Mitochondrial involvement in temporal lobe epilepsy

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Abstract

Mitochondrial dysfunction has been identified as a potential cause of epileptic seizures and therapy-resistant forms of severe epilepsy. Thus, a broad variety of mutation in mitochondrial DNA or nuclear genes leading to the impairment of mitochondrial respiratory chain or of mitochondrial ATP synthesis has been associated with epileptic phenotypes. Additionally, with a variety of different methods impaired mitochondrial function has been reported for the seizure focus of patients with temporal lobe epilepsy and Ammon’s horn sclerosis and of animal models of temporal lobe epilepsy. Since mitochondrial oxidative phosphorylation provides the major source of ATP in neurons and mitochondria participate in cellular Ca²⁺ homeostasis, their dysfunction strongly affects neuronal excitability and synaptic transmission, which is proposed to be highly relevant for seizure generation. Additionally, mitochondrial dysfunction is known to trigger neuronal cell death, which is a prominent feature of therapy-resistant temporal lobe epilepsy. Therefore, mitochondria have to be considered as promising targets for neuroprotective strategies in epilepsy.

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Introduction

Epilepsy is one of the most common neurological disorders affecting about 0.5 to 0.7% of the population worldwide. The hallmark of epilepsy is recurrent seizures, which on a cellular level is characterised by synchronized discharges of large groups of neurons that interrupt normal function. It is well known that epileptic seizures can occur as a presenting sign of mitochondrial dysfunction in the central nervous system. Generalised seizures have been observed in several forms of myoclonus epilepsy, being associated with mutations in the mitochondrial DNA polymerase γ (POLG) (Naviaux and Nguyen, 2004; Zsurka et al., 2008) or mitochondrial trnA¹⁸⁵⁵⁰⁷ genes (Shoffner et al., 1990; Zeviani et al., 1993). Partial seizures are frequently noticed in mitochondrial encephalopathies, including the MELAS syndrome, associated with mutations in the mitochondrial tRNALeu(UUR) gene (Goto et al., 1990, 1991). More recently, evidence for a more general involvement of mitochondria also in sporadic forms of epilepsy has been accumulating (Kann et al., 2005; Kunz, 2002; Kunz et al., 2000). From one hand side, this is related to the fact that mitochondria are intimately involved in pathways leading to neuronal cell death (Krajewski et al., 1999) seen in both experimental and human epilepsy. In addition, there is a growing body of evidence that mitochondrial dysfunction plays a considerable role in the process of epileptogenesis and seizure generation in temporal lobe epilepsy with Ammon’s horn sclerosis—a subclass of therapy-resistant forms of epilepsy.

Mitochondrial dysfunction is associated with inherited forms of epilepsy

Defects of oxidative phosphorylation in the CNS are the characteristic sign of mitochondrial encephalopathies. In a broad variety of
these diseases epileptic seizures have been observed. An overview of the most common mitochondrial disorders presenting with an epileptic phenotype is given in Tables 1 and 2. They are either related to mutations in nuclear genes (Table 1) or are direct consequences of mtDNA mutations (Table 2). Thus, mutations in mitochondrial DNA polymerase γ (POLG) have been associated not only with autosomal dominant (adPEO) (Suomalainen et al., 1997; Van Goethem et al., 2001), but also with a severe epilepsy of childhood, called Alpers–Huttenlocher syndrome (Naviaux and Nguyen, 2004; Zsurka et al., 2008). Apart from severe epilepsy, frequently presenting as epilepsy partialis continua, all Alpers–Huttenlocher patients suffer from various manifestations of a severe mitochondrial disease, with brain and liver being mainly affected. The major biochemical defect is a severe depletion of mtDNA, markedly pronounced in liver and explaining the severe toxicity of valproic acid in these patients (Zsurka et al., 2008). A further mitochondrial disease presenting frequently with epilepsy is the Leigh syndrome. This disease is biochemical heterogeneous and also genetically extremely diverse, involving mutations both in nuclear and mitochondrial genes (cf. Tables 1 and 2).

