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Mitochondrial Dysfunction in Neurodegeneration

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Numerous toxins are known to interfere with mitochondrial respiratory chain function. Use has been made of these in the development of pesticides and herbicides, and accidental use in man has led to the development of animal models for human disease. The propensity for mitochondrial toxins to induce neuronal cell death may well reflect not only their metabolic pathways but also the sensitivity of neurons to inhibition of oxidative phosphorylation. Thus, the accidental exposure of humans to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and to 3-nitropropionic acid has led to primate models of Parkinson's disease and Huntington's disease, respectively. These models were made all the more remarkable when identical biochemical deficiencies were identified in relevant areas of humans suffering from the respective idiopathic diseases. The place of complex I deficiency in Parkinson's disease remains undetermined, but there is recent evidence to suggest that, in some cases at least, it may play a primary role. The complex II/III deficiency in Huntington's disease is likely to be secondary and induced by other pathogenetic factors. The potential to intervene in the cascade of reactions involving mitochondrial dysfunction and cell death offers prospects for the development of new treatment strategies either for neuroprotection in prophylaxis or rescue.

KEY WORDS: Mitochondria; MPTP; Parkinson's disease; Huntington's disease; Alzheimer's disease.

TOXIN MODELS OF PARKINSON'S DISEASE

A variety of toxins have been shown to give rise to selective neuronal loss and/or a parkinsonian syndrome in either humans, other primates, or rodents. These agents include manganese, iron, cyanide, azide, β-carbolines, isoquinolines, and MPTP.

MPTP

Since the original observation of a parkinsonian syndrome in some drug addicts inadvertently taking a synthetic heroin substitute contaminated with MPTP (Ballard *et al.*, 1985), MPTP parkinsonism has become the best known model of Parkinson's disease. Administration of MPTP to primates and other species has been shown to cause selective destruction of nigrostriatal dopaminergic neurons. The specificity for dopaminergic neurons resides in the uptake and conversion characteristics of MPTP. MPTP readily crosses the blood brain barrier and is converted to MPP^+ by the action of monoamine oxidase B which is particularly active in astrocytes in catecholaminergic regions of the brain where it is located on the outer mitochondrial membrane. MPP^+ may be distributed throughout the brain; however, because it is a substrate for the dopamine transporter, MPP^+ is specifically concentrated into dopaminergic neurons. The removal of MPTP toxicity by deprenyl or mazindol demonstrates the importance of the conversion by MAOB and the specific uptake by the dopamine transporter to MPTP neurotoxicity.

Once inside the nerve terminal MPP^+ may either be transported into synaptic vesicles or be concentrated

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into mitochondria. This involves passive Nerstian transport using the electrochemical gradient across the mitochondrial inner membrane. There is extensive evidence that MPP^+ specifically inhibits complex I activity of the mitochondrial respiratory chain (Nicklas *et al.*, 1985; Ramsay *et al.*, 1986), preventing electron flow from NADH to ubiquinone at the same or similar site to the classical complex I inhibitors rotenone and piericidin A (Ramsay *et al.*, 1991). However, MPP^+ is a relatively poor reversible inhibitor of complex I activity, which may explain why it only has a pronounced effect in cells which can concentrate it; in, addition these higher concentrations may need to be sustained for greatest effect. MPP^+ accumulates in the substantia nigra and is eliminated from other regions (Irwin and Langston, 1985). It has been suggested that MPP^+ associates with neuromelanin, maintaining higher concentrations in the nigrostriatal region enhancing the ATP depletion due to mitochondrial inhibition leading to cell death.

