Is huntingtin a modulator of VDAC?
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Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder caused by an abnormal increase in amount of CAG codon in exon 1 of the gene encoding the protein huntingtin (Htt). Studies concerning HD patients as well as studies using HD models including transgenic animals, cell cultures and the yeast Saccharomyces cerevisiae indicate that profound mitochondrial impairment is an early and important event in HD pathogenesis. It is also evident from some of these studies that the severity of mitochondrial abnormalities correlates with increasing number of glutamine repeats in mutated form of huntingtin (mHtt), suggesting mitochondria as a major target of mHtt. However, till now the mechanisms of mHtt effect as well as that of Htt on mitochondria have not been clearly defined. Interestingly, our preliminary pulldown assay with purified and GST tagged Htt and mHtt constructs obtained from M.J. Monteiro and S. cerevisiae mitochondria suggests that VDAC (voltage-dependent anion-selective channel) interacts both with Htt and mHtt. Accordingly, the proposed “mitochondrial targets” of mHtt include processes that are known to be affected by VDAC; i.e. the respiratory chain, transcriptional regulation and protein import, calcium balance, oxidative stress and apoptosis, or might be affected due to interaction between VDAC and the involved proteins; i.e. mitochondrial trafficking and fusion/fission. Moreover, our studies point at VDAC involvement in cytoprotection including neuroprotection. Thus, studies concerning direct interaction between VDAC and Htt/mHtt appear a logical step in studies of HD etiology and their results could be important for the development including neuroprotection. Thus, studies concerning direct interaction between VDAC and Htt/mHtt appear a logical step in studies of HD etiology and their results could be important for the development including neuroprotection. Thus, studies concerning direct interaction between VDAC and Htt/mHtt appear a logical step in studies of HD etiology and their results could be important for the development of new therapeutic strategies concerning HD.

Since experimental approaches have shown that N terminus fragments of mHtt recapitulate several aspects of the full length mutant protein’s toxicity we applied only the first exon of Htt and mHtt encoding gene. First we measured the effect of Htt on reconstituted VDAC. We applied VDAC isoform mix isolated from human neuroblastoma cells and the most abundant isoform of human VDAC isoforms, i.e. VDAC1 expressed and isolated from S. cerevisiae cells. We observed a strong effect of Htt on the channel conductance, particularly in the case of purified VDAC1. Accordingly, our pulldown experiments indicate direct interaction between Htt and human VDAC1. Moreover, Htt did not handicap growth of S. cerevisiae cells expressing human isoforms of VDAC.

Mitochondria from the embryos of brine shrimp (Artemia franciscana) do not undergo Ca2+-induced permeability transition in the presence of a profound Ca2+ uptake capacity. Furthermore, this crustacean is the only organism known to exhibit bongkrekate-sensitive mitochondrial adenine nucleotide exchange, prompting the conjecture that refractoriness to bongkrekate and the absence of Ca2+-induced permeability transition are somehow related phenomena. Here we report that mitochondria isolated from two other crustaceans, brown shrimp (Crangon crangon) and common praw (Palaeomon serratus) exhibited bongkrekate-sensitive mitochondrial adenine nucleotide transport, but lacked a Ca2+-induced permeability transition. Ca2+ uptake capacity was robust in the absence of adenine nucleotides in both crustaceans, unaffected by either bongkrekate or cyclosporin A. Transmission electron microscopy images of Ca2+-loaded mitochondria showed needle-like formations of electron-dense material strikingly similar to those observed in mitochondria from the hepatopancreas of blue crab (Callinectes sapidus) and the embryos of A. franciscana. Alignment analysis of the partial coding sequences of the adenine nucleotide translocase (ANT) expressed in C. crangon and P. serratus versus the complete sequence expressed in A. franciscana reappraised the possibility of the 208–214 amino acid region for conferring sensitivity to bongkrekate. However, our findings suggest that the ability to undergo Ca2+-induced mitochondrial permeability transition and the sensitivity of adenine nucleotide translocase to bongkrekate are not necessarily related phenomena.

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Mitochondrial function in human endothelial EA.hy926 cells
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The endothelium is not considered to be a major energy-requiring organ, but nevertheless endothelial cells have an intensive mitochondrial network. Healthy endothelium is essential for homeostasis of cardiovascular system, while endothelial dysfunction leads to cardiovascular diseases including atherosclerosis, diabetes and heart failure. Endothelial dysfunction is tightly linked to the overproduction of reactive oxygen species, development of oxidant stress and inflammatory response of endothelium. Mitochondria of the vascular endothelium seem to be an important player in these processes. It is considered that, in contrast to numerous cell types, synthesis of ATP in endothelium occurs mainly via a glycolytic pathway and endothelium seems to be relatively independent of the mitochondrial energy supply. In the present work, we study mitochondrial function in the human umbilical vein endothelial cultured cells (line EA.hy926) by measuring cell oxygen consumption in media with various energy fuels, i.e., with different levels of glucose, pyruvate, glutamine or fatty acids. The highest oxygen consumption rate was observed with pyruvate alone or glutamine and the lowest with 25 mM glucose alone. Glucose decreased the oxygen consumption rate with pyruvate and glutamine in a concentration-dependent manner.

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