Mitochondrial dysfunction in neurodegenerative disorders

M. Baron, A.P. Kudin and W.S. Kunz
Department of Epileptology and Life&Brain Center, University Bonn, Sigmund-Freud-Strasse 25, D-53105 Bonn, Germany

Abstract
There is compelling evidence for the direct involvement of mitochondria in certain neurodegenerative disorders, such as Morbus Parkinson, FRDA (Friedreich’s ataxia), ALS (amyotrophic lateral sclerosis), and temporal lobe epilepsy with Ammon’s horn sclerosis. This evidence includes the direct genetic evidence of pathogenic mutations in mitochondrial proteins in inherited parkinsonism (such as PARK6, with mutations in the mitochondrial PINK1 [PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced kinase 1]) and in FRDA (with mutations in the mitochondrial protein frataxin). Moreover, there is functional evidence of impairment of respiratory chain in sporadic forms of parkinsonism, ALS, and temporal lobe epilepsy with Ammon’s horn sclerosis. In the sporadic forms of the above-mentioned neurodegenerative disorders, increased oxidative stress appears to be the crucial initiating event that affects respiratory chain function and starts a vicious cycle finally leading to neuronal cell death. We suggest that the critical factor that determines the survival of neurons in neurodegenerative disorders is the degree of mitochondrial DNA damage and the maintenance of an appropriate mitochondrial DNA copy number. Evidence for a depletion of intact copies of the mitochondrial genome has been provided in all above-mentioned neurodegenerative disorders including ALS and temporal lobe epilepsy with Ammon’s horn sclerosis. In the present study, we critically review the available data.

Introduction
This review summarizes recent functional and genetic findings reporting a mitochondrial impairment in certain neurodegenerative diseases, such as Morbus Parkinson, FRDA (Friedreich’s ataxia), ALS (amyotrophic lateral sclerosis) and temporal lobe epilepsy.

Morbus Parkinson
Morbus Parkinson [PD (Parkinson's disease)] is a neurodegenerative disorder affecting dopaminergic neurons in substantia nigra. Mitochondrial respiratory complex I deficiency and oxidative stress have been reported to occur in these neurons, and cytoplasmic aggregates (‘Lewy bodies’) of α-synuclein and other proteins have been observed in the affected neurons [1].

Among the different mitochondrial abnormalities that have been described in human neurodegenerative diseases, the respiratory complex I deficit appears to be relatively PD-specific. For PD, this deficit has been documented by reduced immunoreactivity for complex I not only in autopsies of the degenerated substantia nigra [2], but also by enzyme activity measurements in apparently unaffected peripheral cells such as fibroblasts [3], platelets and skeletal muscle fibres [4–6]. In spite of controversies about PD pathology in peripheral cells [7], it has been shown that mitochondria from thrombocytes of PD patients transferred to rho-zero cells, which lack mitochondrial DNA, induce a complex I defect [8]. The observation that the degeneration of dopaminergic neurons can be modelled with inhibitors of the mitochondrial respiratory chain such as rotenone [9] or MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [10], which both inhibit complex I, corroborates an involvement of mitochondrial dysfunction in the pathogenesis of PD.

Disorders with monogenetic inheritance resembling the clinical picture of PD while displaying early onset are rare but highly relevant, since they allowed the identification of the respective disease genes: mutations in α-synuclein (PARK1), parkin (PARK2), UCH-L1 (ubiquitin C-terminal esterase L1) (PARK3) and DJ-1 (PARK7) were shown to result in protein misfolding, ubiquitin–proteasome deficits and oxidative stress [11,12]. The first genetic link to mitochondrial dysfunction came from PARK6, where the mitochondrial protein kinase PINK1 [PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced kinase 1] was found mutated [13] by a W437OPA truncation mutation, a G309D missense mutation with postulated loss of ATP binding [14], or several other nonsense mutations, indicating a loss of PINK1 function within mitochondria as the cause of pathogenesis [15].

A common feature of sporadic and inherited forms of PD is increased oxidative stress in dopaminergic neurons. Since
mitochondrial respiratory chain complex I appears to be the main source of ROS (reactive oxygen species) in neurons [16], an important contribution of mitochondria in the neurodegenerative process is very likely. Additional ROS generation by the cytosolic tyrosine hydroxylase and monoamine oxidases might underlie the preferential degeneration of neurons with dopaminergic neurotransmission in PD.

The molecular mechanism explaining the persistent mitochondrial dysfunction in dopaminergic neurons is suggested to be related to clonal accumulation of deleted mtDNA (mitochondrial DNA) molecules at the single cell level [17,18]. This in turn diminishes the residual amount of wild-type mtDNA, thus leading to mitochondrial dysfunction by reduced expression of mitochondrially encoded proteins.