Although MPP^+ has been shown to inhibit complex I activity, decreased ATP synthesis may not be its only effect. There is evidence that free radicals may also be involved. Mouse brain mitochondria incubated with MPTP induced superoxide formation (Rossetti *et al.*, 1988), and transgenic mice overexpressing the SOD1 gene were resistent to MPTP (Przedborski *et al.*, 1992), suggesting oxidative stress contributes to MPTP toxicity. It has been suggested that redox cycling between $MPDP^+$ and MPP^+ is the source of free radicals. An alternative mechanism of free radical release involves the mitochondrial respiratory chain (MRC). The MRC has been known for some time to be a significant source of cellular superoxide as single electrons leak from the respiratory chain to molecular oxygen (Boveris *et al.*, 1972). When the MRC is inhibited by rotenone (Takeshige and Minakami, 1979) or MPP^+ (Hasegawa *et al.*, 1990), there is a marked increase in superoxide generation. However, work by Ramsay and Singer (1992) suggests that while toxin inhibition of complex I may result in lipid peroxidation, superoxide originates from the interaction of the inhibitor with a separate site either within the complex or elsewhere within the MRC.

Although MPP^+ is a reversible inhibitor of complex I, prolonged incubation of mitochondria with MPP^+ under reducing conditions caused a progressive irreversible inhibition of complex I activity which could be prevented by a variety of free radical scavengers (Cleeter *et al.*, 1992). This suggests that not only does MPP^+ cause complex I inhibition, but it can induce oxidative stress which can lead to further inhibition of the complex and further oxidative stress. Consequently complex I deficiency and oxidative stress can persist even after the removal of the toxin, a process observed in dopaminergic neurons in culture (Michel *et al.*, 1990).

There are conflicting reports as to whether excitotoxicity plays any role in MPTP/MPP^+-induced neuronal loss. The pretreatment of rats with the NMDA receptor antagonist MK-801 prevented the loss of dopaminergic neurons following intranigral infusions of MPP^+ (Turski *et al.*, 1991). MPP^+ infused into rat striatum caused decreases in dopamine, GABA, and serotonin levels, which was partly protected by prior removal of the corticostriatal glutamatergic input and protected by MK-801, suggesting that excitotoxic mechanisms may be involved in MPP^+ toxicity (Storey *et al.*, 1992).

However, the pretreatment of mice with MK-801 failed to protect the neostriatal decrease in dopamine levels caused by the systemically given MPTP, or the neuronal loss caused by MPP^+ infusions (Sonsalla *et al.*, 1992). MK-801 failed to protect mesencephalic dopaminergic neurons from MPP^+ toxicity even at concentrations of MK-801 that protected the same neurons from NMDA induced toxicity. This implies that the toxicity of MPP^+ on dopaminergic neurons is not mediated through interaction with the NMDA glutamate receptor (Finiels-Marlier *et al.*, 1993).

There is evidence that calmodulin-dependent activation of neuronal nitric oxide synthase (NOS) may be important in MPTP/MPP^+-induced neuronal death. The selective inhibitor of neuronal NOS, 7-nitroindazole (7-NI), protects against MPTP-induced dopamine depletion in mice (Schulz *et al.*, 1995) and dopamine depletion and loss of tyrosine hydroxylase-positive neurons in MPTP-treated baboons (Hantraye *et al.*, 1996). In addition neuronal NOS knockout mice are resistant to MPTP neurotoxicity, suggesting the importance of NOS and nitric oxide in MPTP/MPP^+ neurotoxicity (Przedborski *et al.*, 1996).

There is increasing evidence that MPP^+ can initiate apoptotic cell death in a variety of cell types including PC12, cerebellar granule cells, and mesencephalic cultures (Dipasquale *et al.*, 1991; Hartley *et al.*, 1994; Mochizuki *et al.*, 1994). This is not a feature restricted to MPP^+, but characteristic features of apoptosis have been reported with other MRC inhibitors including rotenone and antimycin A. The underlying stimulus causing apoptosis is not known, but a functioning MRC is required (Wolvetang *et al.*, 1994), and apoptosis is

most pronounced at lower inhibitor concentrations, with necrosis more prominent at higher inhibitor concentrations (Hartley *et al.*, 1994). This suggests that under condition of mild inhibition where ATP synthesis is not markedly affected, it is possible that free radical generation by the MRC may be important for initiating apoptosis, whereas at higher inhibitor concentrations ATP synthesis is significantly compromised leading to catastrophic consequences for the cell and necrosis.

While MPTP is not a perfect model of idiopathic PD, it does share several important features in common with PD. The differences may reflect the time course of the degeneration, but it may also reflect a different mechanism of cell death.

