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Biochimica et Biophysica Acta 1410 (1999) 159–170

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Review

Mitochondrial involvement in Parkinson's disease, Huntington's disease, hereditary spastic paraplegia and Friedreich's ataxia

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Received 27 August 1998; accepted 1 October 1998

Abstract

Respiratory chain dysfunction has been identified in several neurodegenerative disorders. In Friedreich's ataxia (FA) and Huntington's disease (HD), where the respective mutations are in nuclear genes encoding non-respiratory chain mitochondrial proteins, the defects in oxidative phosphorylation are clearly secondary. In Parkinson's disease (PD) the situation is less clear, with some evidence for a primary role of mitochondrial DNA in at least a proportion of patients. The pattern of the respiratory chain defect may provide some clue to its cause; in PD there appears to be a selective complex I deficiency; in HD and FA the deficiencies are most severe in complex II/III with a less severe defect in complex IV. Aconitase activity in HD and FA is severely decreased in brain and muscle, respectively, but appears to be normal in PD brain. Free radical generation is thought to be of importance in both HD and FA, via excitotoxicity in HD and abnormal iron handling in FA. The oxidative damage observed in PD may be secondary to the mitochondrial defect. Whatever the cause(s) and sequence of events, respiratory chain deficiencies appear to play an important role in the pathogenesis of neurodegeneration. The mitochondrial abnormalities induced may converge on the function of the mitochondrion in apoptosis. This mode of cell death is thought to play an important role in neurodegenerative diseases and it is tempting to speculate that the observed mitochondrial defects in PD, HD and FA result directly in apoptotic cell death, or in the lowering of a cell's threshold to undergo apoptosis. Clarifying the role of mitochondria in pathogenesis may provide opportunities for the development of treatments designed to reverse or prevent neurodegeneration.

Keywords: Parkinson's disease; Huntington's disease; Hereditary spastic paraplegia; Mitochondrion

Contents

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0005-2728/99/$ – see front matter
PII: S0005-2728(98)00164-9

1. Introduction

Mitochondrial respiratory chain dysfunction has now been identified in a number of neurodegenerative disorders. In some, e.g. Parkinson's disease (PD), this may play a role in aetiology and pathogenesis. In others, the mitochondrial deficiency is known to be secondary to a mutation in a non-respiratory chain protein coding gene, e.g. Huntington's disease (HD), Friedreich's ataxia (FA), hereditary spastic paraplegia (HSP). The apparent predilection of respiratory chain or oxidative phosphorylation (OXPHOS) defects for the central nervous system may be explained by this tissue's particular dependence on aerobic metabolism. Mitochondrial involvement in Alzheimer's disease and FA has been selected for special sections in this issue. This review will focus on respiratory chain involvement in PD, HD and the recently discovered mitochondrial involvement in HSP.

2. Parkinson's disease

The clinical characteristics of PD are bradykinesia, rigidity and tremor. Onset is typically in the sixth or seventh decade of life, with a lifetime risk of developing PD of 1 in 40. The main clinical features of PD are the result of degeneration of dopaminergic neurones in the substantia nigra pars compacta. Although other areas may be involved, e.g. locus ceruleus, substantia innominata, as well as other neurotransmitter systems, e.g. cholinergic, it is the dopamine system on which most attention has been focused in terms of aetiology, pathogenesis and treatment. Surviving dopaminergic neurons in the substantia nigra may contain intracytoplasmic inclusions (Lewy bodies). How these inclusions develop is not known but they are found elsewhere in the PD brain as well as in other neurodegenerative diseases including diffuse Lewy body disease and motor neuron disease (amyotrophic lateral sclerosis).

There is increasing evidence for a significant genetic component to PD. Early twin studies found no increase in concordance amongst monozygotic twins, suggesting little if any genetic influence [1–4]. However, such studies were complicated by several confounding factors including ascertainment and subclinical PD in the non-affected twin. The most recent and reliable studies are those that include positron emission tomography (PET) assessment with ^{18}fluoro-dopa [5]. ^{18}Fluoro-dopa PET has the sensitivity to detect decreased dopamine uptake in the striatum that reflects loss of nigrostriatal dopaminergic neurons in patients prior to the development of the clinical features of PD [6]. This technique improves the analysis of concordance rates amongst monozygotic and dizygotic twins and now confirms that a genetic component is likely to contribute to PD [5].

