

Role of Mitochondria in Parkinson's Disease

Neuropathology 2000

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8 September 2000, London, England, UK



Imperial College

OF SCIENCE, TECHNOLOGY AND MEDICINE

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The meeting has been approved by the Royal College of Pathologists for CPD purposes at a rate of 6 credits.

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The Wellcome Trust**Cover**

From „The Villager’s Friend and Physician“ by James Parkinson (possibly portraying himself; for reference see „James Parkinson, 1755-1824: From Apothecary to General Practitioner“ by Shirley Roberts, London: Royal Society of Medicine Press, 1997, 136 p)

Time Table, Friday, 8 September 2000

- 8:50 **Welcome and Introduction**
M.B. Graeber (*London/UK*)
- 9:00 **Neuropathology and differential diagnosis of Parkinson's disease**
P.L. Lantos (*London/UK*)
- 9:40 **The complex I defect in Parkinson's disease**
A.H.V. Schapira (*London/UK*)
- 10:20 **Coffee break**
- 10:40 **Cybrid analysis of mitochondrial DNA in parkinsonian twins**
G. Hofhaus (*Düsseldorf/Germany*)
- 11:20 **Mitochondria and cell death**
G. Kroemer (*Villejuif/France*)
- 12:00 **Lunch break**
- 13:00 **Matrilineal inheritance of Parkinson's disease**
R. Swerdlow (*Charlottesville/USA*)
- 13:40 **Sequence analysis of mitochondrial genes in Parkinson's disease**
S. Kösel (*Martinsried/Germany*)
- 14:20 **Coffee break**
- 14:40 **Free radicals and Parkinson's disease**
P. Jenner (*London/UK*)

- 15:20 **Alternative expression of K-ATP channel subunits by dopaminergic midbrain neurons**
J. Roeper (*Oxford/UK*)
- 16:00 **Anti-apoptotic therapy in Parkinson's disease**
H. Mochizuki (*Tokyo/Japan*)
- 16:40 End of Meeting

The neuropathology and differential diagnosis of Parkinson's disease

P.L. Lantos, Department of Neuropathology, Institute of Psychiatry, King's College London, UK

Macroscopically the core pathology of Parkinson's disease consists of pallor of the brainstem pigmented nuclei particularly the substantia nigra and the locus coeruleus, and microscopically includes the following histological changes: Lewy bodies, loss of pigment, neuronal loss, abnormal neurites, astrogliosis and microglial activation. Lewy bodies are the histological hallmark: they occur not only in the pigmented nuclei of the brainstem, but also in much wider distribution to include other deep grey structures, the autonomic nervous system and the cerebral cortex. Ultrastructurally these inclusions are composed of filamentous, granular and vesicular structures and they immunostain for cytoskeletal proteins, α -synuclein, ubiquitin, chromogranin A and synaptophysin. The widespread occurrence of Lewy bodies in the cerebral cortex results in dementia with Lewy bodies, a more recently defined clinicopathological entity. The immunoreaction for α -synuclein reveals the full spectrum of Lewy body pathology, and idiopathic Parkinson's disease and dementia with Lewy bodies, together with multiple system atrophy now form a disease group referred to α -synucleinopathies. Moreover a mutation link to chromosome 4q21-23 is associated with autosomal Parkinson's disease, and Ala53Thr has been identified in *SNCA*, the gene encoding α -synuclein.

Idiopathic Parkinson's disease clinically may be confused with two other neurodegenerative disorders: multiple system atrophy (MSA) and progressive supranuclear palsy (PSP). However, neuropathological examination reveals a completely different histology: tau-positive neurofibrillary tangles and glial inclusions are the hallmarks of PSP, while glial cytoplasmic inclusions are pathognomonic of MSA.

Cybrid analysis of mitochondrial DNA in Parkinsonian twins

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There is growing evidence that mitochondrial malfunctions caused by mutations of mitochondrial DNA are involved in a variety of disorders - and possibly include neurodegenerative diseases such as Parkinson's (PD). The peculiar genetics of mitochondrial DNA (mtDNA) could explain the late onset of the disease, the lack of inheritance found in the majority of cases and the phenomenon that the onset of the disease can differ between monozygotic twins. In an attempt to identify differences in the mtDNA of PD twins, we investigated twin-pairs in which only one sibling showed symptoms of the disease. We transferred their mtDNA into a permanent human cell line. The resulting transmitochondrial cell lines were analysed with respect to their mitochondrial respiration and revealed differences in one of the pairs. The results can be explained by an as yet undiscovered heteroplasmic mutation in their mtDNA. Analysis of mitochondrial translation points to a mitochondrial tRNA mutation. Work to identify the underlying mutation is underway.