A well known mitochondrial disorder with an epileptic phenotype which is linked to point mutations in the mitochondrial DNA is the MERRF (myoclonus epilepsy with ‘ragged red fibers’) syndrome. This disease has been initially associated with mutations in the mitochondrial tRNA<sup>Thr</sup> (Shoffner et al., 1990; Zeviani et al., 1993). However, as shown in Table 2, a large list of other mitochondrial DNA mutations has been also associated with epileptic phenotypes. The majority of these mutations are located in the mitochondrial tRNA genes, and thus affect the protein biosynthesis of all mitochondrial-encoded subunits of the following complexes of the mitochondrial oxidative phosphorylation pathway: complex I (NADH:CoQ oxidoreductase, containing 7 mitochondrial-encoded subunits), complex III (CoQH<sub>2</sub>:cytochrome c oxidoreductase, containing 1 mitochondrial-encoded subunit), complex IV (cytochrome c oxidase, containing 3 mitochondrial-encoded subunits) and complex V (F<sub>1</sub>F<sub>0</sub>-ATPase, containing 2 mitochondrial-encoded subunits). Quite rarely, also mutations in polypeptide-coding mitochondrial genes have been reported in patients with epilepsy – in the ATPase 6 gene, in the CO I, CO III genes, in the Cyt b gene, and in the ND1, ND2, ND3, ND5, ND6 genes (Table 2). The large variation in the clinical phenotype, even for a given mutation, is a well known feature of mitochondrial diseases. It is very common for these diseases, frequently associated with epilepsy, that the CNS, including the cortex, is significantly affected. Imaging techniques have confirmed that grey matter involvement is an early feature of MERRF and MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes), however also white matter changes are seen at later stages, but usually not in isolation (Cock and Schapira, 1999). A mosaic distribution of mutant mtDNA has been documented for MERRF and MELAS mutations leading in different tissues and even in individual neurons to different wild type/mutant mtDNA ratios (so called heteroplasmy) (D’Mauro et al., 1999). Since the mtDNA mutations are known to cause functional alterations in neuronal oxidative phosphorylation only beyond a certain threshold level ((Schröder et al., 2000), cf. scheme 1), a
highly diverse distribution pattern of the mitochondrial pathology is possible. This phenomenon can explain the large phenotypic variation of these diseases.

**Mitochondrial dysfunction in human temporal lobe epilepsy**

In contrast to the relatively rare mitochondrial encephalopathies being associated with mtDNA mutations, epilepsy is a frequent neurological disorder, usually well controlled by presently available drugs. However, 20 to 30% of patients do not experience seizure control with available medication. The majority of these patients suffer from focal epilepsies, which are frequently consequences of brain trauma, complicated febrile convulsions, status epilepticus, ischemic lesions and brain tumours. The areas of epileptogenesis in these cases are usually characterised by cell loss. One of the most frequent and devastating forms of epilepsy involves the development of an epileptic focus in temporal lobe structures. Brain structures, like the hippocampus, having a low seizure threshold, reside in the temporal lobe and develop in the time course of the disease a severe loss of pyramidal cells in the CA1, CA3 and CA4 subfields. In Fig. 1a (Nissl stain) and Fig. 1c (Timm stain), the characteristic pattern of the segmental neuronal cell loss in human temporal lobe epilepsy (TLE) with Ammon’s horn sclerosis (AHS) is shown in comparison to a lesion-caused TLE (Fig. 1b, Nissl stain and Fig. 1d, Timm stain). While the granular cell layer is relatively preserved the progressive loss of pyramidal cells of the CA1, CA3 and CA4 layers are the neuropathological hallmarks of AHS (Margerison and Corsellis, 1966). This progressive cell loss is suggested to be a major reason that seizures of temporal lobe origin become particularly resistant to antiepileptic drug therapy at later stages of the disease. Moreover, this neuronal cell loss, which resembles features of cell death in other neurodegenerative diseases, is perhaps the reason for the progressive dramatic memory impairment in TLE. Consequently, patients with TLE suffer recurrent seizures and progressive impairment of memory with devastating behavioral and social consequences.