FRDA

Human FXN (frataxin) is a ~17 kDa protein whose deficiency causes FRDA, a neurodegenerative disorder characterized by degeneration of the Purkinje neurons of the cerebellum that causes limb ataxia, loss of proprioception, dysarthria, skeletal abnormalities, hypertrophic cardiomyopathy and increased incidence of diabetes. In the vast majority of patients (96–98%), the defective expression of FXN is due to a homozygous GAA triplet repeat expansion within the first intron of the FXN gene, located on chromosome 9q13 [19]. The hyperexpansion of GAA repeats determines a formation of a triple helix non-B DNA structure, resulting in an inhibition of FXN mRNA transcription [20]. Moreover, missense mutations are present in FRDA compound heterozygotes, representing 2–4% of patients, which carry an intronic GAA expansion on one FXN allele and an exonic point mutation, mainly located at the C-terminal region of FXN, on the other allele [21]. FXN is involved in several aspects of intracellular iron metabolism, such as biogenesis of haem [22] and iron–sulphur clusters [23], iron binding/storage [24] and iron chaperone activity [25]. Consequently, FXN-defective organisms, from unicellular yeast to humans, exhibit a plethora of metabolic disturbances caused by intramitochondrial iron accumulation, such as the loss of iron–sulphur cluster-dependent enzymes [26], reduced oxidative phosphorylation [27] and altered antioxidant defences [28]. In this context, it has to be mentioned that the molecular cause for reduced mitochondrial oxidative phosphorylation is a severe decrease in copy number of mtDNA as a result of increased oxidative stress [26].

ALS

ALS is a devastating motonic disease (incidence 2:100 000) caused by a progressive degeneration of the anterior horn cells of the spinal cord and cortical motor neurons. The primary cause of the neuronal cell death in ALS so far remains unclear. Some early concepts relate the neurodegenerative process to glutamate-induced excitotoxicity [29]. There is compelling evidence for increased oxygen radical damage in brain tissue of patients with ALS [30]. In line with this concept, it was demonstrated that some patients with autosomal-dominant familial ALS (familial ALS) have point mutations in the Cu²⁺/Zn²⁺ SOD1 (superoxide dismutase 1) gene [31]. While the aetiology of the sALS (sporadic ALS) has remained unknown, 20% of ALS cases are associated with a dominantly inherited mutation in this particular gene. Till now, more than 100 different mutations in the SOD1 have been described [32]. Surprisingly, most of these mutant SODs retain full enzymatic activity, and therefore a ‘toxic gain of function’ as cause for the disease has been postulated. Nevertheless, it remained unclear how the mutated SOD1 causes the selective loss of motor neurons. Mouse models, which carry these mutations, develop severe motor neuron disease [33,34], and the most prominent ultrastructural abnormality is the presence of vacuoles in axons and dendrites, which appear to be derived from degenerating mitochondria [33]. Similarly, in anterior horn neurons of patients with sALS, conglomerations of dark abnormal mitochondria were detected [35]. These findings strongly suggest an involvement of mitochondria in the process of degeneration of motor neurons. Abnormal mitochondria also have been observed by COX (cytochrome c oxidase)/SDH (succinate dehydrogenase) double staining of motor neurons [36] in early stages of the disease. Additionally, impaired mitochondrial function has been detected in peripheral tissues of patients with ALS, such as in skeletal–muscle biopsies [37,38] and in cybrids made from thrombocytes of ALS patients fused to rho-zero cells [39]. In addition to certain rare mtDNA mutations being suggested to play a role in the pathogenesis of the disease [40], an important mechanism explaining at least the mitochondrial dysfunction seems to be mtDNA depletion [38], which is most probably, like in the above-mentioned neurodegenerative diseases, also related to increased oxidative stress. Since mtDNA depletion has been directly observed in skeletal–muscle biopsies from sALS patients in early stages of the disease [38], this finding has been implicated to be relevant for the neurodegenerative process [41].