Mitochondria and cell death

G. Kroemer, CNRS-UMR 1599, Institut Gustave Roussy, Villejuif, France

The mitochondrial permeability transition pore complex (PTPC), also called mitochondrial megachannel, is a multiprotein complex formed at the contact site between the mitochondrial inner and outer membranes, exactly at the same localization at which proteins of the Bcl-2/Bax family are particularly abundant. We have obtained four independent lines of evidence suggesting the implication of the PTPC in apoptosis. First, in intact cells, apoptosis is accompanied by an early permeabilization of mitochondrial membranes. In several models of apoptosis, specific agents inhibiting the PTPC abolish the activation of downstream caspases and endonucleases, indicating that PTPC opening is a critical event of the apoptotic process. Second, mitochondria are rate-limiting for caspase and nuclease activation in several cell-free systems of apoptosis. Isolated mitochondria release apoptogenic factors capable of activating pro-caspases or endonucleases upon opening of the PTPC in vitro. Third, opening of the purified megachannel reconstituted into liposomes is inhibited by recombinant Bcl-2 or Bcl-XL, two apoptosis-inhibitory proteins which also prevent PTPC opening in cells and isolated mitochondria. Fourth, an increasing number of endogenous, viral, or xenogeneic effectors directly act on the PTPC to trigger the apoptotic cascade. Altogether, our results suggest that PTPC opening is sufficient and (mostly) necessary for triggering apoptosis.

Matrilineal inheritance of Parkinson's disease

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Cybrid studies suggest mitochondrial dysfunction in Parkinson's disease (PD) results from either acquired or inherited mutation of mitochondrial DNA (mtDNA). If inherited, epidemiologic analysis may reveal evidence of maternal transmission. In one PD clinical database, after correcting for male predominance there was a statistically significant overrepresentation of PD affected mothers, which is consistent with mitochondrial inheritance in some of the ascertained cases. This finding is consistent with a prior PD clinical database study in which the PD-affected parents of prospectively ascertained PD sib-pairs were overwhelmingly maternal, and with reports of multicase, multigenerational families in which PD transmission respects maternal lines. If matrilineal inheritance does indeed occur in PD and is driven by mtDNA aberration, several types of mtDNA aberration are possible. Association studies suggest certain mtDNA polymorphisms may appear more commonly in some PD populations than in non-PD controls. Reports of maternally inherited parkinsonism in families carrying known mtDNA mutations exist. The sporadic epidemiology of most PD further suggests a potential role for low abundance or compound mtDNA heteroplasmies. Further epidemiology and mtDNA studies in PD will hopefully provide increasing insight into this important issue.

Sequence analysis of mitochondrial genes in Parkinson's disease

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Recent molecular genetic and biochemical data support the concept of a genetically heterogeneous basis of Parkinson disease (PD). Pedigree studies as well as biochemical analyses have shown that mutations of the mitochondrial genome play a role in PD pathogenesis in a subset of patients. We have performed a complete sequence analysis of all mitochondrial complex I and tRNA genes in 22 cases of neuropathologically confirmed idiopathic PD. In addition, five pairs of monozygotic twins were studied, four of which were discordant for PD at the time of molecular genetic analysis. DNA, extracted from the substantia nigra of histologically proven PD cases, revealed seven novel missense mutations in complex I subunit genes ND1 (3992 C/T, 4024 A/G), ND4 (11253 T/C, 12084 C/T), ND5 (13711 G/A, 13768 T/C) and ND6 (14582 T/C), four of which affect the ND4 and ND5 genes and are unique to PD. Furthermore, five known missense mutations, three secondary LHON mutations and 43 synonymous polymorphisms were present in PD brains which included 20 novel sequence variants. In addition to six known polymorphisms, two new mutations were found in the genes for tRNA(Thr) (15959 G/A) and tRNA(Pro) (15965 T/C). Among the new mutations, the tRNA sequence variants and the ND4 11253 T/C transition which changes a conserved isoleucine residue into threonine are most likely to increase the susceptibility to PD. In monozygotic twins, two of five new sequence changes represent missense mutations (ND2 4924 G/A, ND3 10192 C/T). However, mitochondrial DNA sequences were identical in diseased and non-affected siblings of each pair. Thus, our data are in line with the view that mutations of mitochondrial DNA may contribute to PD pathogenesis in a minority of PD cases but experimental studies have to be carried out to demonstrate the pathogenicity of certain mitochondrial haplotypes.