Several pedigrees with familial parkinsonism have been described. Inheritance patterns in these families have usually been autosomal dominant [7–10], although maternal inheritance has also been described [11], implying involvement of mitochondrial DNA (mtDNA). An important advance in our understanding of PD came with the identification of mutations in the α-synuclein gene in certain autosomal dominant parkinsonian families [12–14]. Mutations in this gene have not been identified in sporadic patients with PD, nor in other familial cases [15]. Nevertheless, the mechanisms by which the mutation in α-synuclein can result in apparently selective dopaminergic cell death will provide valuable insights into the events that may result in nigral cell loss in sporadic, primary PD. Other chromosomal loci have also recently been identified in certain PD families [16,17], but the gene products and their function remain uncharacterised at present.

Mitochondrial involvement in PD was established with the identification of complex I deficiency in the substantia nigra [18–20]. The complex I defect appears to be selective in terms of the involvement of other components of the OXPHOS system. Analysis

of other areas of the PD brain, including striatum (caudate and putamen), cortex, cerebellum, globus pallidum, tegmentum and substantia innominata, has not identified an OXPHOS deficiency [21–24]. There is no apparent respiratory chain defect in substantia nigra or other brain areas in multiple system atrophy [25], a disorder resembling PD treated with L-dopa and with severe nigral dopaminergic cell loss. This suggests that the complex I deficiency in PD is neither related simply to neuronal degeneration nor to L-dopa toxicity.

At the time of death, the PD substantia nigra has lost probably $>85\%$ of its dopaminergic neurons, these neurons comprising $<5\%$ of the total cell population. Thus a $\sim 35\%$ deficiency of complex I in nigral homogenates must reflect decreased activity in both neurons and glia. Although complex I immunoreactivity is reduced in PD nigral neurons [26] it is not known whether or not the neurons have a more severe defect than glia. However, their greater dependence on aerobic metabolism would suggest that they will be more vulnerable to such a mitochondrial abnormality. Further studies are needed in this area to define in greater detail the extent and effect of the complex I deficiency on dopaminergic neurons.

Lewy bodies are present in high numbers in the cingulate gyrus of diffuse Lewy body (DLB) brains. Analysis of complex I activities in this area did not identify any abnormality, suggesting that the complex I deficiency is not related to Lewy body expression [24]. Complex I activity was also normal in cingulate cortex from AD brains, an area with marked gliosis, implying that gliosis per se is not a cause of the complex I defect in PD. Interestingly, reduced glutathione (GSH) levels are decreased in PD substantia nigra and AD cingulate cortex suggesting that there is oxidant stress in all these areas, but that this is not necessarily associated with complex I deficiency.

Respiratory chain dysfunction has also been sought in tissues outside the brain in PD patients. Data on skeletal muscle remain difficult to interpret [27]. The first study using ^{31}phosphorus magnetic resonance spectroscopy of skeletal muscle found no abnormality of oxidative phosphorylation [28]. The second, however, did demonstrate defects [29]. Respiratory chain function analysis of skeletal muscle mitochondria by polarography or of homogenates has provided conflicting results. These studies have been reviewed but the majority showed no abnormality, although severe defects were identified in a small proportion of patients [30]. Whilst methodological variations may explain some of the differences, it may be that the results of mitochondrial function assays in PD skeletal muscle reflect the heterogeneity of the disease itself (see below). In contrast, analysis of respiratory chain function in platelets from PD patients has provided more consistent results (see [30] for review) [31]. There is a clear consensus for mitochondrial dysfunction in PD platelets; almost all studies have demonstrated complex I deficiency, some having also found defects of complexes II–IV. The severity of the complex I defect does vary between studies (16–55%), but in none was there sufficient sensitivity to allow complex I activity to be used as a bio-marker. The consistency over time of a complex I defect in platelets from PD patients has been demonstrated [31,32]. The presence of a complex I deficiency in platelets but not PD brain areas outside the substantia nigra may at first seem a paradox. However, there are several possible explanations, both methodological and biochemical. Respiratory chain function is most sensitively assessed on isolated mitochondria. This is demonstrated in platelets where homogenates showed no abnormality [22] but enriched mitochondrial fractions did [33]. Frozen post mortem brain tissue does not easily lend itself to mitochondrial isolation and so data derived from this tissue have been based on homogenate analysis. Thus, whilst the most severe defect is readily identified in substantia nigra, it is possible that homogenate analysis is insufficiently sensitive to detect less severe defects in other areas. The cause of the severity of the defect in substantia nigra may be multifactorial including exacerbation through oxidative damage generated via, for example, dopamine autoxidation — through direct dopamine inhibition [34], or perhaps reflecting the effect of a primary aetiological factor, e.g. mtDNA mutation. Alternatively, platelets have pharmacological features in common with dopaminergic neurons, e.g. uptake of MPP^+, presence of monoamine oxidase, which have led some to suggest that platelets may be used as a model for dopaminergic neurons [35]. However, caution must be used in extrapolating pharmacological or biochemical abnormalities from platelets to neurons.