Cyanide and Azide

In humans cyanide toxicity, if it does not prove fatal can lead to a parkinsonian syndrome involving generalized rigidity and bradykinesia (Rosenberg *et al.*, 1989, Uitti *et al.*, 1985). In one case this was associated with degeneration of the globus pallidus and putamen; there was a complete loss of nerve cells in the zona reticularis of the substantia nigra, but the zona compacta was intact (Uitti *et al.*, 1985). In a second patient MRI revealed lesions in the globus pallidus and posterior putamen and 6-fluorodopa uptake was similar to that found in idiopathic PD patients (Rosenberg *et al.*, 1989). Cyanide given to monkeys resulted in bilateral lesions of the basal ganglia (Hurst, 1942), and azide injected intramuscularly into monkeys resulted in lesions to the putamen and changes to the substantia nigra (Mettler, 1972). Cyanide and azide are both inhibitors of cytochrome oxidase (complex IV) of the mitochondrial respiratory chain; however, in the absence of a mechanism to target these inhibitors to the basal ganglia the sensitivity of this region to these inhibitors presumably reflects the sensitivity of this region to impaired ATP supply.

Iron

Stereotactic infusions of iron into the substantia nigra of rodents result in the loss of neurons in the SN and decreased striatal dopamine levels (Ben Schacher and Youdim, 1991). A single low dose iron infusion induced progressive histologic, neurochemical, and rotational behavioral abnormalities (Sengstock *et al.*, 1992). PC12 cells exposed to increased iron concentrations stop dividing and exhibit decreased activities of complexes I and IV of the mitochondrial respiratory chain (Hartley *et al.*, 1993).

Manganese

Manganese toxicity presents clinical symptoms similar to those of Parkinson's disease. The symptoms are progressive and responsive to L-dopa therapy, although unlike idiopathic PD patients they do not develop the on-off fluctuations and dyskinesia (Huang *et al.*, 1993). Rhesus monkeys injected intravenously with manganese developed a parkinsonian syndrome characterized by bradykinesia, rigidity, and dystonia which was not L-dopa responsive. Neuronal loss primarily occurred in the globus pallidus and substantia nigra pars reticulata but the nigrostriatal dopaminergic system was spared (Olanow *et al.*, 1996). This suggests that manganese-induced parkinsonism is quite distinct from either idiopathic PD or MPTP-induced parkinsonism.

β-Carbolines

β-Carbolines can be produced by the condensation of tryptophan and aldehydes and have been suggested as possible endogenous PD neurotoxins. There is some doubt about whether β-carbolines inhibit MRC activities. While β-carbolines were reported to inhibit the activities of complexes I and II (Fields *et al.*, 1992) this was not fully substantiated by Krueger *et al.*, (1993) who reported that 2,9-dimethylharmaninium and 2,9-dimethylnorharmaninium preferentially inhibited NADH-linked substrate oxidation; however, when submitochondrial particles were used, complex I activity was only partially inhibited, suggesting that the β-carbolinium compounds were acting indirectly upon the MRC (i.e., affecting substrate transport).

Isoquinolines

Isoquinoline derivatives structurally related to MPTP are formed within the brain by a condensation of biogenic amines with aldehydes. They have been detected in human brains (Kohno *et al.*, 1986; Makino *et al.*, 1990; Niwa *et al.*, 1991; Ohta *et al.*, 1987) although the levels were not significantly raised in PD brains.

A variety of isoquinoline derivatives have been shown to inhibit mitochondrial complex I activity, with some as potent as MPP^+ (McNaught *et al.*, 1995a; Suzuki *et al.*, 1988, 1992). However, with intact liver mitochondria the inhibition of NADH-linked substrates was less potent than MPP^+, suggesting that they do not generally gain entry to mitochondria as effectively as MPP^+ (McNaught *et al.*, 1996a), although the potency was reported as similar to that of MPP^+ in a separate report using brain mitochondria (Suzuki *et al.*, 1990). There are also reports that isoquinolines inhibit α-ketoglutarate dehydrogenase (McNaught *et al.*, 1995b; Suzuki *et al.*, 1988) and monoamine oxidase activities (Thull *et al.*, 1995).

