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Mitochondrial respiratory failure in skeletal muscle from 26 patients with Parkinson's Disease.

O. Blin*, C. Desnuelle, O. Rascol, M. Borg, H. Peyro Saint Paul, J.P.Azulay, F. Billé, D. Figarella, F. Pontier, J.F. Pellissier, J.L. Montastruc, G. Serratrice, Marseille, Nice, Toulouse, France

We studied mitochondrial respiratory chain function in skeletal muscle from patients with idiopathic Parkinson's disease (PD) as compared with age-matched controls in order to determine the occurence of mitochondrial respiratory chain abnormalities in de novo and treated PD patients. 26 patients with well established clinical diagnosis of PD (UKPDSBB criteria), aged 41-81, Hoehn & Yahr stage 1-5, UPDRS score 10-95, Schwab & England score 10-90%, Covi-Raskin scores 0-12, duration of the disease 1-23 years, were included in the study after they gave informed consent and approuval of the local Ethic Committee was obtained. 6 of 26 have never been previously treated by L-Dopa. Mitochondrial fractions were isolated from deltoid and biceps brachialis muscle samples. Age-matched control values were obtained from subjects evaluated for neuromuscular disease in whom no abnormality was found. The individual activity of complex I (NADH:