Cell death mechanisms in Parkinson's disease

P. Jenner, Neurodegenerative Diseases Research Centre, GKT School of Biomedical Sciences, King's College, London, UK

The mechanisms underlying cell death in Parkinson's disease remain unknown but oxidative stress, excitotoxicity and mitochondrial dysfunction may be important. Free radical action leads to oxidative damage in Parkinson's disease as shown by increased lipid peroxidation, DNA oxidation and protein carbonyl formation. The latter may be important as increased protein oxidation occurs all over the brain in Parkinson's disease yet leads only to pathology in selected brain areas. Similarly, in some cases of familial Parkinson's disease, there is also a general expression of mutant alpha-synuclein which again leads to selective nigral cell death. This leads to the concept that alterations in proteolysis in both sporadic and familial Parkinson's disease may be important in inducing nigral pathology. Indeed, over-expression of mutant alpha-synuclein in cell lines increases vulnerability to toxic insults produced by mitochondrial inhibitors and free radical species. Indeed, following inhibition of proteasomal function, cell vulnerability to the presence of mutant proteins increases and there is evidence that proteasomal function may be inhibited in substantia nigra in Parkinson's disease. Altered proteolysis may result in the formation of intracellular vesicles in which proteinous material is stored to prevent cellular toxicity and these may be the reason why Lewy bodies form in Parkinson's disease. Why alterations in proteolysis should selectively affect the substantia nigra is not clear but the high levels of basal oxidative stress and protein oxidation may lead to damage to the proteasome. This coupled to the presence of a high load of abnormal or mutant protein may serve to prevent normal proteolysis so starting the cascade of events underlying cell death in Parkinson's disease.

Alternative expression of K-ATP channel subunits by dopaminergic midbrain neurons

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Mitochondrial dysfunction, in particular complex I (CX I) inhibition, is a trigger mechanism for dopaminergic (DA) neurodegeneration in Parkinson's Disease. However, the pathophysiological response of DA neurons to CX I inhibition remains to be defined. In this context, ATP sensitive potassium (K-ATP) channels are attractive targets as they directly couple the metabolic state of neurons to their functional electrical activity. The K-ATP channels consist of pore-forming Kir6.1 or Kir6.2 subunits in combination with sulfonylurea receptor isoforms (SUR1, SUR2A or SUR2B). With patch-clamp and single-cell RT-PCR techniques we have previously shown that mouse dopaminergic (DA) neurons in the Substantia Nigra (SN) possess different types of functional K-ATP channels composed of Kir6.2 in combination with SUR1 or SUR2B subunits. This alternative SUR expression was correlated with major differences in acute response to mitochondrial complex I inhibition. Only SUR1-K-ATP channels were activated by partial complex I inhibition, which completely suppressed normal electrical activity (Liss et al, 1999a). In the *weaver* mouse, a genetic model of dopaminergic degeneration, surviving DA neurons exclusively expressed SUR1 and displayed tonically activated K-ATP channels (Liss et al., 1999b). We now studied the response of DA neurons to CX I inhibition, induced by MPP⁺ or rotenone, in K-ATP (Kir6.2) knockout (-/-) and control (+/+) mice. Only in control mice, MPP⁺ induced membrane hyperpolarization and complete loss of pacemaker activity in SUR1-expressing DA neurons. In contrast, CX I inhibition did not lead to membrane hyperpolarization in Kir6.2 KO-mice. These results demonstrate that alternative K-ATP channel expression controls the acute response to mitochondrial dysfunction in DA neurons and might be a molecular mechanism for the differential vulnerability of DA neurons in Parkinson's Disease.

Liss B., Bruns R. & Roeper J. (1999a) Alternative sulphonylurea receptor expression defines metabolic sensitivity of K-ATP channels in dopaminergic midbrain neurons. *The EMBO Journal* 18: 833-846.

Liss B., Neu A. & Roeper J. (1999b) The *weaver* mouse gain-of-function phenotype of dopaminergic midbrain neurons is determined by coactivation of *wvGirk2* and K-ATP channels. *J. Neuroscience* 19: 8839-8848.