A variety of isoquinoline derivative compounds have been shown to be toxic to PC12 cells (Maruyama *et al.*, 1993; McNaught *et al.*, 1996b), with their toxicity reflecting their affinity for the dopamine transporter, suggesting that their ability to concentrate into the neurons is more important than their ability to inhibit mitochondrial function (McNaught *et al.*, 1996a,b). N-methylated and oxidized isoquinoline derivatives were generally more toxic (Maruyama *et al.*, 1993), and there is evidence that the substantia nigra region contains the activities for N-methylation (methyltransferase) and oxidation of isoquinolines (monoamine oxidase) (Naoi *et al.*, 1993). Consequently structures similar to MPP^+ could accumulate in the SN region and contribute to the neuronal loss in PD.

There is evidence that some isoquinoline derivatives cause specific pathology when given to rodents. Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) and related compounds were injected into the rat striatum; however, only N-methyl-(R)-salsolinol induced behavioral changes similar to PD, including stiff tail and twitching at rest (Naoi *et al.*, 1996). Dopamine and noradrenaline were decreased in the striatum and more markedly in the substantia nigra with a decrease in the number of tyrosine-hydroxylase containing neurons. This compound has been shown to be present in human brains (Niwa *et al.*, 1991), and its selective toxicity to dopaminergic neurons indicates it is a potential candidate in PD.

Several other *in vivo* models of isoquinoline administration have also been shown to target the dopaminergic region of the brain. These include the injection of 1-benzyl-1,2,3,4-tetrahydroisoquinoline to mice, a compound found in human CSF and raised in some patients with PD, which induced bradykinesia and responded to L-dopa (Kotake *et al.*, 1995). Equally, long-term administration of tetrahydroisoquinolines to monkeys produced a parkinsonian syndrome causing decreased dopamine levels and decreased tyrosine hydroxylase activity in the nigrostriatal region (Nagatsu and Yoshida, 1987; Yoshida *et al.*, 1990). Intraventricular infusions of 1,2,3,4-tetrahydro-2-methyl 4,6,7-isoquinolinetriol into rats caused a decrease in striatal dopamine and decreased noradrenaline in the locus coeruleus with a similar potency as MPTP (Liptrot *et al.*, 1993). N-methyl-tetrahydroisoquinoline and N-methyl-tetrahydroisoquinolinium ion were both toxic to mesencephalic dopaminergic neurons, but less toxic than MPP^+ (Nishi *et al.*, 1994).

Consequently not only are isoquinoline derivatives found in the brain, but many have been shown to inhibit complex I activity and cause selective damage to the nigrostriatal region and are therefore good candidates for causes of PD.

Toxin Models of Huntington's Disease

Excitotoxic lesions generated in primates using NMDA receptor agonists like quinolinic acid cause neuronal loss similar to that found in HD, emphasizing the potential role of excitotoxicity in HD (Beal *et al.*, 1986).

Intrastriatal injections of the reversible succinate dehydrogenase inhibitor, malonate, into rats caused a decrease in ATP levels and increased lactate and excitotoxic lesions which could be prevented by NMDA antagonists (Beal *et al.*, 1993; Greene *et al.*, 1993; Henshaw *et al.*, 1994).

3 nitropropionic acid (3NP), an irreversible inhibitor of succinate dehydrogenase (complex II) of the mitochondrial respiratory chain, when administered to rats or primates can also result in selective neuronal loss. In China children exposed to 3NP from a fungal contaminant of sugar cane developed gastrointestinal disturbances followed by encephalopathy and coma, which was followed by dystonia upon recovery. In mice 3NP caused bilateral symmetric lesions of the caudate and putamen not dissimilar to that seen with excitotoxins (Gould and Gustine, 1982; Hamilton and Gould, 1987). When administered chronically, 3NP resulted in striatal neuronal loss and similar to HD NADPH diaphorase neurons were spared (Beal *et al.*, 1993), and in primates chronic administration produced apomorphine-inducible movement disorder involving choreiform and dystonic movements similar to HD with lesions similar to those found in HD (Brouillet *et al.*, 1993). NMDA antagonists could partially

protect cerebellar granule cells from the toxicity of 3NP, suggesting excitotoxicity was involved in 3NP toxicity although it is not the only mechanism.