Whatever the cause of the complex I deficiency in PD platelets, its presence may be used as a tool to dissect out some of the aetiological factors which may be relevant to this disease.

Each mitochondrion contains 2–10 molecules of mtDNA. MtDNA encodes 22 transfer RNAs (tRNA), two ribosomal RNAs and 13 proteins: seven subunits of complex I, cytochrome *b* of complex III, COI, COII and COIII of complex IV (cytochrome oxidase) and subunits 6 and 8 of complex V (ATPase). MtDNA is inherited through the maternal line. In excess of 80 mtDNA mutations have been associated with human disease [36]. Mutations may occur in protein coding or RNA genes; they usually co-exist (probably intramitochondrially) with normal wild-type molecules. The proportion of mutant molecules may vary from one tissue to another. The level of mutant load required to induce a respiratory chain deficiency and cell dysfunction is usually high, but this may vary depending on the tissue's dependence on oxidative phosphorylation.

The presence of complex I deficiency in PD clearly raised the possibility that this defect might be determined by a mutation in one of the mtDNA complex I genes. However, most cases of PD are sporadic and do not appear to follow a maternal pattern of inheritance as might be expected from an mtDNA mutation. However, it is now clear that the majority of patients with 'mitochondrial myopathy' and a defined mtDNA mutation appear as sporadic cases [37]. Even 40% of patients with the archetypal maternally inherited disease, Leber's hereditary optic neuropathy, have no family history [38]. Thus a history of maternal inheritance in a disease is not a sine qua non for mtDNA involvement. Several studies have sequenced mtDNA from PD patients but no consistent mutation has been identified. Some alterations in sequence have been found in greater frequency in PD patients than controls, but this may to some extent reflect different ethnicity and haplotype background [39–45].

The presence of the complex I defect in PD patients offers an opportunity to determine whether this is caused by a mtDNA defect. Platelets are derived from bone marrow megakaryocytes and thus have respiratory chain complexes with both nuclear and mtDNA encoded subunits. Platelets circulating in the blood do not have nuclei. It is possible to generate cells without mtDNA (ρ^0 cells) by exposure over several passages to, for instance, ethidium bromide or DDC [46]. Such cells can grow and divide in medium supplemented with pyruvate and uridine. ρ^0 cells can serve as a recipient of donor mtDNA which, following fusion of an enucleated cell (or platelet) containing mitochondria, can be replicated in the cybrid cells. Following this fusion and growth, the resulting cybrids will have mtDNA and mtDNA encoded respiratory chain subunits from the donor, and nuclear DNA encoded subunits from the host ρ^0 cells (see Fig. 1). In this model, if a defect of respiratory chain function is present in the donor mitochondria and perpetuated in the cybrids following fusion, then it must be caused by an abnormality of the donor mtDNA, as this is the only component

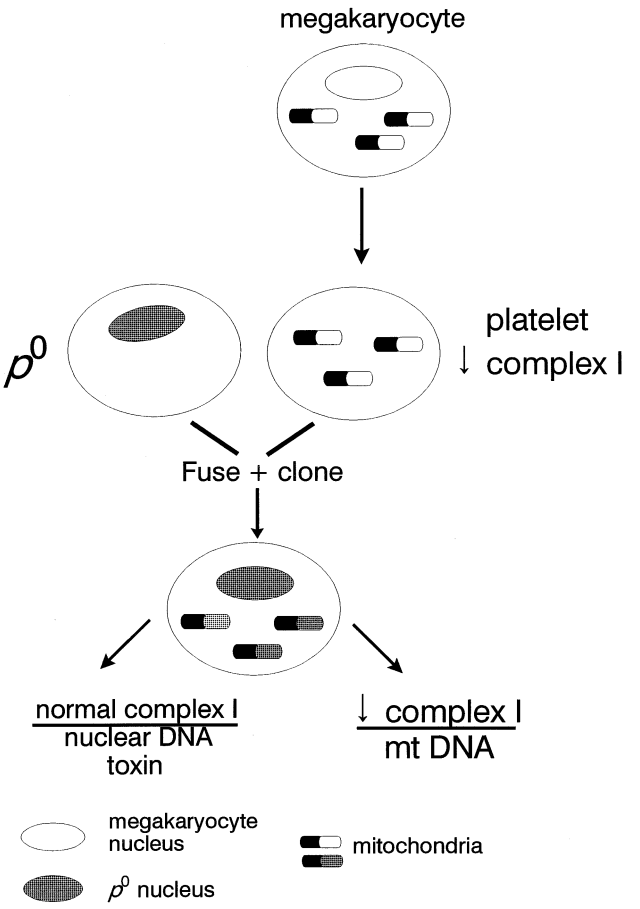

Fig. 1. Schematic illustration of the principle of the ρ^0 cybrid studies used in the identification of an mtDNA contribution to the complex I defect in PD.