It is well documented that both nerve cells and glia undergo necrotic and apoptotic cell death during seizures (Bengzon et al., 1977). Neuropathological investigations have repeatedly pointed to a similarity between ischemic and seizure related alterations of neurons characterised by swollen and often disrupted mitochondria (Meldrum, 1993). In patients with temporal lobe epilepsy and AHS, mitochondrial ultrastructural pathology was described as characteristic feature of hilar neurons (Blümcke et al., 1999), comprising mainly of inhibitory interneurons. In addition to the neuropathological abnormalities also functional defects of mitochondria have been reported in the areas of epileptogenesis. Thus a severe impairment of respiratory chain complex I activity was observed for the CA3 hippocampal subfield from patients with AHS and in the parahippocampal gyrus of patients with parahippocampal lesions (Kunz et al., 2000). In these reports mitochondrial abnormalities have been observed only close to or directly in the epileptic focus, while the investigated surrounding brain tissue (e.g. the parahippocampal gyrus of patients with clearly pronounced hippocampal pathology and a hippocampal seizure focus) showed no mitochondrial pathology.

**Scheme 1.** Hypothetical common pathway of seizure generation in mitochondria-related genetic forms of epilepsy and temporal lobe epilepsy with Ammon’s horn sclerosis. Possible sites of intervention by mitochondria-targeted therapies are depicted with numbered red arrows: 1 — Intervention with mitochondrial Ca²⁺ overload. A therapeutic agent affecting Ca²⁺ overload is the antiepileptic drug topiramate (Kudin et al., 2008). 2 — Intervention with elevated ROS production. Potential therapeutic agents with effects on ROS production are coenzyme Q and idebenone (Chaturvedi and Beal, 2008). 3 — Intervention with segregation of mtDNA mutations. The ketogenic diet has been suggested to slow down the segregation of pathogenic mtDNA mutations (Santra et al., 2004). OXPHOS — oxidative phosphorylation; ROS — reactive oxygen species.
Mitochondrial dysfunction in the hippocampal CA3 area of patients with AHS has been confirmed on basis of the following independent observations:

1. In studies applying interictal [18F]fluorodeoxyglucose positron emission tomography the degree of hippocampal glucose hypometabolism in AHS patients determined in vivo was strongly correlated to the respiratory activity of the CA3 subfield determined in vitro (Vielhaber et al., 2003). On the other hand, almost no correlation was observed to the respiratory activity of the other hippocampal subfields.

2. Applying high resolution NMR spectroscopy the hippocampal loss of N-acetyl aspartate (NAA) was found to be restricted to the CA3 subfield, but was not detectable in other subfields, like CA1 experiencing an even more intense cell loss. Furthermore, in agreement with the region-specific mitochondrial impairment a considerable increase of lactate and succinate was observed in the CA3 subfield (Vielhaber et al., 2008).

3. An about two-fold decrease in the copy number of mitochondrial DNA and of aconitase activity was observed in the hippocampal CA3 subfield of patients with AHS, providing the molecular cause for the observed decrease in activity of mitochondrial respiratory chain (Baron et al., 2007).

4. Pronounced drops of NAD(P)H fluorescence transients compatible with an impairment of mitochondrial function were observed in human tissue from patients with TLE (Kann et al., 2005). These findings were interpreted in support of the hypothesis that the hypometabolism in the epileptic focus is more of a reflection of dysfunction in cellular energy metabolism rather than a neuronal cell loss (see also Henry et al., 1994; O’Brien et al., 1997).

Taken together these findings strengthen the viewpoint of a putative underlying metabolic dysfunction as an important pathophysiological mechanism in human temporal lobe epilepsy with Ammon’s horn sclerosis. As a potential molecular cause of the detected local impairment of mitochondrial respiratory chain a decrease of the mtDNA copy number, very similar to Alpers–Huttenlocher syndrome, can be delineated (Baron et al., 2007). This similarity supports the viewpoint that mitochondrial dysfunction in the seizure focus of AHS patients is not only relevant for the progressive neuronal cell death, but directly involved in seizure generation as depicted in the hypothetical Scheme 1. Decreased aconitase activities in the CA3 hippocampal subfield of AHS patients indicate a possible role of oxygen radicals in causing neuronal mtDNA damage occurring selectively in the areas of epileptogenesis (Baron et al., 2007).
Mitochondrial involvement in experimental epilepsy