In the presence of MRC inhibitors, no toxic concentrations of excitatory amino acids become toxic (Novelli *et al.*, 1988; Zeevalk and Nicklas, 1991). Mitochondrial respiratory chain inhibition will result in decreased cellular ATP synthesis, which will affect cellular functions. In the neuron the Na^+/K^+ ATPase has a major requirement for ATP, and consequently MRC inhibition may result in impaired function and a partial depolarization of the neuron, which may alleviate the voltage dependent Mg^{2+} block of the NMDA receptor. Under these conditions relatively low concentrations of glutamate could cause persistent receptor activation, resulting in increased intracellular Ca^{2+} concentrations and activation of a variety of cellular functions leading to excitotoxicity.

PARKINSON'S DISEASE

MPTP inhibition of complex I through its metabolite MPP^+ was a clue to investigating mitochondrial function in Parkinson's disease (PD). The first report of a complex I defect in PD studied nine PD and nine control substantia nigra samples and found a ~35% defect in activity (Schapira *et al.*, 1989a, b, 1990a). Cumulative data from this same research group have now looked at a total of in excess of 40 PD and 40 matched control substantia nigra samples and found a specific complex I deficiency reduced by ~35% of control mean. To date no abnormality of complexes II/III or IV function has been identified. A further study has also found a selective complex I deficiency to a similar degree in PD substantia nigra (Janetsky *et al.*, 1994).

The identification of the complex I defect in PD substantia nigra raised numerous questions regarding its anatomical specificity within the PD brain, its selectivity for CNS tissue, as well as its relationship to other neurodegenerative disorders and the possible effects of drug treatment. The anatomical specificity of the complex I defect in PD brain has been addressed in several papers (Mann *et al.*, 1992; Mizuno *et al.*, 1990; Schapira *et al.*, 1990b). In summary, no mitochondrial defect has been found in frontal cortex, caudate, putamen (Cooper *et al.*, 1995), tegmentum, or substantia innominata (Gu *et al.*, personal communication). Thus, in anatomic terms the mitochondrial defect is highly selective for the substantia nigra in PD brain. The absence of complex I deficiency in the substantia innominata implies that the mitochondrial abnormality is insufficient to be the sole cause of all types of neuronal cell death in PD. The substantia innominata is a cholinergic area degenerate in PD. This observation has led to the suggestion that the mitochondrial abnormality is part of a "two hit" etiology for PD.

The biochemical studies performed to date have been undertaken on tissue homogenates and thereby reflect the mitochondrial defect in both neurons and glia. Because neurons constitute only a very small proportion of the substantia nigra cell population at the time of death (probably <3%), it is likely that glial mitochondrial function is also impaired. An immunohistochemical study of complex I in PD has suggested that there is decreased immunoreactive material in dopaminergic neurons (Hattori *et al.*, 1991).

The lack of any mitochondrial abnormality in the substantia innominata in PD suggests that the defect identified in substantia nigra is not simply the result of neuronal degeneration. This is further supported by the fact that no mitochondrial defect is seen in the substantia nigra from patients with multiple system atrophy (MSA) (Gu *et al.*, 1997; Schapira *et al.*, 1990b). The substantia nigra is severely degenerate in MSA and so one would expect that if the mitochondrial defect were simply a reflection of cell loss, then the same abnormality should be identified in this tissue.

A further consideration is whether the complex I deficiency in PD could be the result of L-dopa therapy. Indeed L-dopa given to normal rats has been shown to produce a mild and reversible deficiency of complex I activity (~20%; Przedborski *et al.*, 1993). L-Dopa induced the same biochemical defect in the rat striatum as well as substantia nigra. However, evidence from MSA patients who had taken L-dopa in comparable quantities and duration as PD patients together with the absence of any mitochondrial defect in PD striatum argues against L-dopa being the cause of the complex I defect in PD. Nevertheless, it is possible that this therapy may enhance a pre-existing mitochondrial abnormality.