remaining from the donor cells in the cybrids. We have applied this model to investigate the origin of the complex I deficiency in PD platelets [32].

We hypothesised that those PD patients with the lowest platelet complex I activity would be those most likely to bear any putative mtDNA mutation. Platelets from a preselected group of patients with the lowest complex I activities were fused with A549 ρ^0 cells and then grown as mixed or clonal cybrids. Respiratory chain function analysis in the mixed cybrids from controls and PD patients identified a significant complex I deficiency in the latter (25%; $P=0.007$). The maintenance of the complex I defect from platelets to cybrids must therefore be the result of a defect in the host PD mtDNA. Interestingly, the individual donor platelet complex I activity correlated with the complex I activity in the cybrid cells with that donor's mtDNA ($r=0.86$, $P<0.001$). This correlation allowed the PD and control populations to be more clearly separated (Fig. 2).

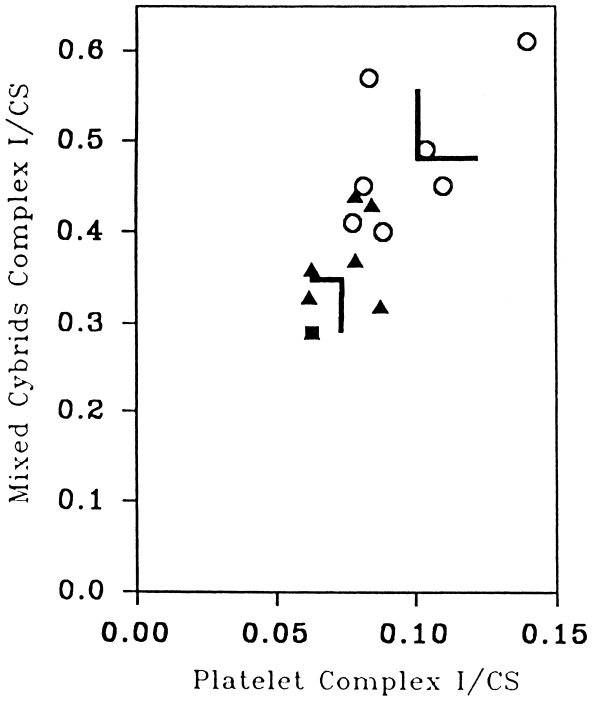

Fig. 2. Relationship between the complex I/CS ratios in the control (○) and PD patient (▲) platelet mitochondrial fractions and their respective Cxl/CS ratio in the platelet A549 ρ^0 mixed cybrid lines. The patient studied further by clonal cybrid analysis is indicated (■). The mean ± S.D. for the control and PD group are indicated. Spearman correlation: $r=0.86$, $P<0.001$ (see ref. [32]).

Further studies were then undertaken on one of the PD patients to determine whether the mtDNA defect was heteroplasmic. In studies with the A3243G $tRNA^{Leu(UUR)}$ mutation of mtDNA associated with encephalomyopathy, we found that the heteroplasmic mutation segregated randomly in the A3243G nuclear background to produce a range of clonal lines with varying mutant load (Gu et al., unpublished observations). Mutant load correlated with biochemical defects in complexes I and IV in that ≥90% mutation was required before deficiencies appeared. This was also apparent on staining for cytochrome oxidase cytochemically or immunocytochemically where there was heterogeneity between the individual cells reflecting different mutant loads between them. Similar clonal studies on the PD patient's cybrids produced identical results. Biochemically, there was a 25% deficiency of complex I ($P=0.005$) and 20% deficiency of complex IV ($P=0.005$). Staining the clones for cytochrome oxidase activity and with a monoclonal antibody to the COI mtDNA encoded subunit likewise demonstrated heterogeneity between cells of the same clone. These results are identical to those obtained with the A3243G mtDNA mutation and suggest that the mtDNA defect in the PD patients may also be heteroplasmic.