An animal model which has been proven to be appropriate to study human TLE with Ammon’s horn sclerosis is the pilocarpine-treated chronic epileptic rat. In this model, the animals are treated systemically with a dose of the muscarinic agonist pilocarpine that induces an acute limbic status. The status epilepticus is usually terminated after 40 min with diazepam. This acute intoxication is followed by a ‘latent’ (i.e. seizure free) period lasting usually 1–2 weeks, followed by a chronic epileptic condition with spontaneous seizures, resembling human TLE (Turski et al., 1983). From the point of view of hippocampal pathology, pilocarpine-treated rats display changes closely resembling the AHS condition that is seen in the majority of TLE patients. As mentioned above, it consists of segmental loss of pyramidal neurons in the CA1, CA3, and CA4 sectors of the Ammon’s horn, and additionally gliosis and recurrent mossy fiber sprouting. In the recent literature, there are accumulating hints for the contribution of oxygen radicals in the process of epileptogenesis and in chronic experimental epilepsy showing progressive neuronal cell death. There is evidence for the increased generation of oxygen radicals in status epilepticus induced by kainate or pilocarpine (Frantseva et al., 2000; Liang et al., 2000) and in the low-magnesium model of epileptiform activity (Kann et al., 2003; Kovacs et al., 2002; Schuchmann et al., 2002). These oxygen radicals are primary generated by mitochondrial respiratory chain (Kudin et al., 2004; Kudin et al., 2004, 2005; Skulachev, 1996). During inhibition of respiratory chain, considerable amounts of superoxide are produced, which can overload the endogenous protective enzymes (glutathione peroxidase, superoxide dismutase, catalase) resulting in an oxidative damage of proteins, phospholipids and mitochondrial DNA. Since an impaired oxidative phosphorylation due to Ca2+ cycling at the inner membrane of hippocampal mitochondria has been demonstrated in kainate-treated chronic epileptic rats (Kunz et al., 1999). Similarly, impaired cellular Ca2+ homeostasis due to substantial alterations of mitochondrial Ca2+ handling was the predominant feature of cybrid cells harbouring the mitochondrial T8356C mutation being associated with MERRF (Brini et al., 1999).

For temporal lobe epilepsy with Ammon’s horn sclerosis it has to be underlined that the mitochondrial pathology is a prominent feature of not only the entire CA3 hippocampal subfield (Baron et al., 2007; Kunz et al., 2000; Vielhaber et al., 2003, 2008) but also of hilar neurons (Blumcke et al., 1999), comprising mainly of mitochondria-rich inhibitory interneurons. Therefore it is reasonable to assume, that the compromised energy metabolism of this particular neuronal population might explain the dysbalance between neuronal excitation and inhibition characteristic for epilepsy (cf. Scheme 1).

Mechanism of mitochondrial dysfunction caused hyperexcitability

Beside alterations of mitochondrial substrate oxidation and ATP synthesis due to disease-associated mutations seen in mitochondrial encephalomyopathies, also the direct partial inhibition of enzymes of mitochondrial respiratory chain – of cytochrome c oxidase by cyanide (Yamamoto, 1996), and of succinate dehydrogenase by 3-nitropropionic acid (Urbanska et al., 1998) – evoke seizures. The potential direct links between the observed impairment of mitochondrial function and the increased neuronal excitability causing epileptiform activity are (i) decreased intracellular ATP levels and (ii) alterations of neuronal calcium homeostasis.