There have also been reports of mitochondrial abnormalities in skeletal muscle and in platelets from patients with PD (see Schapira, 1994 for review). The studies on skeletal muscle are conflicting and no clear consensus has emerged. *In vivo* magnetic resonance spectroscopy has also failed to produce clear results, with one study showing no abnormalities (Taylor *et al.*, 1994) and another showing multiple defects (Penn *et al.*, 1995). In contrast the platelet studies have, in

the majority, shown a specific complex I deficiency in PD. However, the sensitivity and specificity of the defect is insufficient on a group-to-group analysis to provide any basis for it to be considered a biochemical marker. The implications for identifying a mitochondrial defect in peripheral tissues are important and would suggest either an underlying genetic component resulting in an abnormality of complex I activity or alternatively the effect of a widespread toxin action. In this respect it is important to acknowledge that dopaminergic neurons and platelets have some properties in common, e.g., the presence of monoamine oxidase B and the ability to take up MPP^+. Thus, platelets might express biochemical and pharmacological properties which render them and dopaminergic neurons susceptible to similar toxic influences.

Several studies have sought to address the question of whether the mitochondrial defect might be primary or secondary. A primary effect may be determined by a molecular genetic defect of one or more complex I genes. Although only 7 of the 41 subunits of human complex I are encoded by mitochondrial DNA (mtDNA), the ease with which this molecule can be investigated led to a number of studies on mtDNA structure in PD. Although an initial report suggested that the 4.9-kb "common" mtDNA deletion was present in increased quantities in Parkinson's substantia nigra (Ikebe *et al.*, 1990), this was subsequently found not to be the case when control brains were matched for age (Lestienne *et al.*, 1991; Schapira *et al.*, 1990c). MtDNA has been sequenced in PD patients and polymorphisms identified (Ozawa *et al.*, 1991; Shoffner *et al.*, 1993). However, the relevance of these is uncertain and their findings have not been reproduced in all studies (Lücking *et al.*, 1995). A recent study utilizing rho^0 and PD platelet fusion cybrids has demonstrated that the complex I defect present in PD platelets may be transferred to the fusion cybrids (Swerdlow *et al.*, 1996). This result implies that the mitochondrial defect is determined by mtDNA. Results from our own laboratory support this finding (Gu *et al.*, personal communication).

To date the only nuclear gene abnormality associated with PD is linkage to an unidentified gene on chromosome 4q21–23 in a large American/Italian kindred with autosomal dominant parkinsonism (Polymeropoulos *et al.*, 1996). The causative gene defect has not been determined but will clearly provide valuable insight into the biochemical mechanisms that can result in selective dopaminergic cell death, at least in this particular family.

There is persuasive evidence that oxidative stress and damage are also involved in PD (see Olanow, 1993 for review). As described above, there is a close and reciprocal relationship between mitochondrial dysfunction and free radical generation. Generally free radicals induce a complex IV deficiency followed by a complex I defect. On this basis it is unlikely that the mitochondrial abnormality in PD is caused solely by free radicals. Alternatively, complex I inhibition and increased superoxide generation may exacerbate the complex I defect. These factors could induce a self-amplifying cycle in PD substantia nigra.

HUNTINGTON'S DISEASE

Huntington's disease (HD) is caused by an unstable CAG trinucleotide repeat in the IT15 gene on chromosome 4. This encodes an unknown protein (huntingtin) of approximately 340 kDa. Huntingtin is widely expressed in peripheral tissues as well as in the brain and both mutant and wild type forms are present in HD patients.

Evidence for an abnormality of energy metabolism in HD has been available for some time. Both striatal and cerebral cortex glucose metabolism is decreased in HD and appears to precede a bulk tissue loss (Jenkins *et al.*, 1993; Koroshetz *et al.*, 1994). Elevated lactate pyruvate ratios in HD CSF have also been observed although these appear to be related more to a reduction in pyruvate than an elevation in lactate levels (Koroshetz *et al.*, 1993).