In summary, these results suggest that in PD patients preselected by their low complex I activity, a mtDNA defect caused the mitochondrial deficiency, and that the mtDNA defect may be heteroplasmic. Swerdlow et al. [47] used the ρ^0 model in PD and have also found a complex I defect in mixed cybrids but from a group of unselected patients. The ρ^0 model results do not indicate whether the PD associated mtDNA defect is inherited or somatic. It is possible, for instance, that somatic mtDNA mutations may have arisen in the patient's bone marrow or blood from some toxic influence (genetic or environmental). These mutations, likely to be multiple if secondarily generated, would produce the platelet complex I deficiency and be perpetuated in culture to cause the defect in mixed and clonal cybrids. The distinction between inherited and somatic mutations in this instance may be determined either by the identification of a particular significant mtDNA base change and

following its maternal lineage, or using PD nuclear backgrounds in the ρ^0 model to see if they induce secondary mtDNA changes.

The heterogeneity in expression of cytochrome oxidase activity and subunits was paralleled by changes in mitochondrial membrane potential ($\Delta\Psi_m$) as measured by JC-1. Whilst some of the clones with the PD mtDNA had apparently normal $\Delta\Psi_m$, other cells had low $\Delta\Psi_m$. To some extent this is an indirect measure of OXPHOS and reflects the biochemical defects outlined above. Nevertheless, the demonstration that the PD mtDNA defect can induce a fall in the $\Delta\Psi_m$ has important implications for its role in lowering the apoptotic threshold of cells.

2.1. Environmental factors

MPTP was identified as the contaminant of a meperidine analogue 'designer drug' responsible for inducing parkinsonism in a small group of drug addicts in California.

MPTP appears to induce clinical features similar to, but not identical with, idiopathic PD, but which still remain responsive to dopamine receptor activation. Pathological study of a brain from an MPTP addict with parkinsonism showed severe destruction of dopaminergic neurons in the substantia nigra [48]. ^{18}Fluoro-dopa positron emission tomography in patients with MPTP parkinsonism indicated progression of nigrostriatal cell loss over seven years at a rate faster than aging and comparable with idiopathic PD [49]. This implies that the toxicity of this compound continues long after initial exposure and indicates the presence of ongoing biochemical abnormalities causing cell death. MPTP is a protoxin, metabolised to its active derivative 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B (MAO-B), the distribution of which therefore determines the site of toxicity. MPP^+ is a specific reversible inhibitor of complex I and results in a fall in ATP levels. MPP^+ probably interacts with complex I at the same site as rotenone and piericidin A [50]. MPP^+ induces more severe and irreversible inhibition of complex I if cytochrome oxidase (complex IV) is inhibited [51]. This inhibition was prevented with free radical scavengers indicating oxidative damage of complex I under these conditions. Complex I inhibition results in increased free radical generation from the respiratory chain and so the MPP^+ model suggests that a self amplifying cycle of complex I deficiency and damage may result in progressive cell damage. Such a situation would fit well with the progressive striatal lesion in MPP^+ exposed patients as determined by ^{18}fluoro-dopa PET. There is increasing evidence that MPP^+ generates free radicals and oxidative damage in addition to causing complex I inhibition [52,53]. For instance, the nitric oxide synthase inhibitor 7-nitroindazole has been shown to protect monkeys [54] and rodents [55]. However, there is also evidence that 7-nitroindazole also inhibits MAO-B, and this would prevent conversion of MPTP to MPP^+ [56].

1,2,3,4-Tetrahydroisoquinoline (TIQ) and 2-methyl-TIQ are both structurally related to MPTP and both have been found in human brain, including a patient with PD in whom the level of TIQ was greater than controls [57,58]. TIQ can be toxic to dopaminergic neurons and induce motor deficits in monkeys. Interestingly, TIQ is also a complex I inhibitor [59]. *N*-Methyl TIQ is a methylation product of TIQ and is a substrate for MAO-B, producing *n*-methylisoquinolinium ($NMIQ^+$). $NMIQ^+$ inhibits tyrosine hydroxylase and MAO. It is found in certain foodstuffs and can cross the blood brain barrier, but can also be formed by condensation reaction in the brain.

3. Huntington's disease

HD is a neurodegenerative disorder characterised by ataxia, chorea and dementia. Onset is usually in adulthood, although a juvenile form is recognised. HD is an autosomal dominant disease now known to be caused by an abnormal CAG expansion within the IT15 gene on chromosome 4. This gene encodes huntingtin, a widely expressed 349-kDa protein of unknown function. Cell culture models expressing mutant huntingtin develop intraneuronal inclusions [60] of a similar type to those seen in brains from HD patients [61–64].