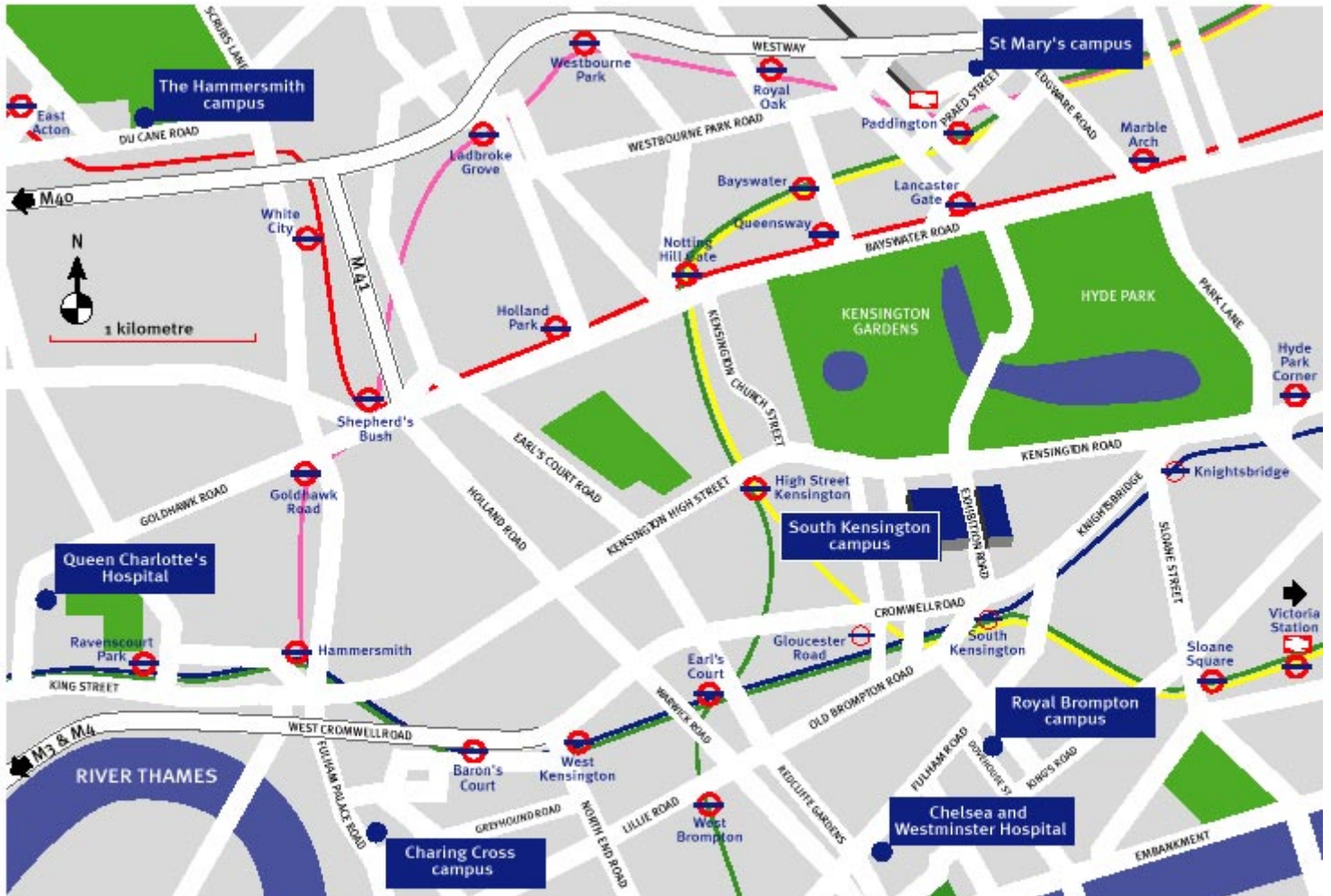
Anti-apoptotic therapy in Parkinson's disease

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The etiology of Parkinson's disease is still unknown. The molecular mechanisms that underlie the loss of dopaminergic neurons have not been determined, although it is highly probable that apoptosis is involved. New insights into the intracellular events leading to apoptosis of dopaminergic neurons, especially the role of the mitochondria, may yield additional potential therapeutic targets. Drugs that act at the mitochondrial stage of apoptosis may become new forms of therapy. Recently, the cascade of caspase-1, which is separate from the mitochondrial pathway, has also been suggested to play an important role in cell death and neurodegeneration. Therefore, anti-apoptotic therapy for Parkinson's disease, that inhibits caspase-1 enzymes may slow down the degenerative process. For effective anti-apoptotic therapy, we have to focus on the delivery system. Gene transfer with a viral vector may be a useful method for delivering anti-apoptotic therapy for Parkinson's disease in the future.

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West London locations



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