A high impact of neuronal ATP levels can be postulated since epileptic seizures are observed in Leigh syndrome patients harbouring the mutations T8993G and T8993C in the ATPase 6 gene (Canafoglia et al., 2001; De Vries et al., 1993). Under these conditions mitochondria still have a high membrane potential enabling normal mitochondrial ion transport. In line with this, normal mitochondrial calcium handling properties at decreased cellular ATP levels were observed in cybrids with the T8993G NARP mutation (Brini et al., 1999). It has to be mentioned that mitochondrial oxidative phosphorylation provides the major source of ATP in neurons and adequate ATP levels are essential to maintain the neuronal plasma membrane potential via the sodium–potassium ATPase which consumes about 40% of the energy (Astrup et al., 1981). Therefore, a decreased neuronal plasma membrane potential due to lower cytosolic ATP levels is probably responsible for epileptic seizures observed in Leigh syndrome patients harbouring ATPase 6 gene mutations.

It is also well established that mitochondria are an important intracellular Ca2+ sequestration system (Tang and Zucker, 1997). Due to this feature, mitochondria also can modulate neuronal excitability and synaptic transmission (Bindokas et al., 1998; Tang and Zucker, 1997) which is altered in epilepsy. In agreement with this concept, impaired oxidative phosphorylation due to Ca2+ cycling at the inner membrane of hippocampal mitochondria has been demonstrated in kainate-treated chronic epileptic rats (Kunz et al., 1999). Similarly, impaired cellular Ca2+ homeostasis due to substantial alterations of mitochondrial Ca2+ handling was the predominant feature of cybrid cells harbouring the mitochondrial T8356C mutation being associated with MERRF (Brini et al., 1999).

Brain energy metabolism as potential target of neuroprotective strategies in epilepsy

Apart from a possible role in seizure generation, mitochondrial dysfunction is known to trigger neuronal cell death highly relevant for therapy-resistant temporal lobe epilepsy. Therefore, mitochondria have to be also considered as a promising target for potential neuroprotective strategies in epilepsy. For certain neurodegenerative diseases with established mitochondrial pathology, like amyotrophic lateral sclerosis and Chorea Huntington, neuroprotective strategies have been suggested as a potential way of therapy. The proposed treatment is buffering of neuronal energy levels by systemic creatine administration. The supplemented creatine passes the blood-brain barrier and increases the total pool of phosphocreatine/creatine available for buffering of the neuronal ATP levels by creatine kinase. Creatine supplementation has been shown to protect motor neurons in a transgenic animal model of amyotrophic lateral sclerosis (Klivenyi et al., 1999) and striatal neurons in an animal model of Huntington’s disease (Ferrante et al., 2000). The buffering of brain energy levels with creatine appeared to be effective not only in the mentioned neurodegenerative disorders but also in hypoxia-induced or traumatic brain injury (Sullivan et al., 2000). Moreover, 3 g/kg creatine was observed to reduce hypoxia-induced seizures in rat and rabbit pups (Holtzman et al., 1999). On the other hand, creatine was found to have even a negative effect in the pilocarpine-treated rat — the experimental model of temporal lobe epilepsy (Vielhaber et al., 2003). Therefore, the potential neuroprotective effect of creatine treatment in human epilepsy remains still to be critically proven.

Further strategies aiming on neuroprotection in epilepsy include the use of the ketogenic diet, which appears to increase mitochondrial glutathione levels (Jarrett et al., 2008). Additional potential beneficial effects include a reduced segregation speed of pathogenic mtDNA mutations by a yet unknown mechanism (Santra et al., 2004; cf. Scheme 1, arrow 3). Additionally, the use of certain novel antiepileptic drugs appears to cause protection of neurons against calcium overload by sustained seizure activity (cf. Scheme 1, arrow 1). Among these drugs, topiramate has been shown to develop a substantial neuroprotective potential (Willmore, 2005). This effect appears to be
directly related to an inhibitory action on the mitochondrial permeability transition (Kudin et al., 2004).

Finally, the intervention with the elevated mitochondrial ROS production during seizure activity appears to be an important therapeutic aim (cf. Scheme 1, arrow 2). Potential therapeutic agents with effects on ROS production are coenzyme Q and idebenone (Chaturvedi and Beal, 2008).

Taken together, mitochondrial dysfunction is proposed to be of high relevance for seizure generation in temporal lobe epilepsy with Ammon’s horn sclerosis. Additionally, mitochondria should be considered as a promising target for future neuroprotective strategies in epilepsy.

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References