As with PD, the pathology of HD focuses primarily on a select neuronal population – the γ-aminobutyric acid containing spiny neurons of the caudate nucleus, in the case of HD. Other areas such as the putamen and cerebral cortex are less affected, and the cerebellum is relatively spared. An important

clue to the pathogenesis of mutant huntingtin expression will be an understanding of how this widely expressed protein can result in such specific neuronal damage when present in mutant form.

Excitotoxicity has been suggested as playing an important role in HD. This is dependent upon glutamate excitation of *N*-methyl-D-aspartate (NMDA) receptors, inward flow of calcium, activation of nitric oxide synthase and production of nitric oxide ($NO^{\bullet}$) [65]. $NO^{\bullet}$ and particularly peroxynitrate ($ONOO^{-}$), the product of its reaction with superoxide ($O_2^{\bullet -}$) are damaging radical species. A critical factor in this pathway is a defect in energy metabolism which releases the magnesium blockade of NMDA receptor activation and renders ambient levels of glutamate toxic. There is now both in vivo and post mortem evidence for a defect of energy metabolism in HD. Striatal and cerebral cortex glucose metabolism is decreased in HD and appears to precede bulk tissue loss [66,67]. Elevated lactate levels in occipital cortex have been identified [68] and recently the length of CAG repeat has been found to correlate with lactate levels in HD striatum [68]. Another study of lactate levels by magnetic resonance spectroscopy, however, did not find any difference between HD and controls [69]. Direct measurement of respiratory chain activity in HD caudate demonstrated a severe deficiency in the activities of complexes II and III (56%, $P < 0.0005$) and a 33% ($P < 0.01$) deficiency of complex IV [70]. A similar but less severe pattern of deficiency was also seen in HD putamen, but not cerebral cortex or cerebellum [71,72]. A 92% deficiency of aconitase activity has also been found in HD caudate, with a 73% defect in putamen, 48% in cerebral cortex but normal levels in cerebellum. Interestingly this distribution of aconitase deficiency mimics more closely the pathology of HD. Aconitase is an iron-sulphur (FeS) containing enzyme which participates in the tricarboxylic acid cycle and in iron homeostasis. Aconitase activity is particularly susceptible to inhibition by $O_2^{\bullet -}$, and also by $NO^{\bullet}$/$ONOO^{-}$ [73–75]. Complexes II and III are FeS containing enzymes and are also sensitive to inhibition by $NO^{\bullet}$ [72]. The pattern of enzyme loss in HD brain therefore supports the involvement of $NO^{\bullet}$ and excitotoxicity in pathogenesis.

Respiratory chain activities were normal in HD platelets [70], although another study had found a complex I deficiency [76]. Mutant huntingtin is also expressed in HD fibroblasts but respiratory chain and aconitase activities were not significantly different from control [72].

Respiratory chain enzyme activity in three of four HD patients, in whom muscle biopsies had been performed, showed decreases in complex I activity [77]. Thus, whilst there is consensus on a mitochondrial deficiency in HD striatum. The situation in tissues outside the brain is less clear. If a mitochondrial defect were unequivocally demonstrated in a tissue which lacked the biochemical and pharmacological properties peculiar to the striatum, then a more direct link between the CAG repeat in the huntingtin mutation and the mitochondrial defect could be made [78].

A mouse model of HD has been created with a transgene containing approximately one kilobase of the human HD promoter region, exon one, carrying CAG repeat expansions of 155–156 units, and 262 base pairs of intron one [79]. The mouse line with 141–157 CAG repeats exhibits progressive neurological disease from age two months [80]. Neuronal intranuclear inclusions (NII) in the mouse striatum are also correlated with frequent indentations of the nuclear membrane and an increase in nuclear pores, observations almost identical to those found in HD brains (see above). The NIIs stain positively for huntingtin and ubiquitin [80]. NIIs appear first in the cerebral cortex, precede the onset of neurological features, and spare the NADPH diaphorase positive neurons of the striatum. The rate of formation of NIIs appears largely dependent upon mutant protein expression levels. These results indicate that the N-terminal fragment of huntingtin bearing an abnormal CAG repeat is sufficient to induce a neurological disease in mice which resembles HD, and induces morphological changes (NIIs) identical to those found in HD and which parallel, but antedate, neuropathological changes.