Of particular interest is the recent demonstration of severe deficiency (56%, $p < 0.0005$) in the activities of complexes II and III and a milder (33%, $p < 0.01$) deficiency in cytochrome oxidase activity in HD caudate nucleus (Gu *et al.*, 1996). This finding provides a direct biochemical parallel between the complex II mitochondrial toxins (malonate, 3NP) which are used to produce animal models of HD. The recent description of abnormal magnetic resonance spectroscopy in skeletal muscle from patients with HD (Koroshetz *et al.*, 1997) raises the possibility that the CAG triplet repeat might be responsible for the mitochondrial defect, as one would expect that the peripheral expression of a mitochondrial deficiency in a clinically unaffected tissue would be related to the widely distributed mutant protein. However, another study using immunostaining with antibodies to the ND1 subunit of complex I, cytochrome oxidase subunit 2 and SDH showed

no abnormality in distribution or intensity between controls and HD patients (Chen and Okayama, 1987).

ALZHEIMER'S DISEASE

As in HD, there is evidence for a defect of energy metabolism in Alzheimer's disease (AD) (Blass and Gibson, 1993; Brown *et al.*, 1989; Duara *et al.*, 1986; Haxby *et al.*, 1986; Sims *et al.*, 1987). For instance, there is evidence from positron emission tomography (PET) that glucose metabolism is depressed in the parietal, temporal, and posterior cingulate cortices and this glucose utilization declines progressively with time (McGeer *et al.*, 1990; Mielke *et al.*, 1994; Smith *et al.*, 1992). Of particular interest is the recent demonstration by PET that cognitively normal individuals homozygous for the apoliprotein $\epsilon 4$ allele, which carries a substantial risk of subsequent development of AD, had significantly reduced rates of glucose metabolism in a pattern identical to AD patients (Reiman *et al.*, 1996).

Deficiencies of complexes II and IV have been described in AD hemibrains (Parker *et al.*, 1994) although other studies have shown either less severe defects in a different distribution or no abnormalities at all (Cooper *et al.*, 1993; Kish *et al.*, 1992). A further more comprehensive study published recently found a decline in the activities of complexes I–IV in occipital cortex and less severe deficiency in frontal and temporal cortex (Mutisya *et al.*, 1994). These findings were generally confirmed by a further study looking at frontal and parietal cortex in AD patients (Chagnon *et al.*, 1995). Cytochrome oxidase staining has also been found to be decreased in AD hippocampus (Simonian and Hyman, 1993) together with a reduction in mRNA levels of the mitochondrial encoded subunit 2 of cytochrome oxidase (Simonian and Hyman, 1994). Thus there is a consensus for a mitochondrial deficiency in AD temporal cortex although the defective site of the respiratory chain does not appear to be as specific as, for instance, that seen in PD or HD.

One report has suggested that a mtDNA polymorphism at position 4336 in a tRNA gene for glycine was increased in AD and PD brain (Hutchin and Cortopassi, 1995; Shoffner *et al.*, 1993), but this has not been reported by other studies. There is also evidence for increased oxidative damage to mitochondrial DNA in AD brain (Mecocci *et al.*, 1994) although this abnormality does not appear necessary to parallel the site of the cytochrome oxidase deficiency. There is also extensive evidence for the presence of oxidative stress and damage in AD (Schapira, 1996). The pattern of mitochondrial deficiency in AD is more in keeping with the secondary effect of free radicals than, for instance, the mitochondrial defect in PD or HD.

CONCLUSION

There is accumulating evidence for mitochondrial dysfunction in a number of neurodegenerative disorders. In some this is likely to be the secondary effect while in others the relationship may be more primary. The remarkable similarity in the biochemical defects induced by specific neurotoxins (MPP^+, 3NP, malonate) and the biochemical defects identified in the corresponding human disorders (PD, HD) suggests a primary role in these circumstances. The role of mtDNA or of nuclear encoded respiratory chain subunits in these disorders has yet to be established, but some evidence is now available that mtDNA may be responsible for the complex I deficiency in at least a proportion of PD patients.

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