any purpose beside acetylation of sirtuin to activate transcription factors?. The availability of two different systems may allow control of different functions. As with other plasma membranes VDAC1 is in the human erythrocyte plasma membrane (27). If VDAC is similar to MTP, opening and closing will be regulated by ionic environment and the redox state of NAD/NADH and glutathione and an increase in NAD under certain ionic environments would lead to changes in gating potential and pore opening (25).

4.2 *E. coli* mutants: Ferricyanide reduction

The effect of introduction of *E. coli* mutants deficient in coenzyme Q provided a different way of determining the coenzyme Q requirement for reduction by whole cells. Since *E. coli* has porin in the plasma membrane it could be expected to reduce ferricyanide. The problem was interference by electrons from the primary energy producing system. To avoid this a mutant of the primary ndh NADHdehydrogenase was tested for ferricyanide reduction and showed no effect. When a mutant without aminolevulinic acid to eliminate heme synthesis and prevent action by the main energy coupled system was tested, the ferricyanide reduction increased, so the system involved is not the main energy conversion one. Since it is located in a channel the most likely function would be to control transport through the channel.

This was consistent with a porin ompF electron transport. With this background coenzyme Q deficient mutants showed decreased ferricyanide reduction. Activity was restored with added coQ 8 which is native to *E. coli* (Table 3). A vitamin K mutant showed no loss in activity. The net result is a redox system dependent on coenzyme Q.

Evidence for a non-heme iron site available on the outside of the ccl 39 cells is based on impermeable chelator inhibition of ferricyanide reduction.and restoration by added iron (Tables 4-5). Since this iron site controls DNA synthesis it may indicate an important function for the porin electron transport (28).

Three *E. coli* mutants deficient in coenzyme Q were tested for ferricyanide reduction. All had much slower reduction, which could be stimulated by added Q8 or Q2 indicating a requirement for coenzyme Q in the porin electron transport (Table 3).

4.3 Favorable effects on autism symptoms

Consistent with the effects on autism the site of action of coenzyme Q function is at the porin, which has been identified by the antibodies. Both ferricyanide and diferrictransferrin stimulate the growth of serum deficien

Table 3. Ferricyanide reduction by *E. coli* mutants

Deficiency	Rate of Ferricyanide Reduction nmol/min×10^{-7} cells
None	0.15
Hemes	0.22
Coenzyme Q Ubi AB-	0.04
Coenzyme Q Ubi F-	0.05
Iron Fep-	0.07
+ 0.04 mMol CoQ Ubi F-	0.15

Activity measured at midlog stage (33)

Table 4. Inhibiton of ferricyanide reduction by extraction of iron by chelator and restoration with added iron CCL 39 cells

Treatment	Rate of Ferricyanide Reduction n mol/min×10^{6} cells
Control	1.92
Chelator extraction	0.74
Treated + Iron	1.97

Data from Ref, (28)

Table 5. CoQ analog inhibition of ferricyanide reduction by HeLa cells treated with BPS 24 hr

Inhibitor	Ferricyanide reduction nmol/ min/ gww -1
None	750
None +coQ 10 micro mol	600
Piericiidin 10-7 M	400
Piericidin 10-7 M + co Q 10 mjcro molar	780
Mono ethoxy coenzyme Q 90micro M	520
Mono ethoxyconzyme Q + co Q 10 micro M	800

Unpublished

transformed cells so the system involved could be either the oxidase or the dehydrogenase (29).

A study of the effect of ubiquinol on autistic children shows improvement in all 8 categories tested, which supports the requirement for coenzyme Q at a site in VDAC which responds to the antibody inhibition (12). The redox state of the electron carriers in VDAC or other porins is indicated to control apoptosis and transport function. (30).

5. ACKNOWLEDGEMENTS

We thank Mr. Eric J. Teske for technical assistance in preparation of the manuscript.

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Abbrevations: AFR: ascorbate free radical, CoQ: Coenzyme Q, ECTO-NOX external NADH Oxidase, QH2: ubiquinol, GSSG: oxidized glutathione, GSH: reduced glutathione, VDAC:Voltage Dependent Anion Channel, UCP: Uncoupler Protein, Ndh: NADH Dehydrogenase, OMP: Outer membrane porin

Key Words: Coenzyme Q, autism, NADH, Sirtuin, ferricyanide reductase, ascorbate free radical, VDAC, cell membrane, Review

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