The mouse model of HD provides an interesting opportunity to determine the role that mitochondrial dysfunction might play in HD. It could be predicted, for instance, that a mitochondrial deficiency in the mouse model brain that was present prior to cell loss might be part of the cascade of biochemical events that begins with mutant huntingtin expression and ends with neuronal cell death. Similarly, the cell cul-

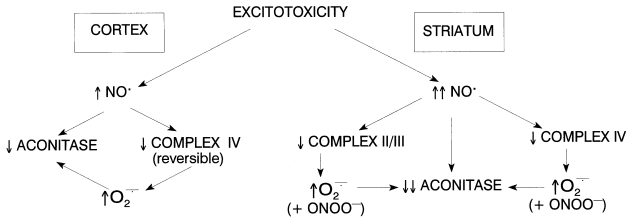

Fig. 3. Schematic representation of the proposed biochemical events causing mitochondrial dysfunction in Huntington's disease.

ture models of mutant huntingtin expression now being developed should again provide the means to investigate the role of mitochondrial dysfunction in HD pathogenesis.

Nevertheless, the pattern of enzyme defects, their severity and their anatomical distribution support the role of excitotoxicity in HD. We have suggested previously that a small increase in $NO^{\bullet}$ generation from excitotoxicity can lead to reversible complex IV inhibition [81]. This in turn would lead to increased $O_2^{\bullet -}$ generation from the respiratory chain in the presence of an inhibitor of the chain [51]. The $O_2^{\bullet -}$ thus produced, together with the $NO^{\bullet}$ generated from excitotoxicity will inactivate aconitase. This pattern of apparently isolated aconitase inhibition may reflect events in the HD cortex, where excitotoxicity will be limited in comparison to the striatum. Higher levels of $NO^{\bullet}$ generation, such as might occur in the striatum, will result in greater $NO^{\bullet}$, $O_2^{\bullet -}$ and $ONOO^-$ production. This will cause inhibition of complex II and III in particular. These events then lay the foundation for the creation of a self-amplifying cycle of respiratory chain inhibition and free radical generation. Oxidative damage to protein, lipids and DNA together with impaired synthesis resulting from this cycle will reduce cell viability and could induce cell death by apoptosis or necrosis [82]. These events are summarised in Fig. 3.

4. Hereditary spastic paraplegia

Hereditary spastic paraplegia (HSP) has a prevalence of approximately 1 in 10 000. Autosomal dominant, autosomal recessive and X-linked forms of HSP have been described [83]. Three loci on chromosomes 14q, 2p and 15q have been associated with an autosomal dominant form [84–86], whilst a locus on 8q is found in recessive families [87]. Patients with HSP may present from childhood to adulthood with gradual onset and progression of leg stiffness and weakness. Additional features such as a neuropathy, ataxia, retinitis, optic atrophy and deafness may be identified in some patients.

To date, the genes responsible for HSP were only known in X-linked forms where mutations in the L1CAM and PLP genes on Xq28 and Xq22, respectively, were described [88,89]. However, a new gene defect has recently been found in pure HSP families with autosomal recessive inheritance linked to a locus on 16q24.3 [90]. The gene product has been termed paraplegin and has 795 amino acids [91]. Paraplegin contains an N-terminus targeting sequence and has been shown to be imported into mitochondria with a processed molecular mass of 81 kDa. Different mutations in the paraplegin gene have also been identified in two additional recessive HSP families supporting the role of the defective protein in HSP. Muscle biopsies from two severely affected and two mildly affected patients with paraplegin mutations showed mitochondrial abnormalities. The more severe cases had ragged red, succinate dehydrogenase positive fibres which stained negatively for cytochrome oxidase (complex IV); mitochondrial paracrystalline inclusions were seen in some fibres. These morphological changes are typical of myopathies associated with mitochondrial DNA mutations. Similar abnormalities were seen in the remaining two patients, but were present in lower frequency. Future studies will be required to define the biochemical deficiency associated with mitochondrial structural changes and to determine the role of paraplegin in mitochondrial function.

5. Friedreich's ataxia

Friedreich's ataxia (FA) is an adolescent autosomal recessive disorder with a prevalence of approximately 1 in 50 000. The main clinical features include progressive ataxia, dysarthria, skeletal deformities, hyporeflexia, pyramidal features and a hypertrophic cardiomyopathy. Most patients are wheelchair-bound within 10–15 years of onset and die from progressive cardiac failure. Pathological processes in FA

primarily affect the central and peripheral nervous systems and consist of a distal axonopathy of 'dying-back' type affecting the large sensory axons of the dorsal root ganglia and the spinocerebellar and pyramidal tracts in the cord, with subsequent loss of the parent cell bodies.