Temporal lobe epilepsy

Epilepsy is one of the most common neurological disorders, affecting approx. 0.5–0.7% of the human population worldwide. The hallmarks of epilepsy are recurrent seizures, which on a cellular level consist of synchronized discharges of large groups of neurons that interrupt normal function. One of the most frequent and devastating forms of epilepsy involves the development of an epileptic focus in temporal lobe structures. Prolonged seizures (status epilepticus), induced in experimental models by kainic acid or pilocarpine, are known to activate neuronal cell death mechanisms in temporal lobe structures similar to other neurodegenerative disorders. This neuronal cell death is also observed in human temporal lobe epilepsy and is one of the most important aspects of epilepsyogenesis. Specifically in the hippocampus, the loss of CA1 and CA3 pyramidal neurons, with relative sparing of the granular neurons of the dentate gyrus and some types of interneurons, is the histopathological hallmark of Ammon’s horn sclerosis.
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(A) Distribution of the mitochondrial marker enzyme citrate synthase in hippocampal subfields of patients with temporal lobe epilepsy. Closed bars, lesion patients ($n = 7$); open bars, patients with Ammon’s horn sclerosis ($n = 19$). For experimental details, see [44]. (B) Mitochondrial DNA copy numbers in hippocampal subfields of patients with temporal lobe epilepsy. Closed bars, lesion patients ($n = 7$); open bars, patients with Ammon’s horn sclerosis ($n = 19$). The copy numbers were determined by real-time PCR using the nuclear Kir 4.1 gene as single copy reference. (A, B) The PCRs were performed on an iCycler™ (Bio-Rad). The PCR conditions were as follows: 3 min at 95°C, 45 cycles with 15 s at 95°C as first segment and 1 min at 60°C as second segment, 1 min at 95°C, 1 min at 55°C, 80 cycles with increasing temperature 0.5°C each 10 s from 55 to 95°C (melting curve), and infinite hold at 16°C. The cycle number values were determined from fits of experimental data to a sigmoidal curve by using the equation: $y = y_0 + a(1 - \exp(-bx))$. The inflection point equals the cycle number value used in the copy number calculations. Triplicate experiments were performed and arithmetic means and standard deviations were calculated. The primer efficiency was calculated from serial dilutions. The copy number values were verified by using a calibration with counted fibroblasts and dilution series of mitochondrial PCR fragments.

This profile of hippocampal neuronal cell death is visible in the activity distribution of the mitochondrial marker enzyme, citrate synthase, shown in Figure 1(A) (for details see [42]). Since neurons contain the highest amounts of mitochondria, the most affected subfield CA1 showed a 50% decreased activity, while the less severely affected CA3 subfield had an approx. 30% diminished activity. The underlying mechanism of this regional selectivity of neuronal cell death remains to be elucidated yet. The probably most important factor, preceding neuronal cell death after status epilepticus, is the increased level of ROS observed in various models of experimental epilepsy, such as after kainate-induced hippocampal damage, after pilocarpine treatment or in low Mg$^{2+}$-induced epileptiform activity of brain slices and slice cultures (cf. [43]). Mitochondrial respiratory chain complex I is very likely to be the most important source of production of these ROS [16]. Moreover, increased production of ROS is a feature of partially respiratory chain complex I-inhibited mitochondria [16], and it is noteworthy to mention in this context that a severe impairment of respiratory chain complex I activity is present in the focus of epileptic activity: the CA3 neurons of the hippocampus from patients with Ammon’s horn sclerosis and in the parahippocampal gyrus of patients with parahippocampal lesions [42]. Similar observations were made in the vulnerable CA1 and CA3 hippocampal subfields of pilocarpine-treated chronic epileptic rats [44]. As a potential cause of the detected impairment of mitochondrial respiratory chain in the rat model, a decrease in the mtDNA copy number was delineated [44]. As shown in Figure 1(B), similar results can be obtained by determinations of mitochondrial DNA copy number in human hippocampal subfields from patients with temporal lobe epilepsy and Ammon’s horn sclerosis (open bars) in comparison with lesion patients (filled bars). In analogy to the rat study [44], reduced mtDNA copy numbers are detectable for both the CA1 and the CA3 regions. Whereas for the CA1 region a considerable decrease in the content of mitochondria is very likely to be responsible for this observation (cf. Figure 1A), the 2-fold lower mtDNA copy numbers in the CA3 region cannot be explained by a lowered mitochondrial content. Thus, as observed in the pilocarpine model of temporal lobe epilepsy [44], mtDNA depletion is a feature of CA3 neurons in Ammon’s horn sclerosis. Since oxygen radicals are known to create mtDNA strand breaks, which facilitate mtDNA breakdown, this finding implies a role of oxygen radicals in causing neuronal mtDNA damage occurring selectively in brain areas generating epileptic activity.

**Mitochondrial DNA depletion is a frequent cause of bioenergetic defects in human neurological disease**

In addition to neurodegenerative diseases, which show the feature of mtDNA depletion in postmitotic cells, there also exists a broad spectrum of genetic syndromes presenting with neurological phenotypes, which are associated with reduced mtDNA copy numbers due to mutations in various nuclear genes involved in mtDNA maintenance. These include genes for the deoxyguanosine kinase [45], thymidine kinase [46], the muscle-, brain- and heart-specific isoforms...
of the adenine nucleotide translocase [47], the mitochondrial helicase Twinkle [48] and the mitochondrial polymerase γ [49,50]. This clearly underlines that the maintenance of the correct mtDNA copy number is a critical factor for proper neuronal functioning.

In summary, the reduction of intact mtDNA copies by oxidative stress-related mutagenesis appears to be the molecular cause of the observed mitochondrial dysfunction in the above-mentioned neurodegenerative diseases. Interestingly, comparable clinical phenotypes, associated with molecular cause of the observed mitochondrial dysfunction [49,50]. This clearly underlines that the maintenance of the

References