The molecular genetic defect in 98% of patients with Friedreich's ataxia has been identified as an unstable GAA triplet repeat in intron 1 of the frataxin (X25) gene [92] resulting in a deficiency of frataxin protein presumably by interfering with RNA processing [93]. RNA analysis has shown frataxin transcription to be highest in the heart, spinal cord and dorsal root ganglia which correlates well with the pattern of degeneration observed in the disease.

The function of frataxin is not known. However, in yeast, deletion of the frataxin gene homologue, YFH1, which shares a highly conserved C-terminal domain with frataxin, resulted in severe growth deficit on fermentable carbon sources and an inability to grow on glycerol and ethanol implying a defective oxidative phosphorylation system. The connection with mitochondria was strengthened by the presence of a predicted N-terminal mitochondrial targeting sequence [94], and the mitochondrial localisation of frataxin was confirmed using fluorescence microscopy [95,96], and its association with mitochondrial membranes has recently been confirmed [93]. The role of frataxin in mitochondria is not known, however, several theories have been proposed. The damage and/or loss of mtDNA in yeast lacking the YFH1 gene have led to the suggestion that frataxin may be required for mtDNA replication or maintenance [97], although loss of mtDNA is a relatively common observation in yeast PET mutants [98–101]. Our own studies suggest that mtDNA levels are also decreased in FA myocardium (J. Bradley, personal communication). Increased blood lactate levels in patients with FA support the hypothesis that oxidative phosphorylation is impaired [102]. There are conflicting reports that certain mitochondrial enzyme activities are decreased in FA cells. These have included pyruvate dehydrogenase, glutamate dehydrogenase, α-ketoglutarate dehydrogenase and complex I–III activities [103–106]. A recent study of two FA patients found decreased activity of complexes I–III and aconitase in heart homogenate [107]. Respiratory chain and aconitase activities were studied in skeletal muscle, lymphocytes and fibroblasts from a single FA patient and were found to be normal. The similarity between a deficiency of the antioxidant vitamin E and Friedreich's ataxia has led to the suggestion that frataxin may be involved in the antioxidant defence of the cell [93]. The connection with abnormal mitochondrial activities is further strengthened by the fact that vitamin E deficiency in rats results in markedly decreased complex I and IV activities [108] although there is no evidence of large scale mtDNA abnormalities in this model (unpublished data).

Frataxin has been suggested to be involved in mitochondrial iron metabolism. The evidence for this comes from the fact that the yeast frataxin homologue, YFH1, has been shown to suppress a mutant unable to grow on iron limited medium [109]. In addition, in iron rich medium the high affinity iron transport system is not usually detectable, but in cells with a deletion of the YFH1 gene this transport system was induced even at high iron concentrations resulting in increased iron uptake [109]. In this mutant there is an increase in the mitochondrial iron levels, but this is not a simple reflection of the increased cellular iron levels which implies frataxin may be involved in mitochondrial iron metabolism [109]. As predicted from Fenton chemistry these cells were very sensitive to H_2O_2 treatment and therefore are vulnerable to oxidative stress and damage [109].

Mitochondrial dysfunction caused by deposition of iron could account for the features of FA. The mitochondrial respiratory chain is a major generator of cellular free radicals. Oxidative stress and damage would be exacerbated in the presence of free iron which, by Fenton chemistry, could generate hydroxyl ions. The proximity of mtDNA and the mitochondrial respiratory chain to the site of free radical generation may contribute to the abnormalities of mtDNA and mitochondrial respiratory chain function that might result. Neurons and cardiac cells are primarily involved in FA pathology. They are highly dependent upon oxidative phosphorylation and consequently would be particularly susceptible to defects of the respiratory chain. However, it is not yet apparent why all neurons are not involved. This could reflect differential frataxin expression, with those tissues involved having the highest frataxin expression, or it may depend on the length and

diameter of the axons that the neuronal perikarya have to support. The neurons initially affected consistently possess long large diameter axons. Failure of the cell bodies to support these axons could result in the distal axonopathy that occurs. In humans, the phenotype observed in the yeast model is unlikely to be compatible with life. The milder phenotype is likely to reflect residual frataxin levels in the patient's cells or alternative mechanisms performing the frataxin role. Null mutants for frataxin have not yet been identified in humans, possibly because they may be fatal. However, there is a correlation between genotype and phenotype with larger GAA repeats being associated with lower frataxin mRNA and protein expression and earlier onset and increased severity of the disease [93,110–112].

Acknowledgements

The author's work described in this paper was supported by the Medical Research Council, the Wellcome Trust and the Parkinson's Disease Society.

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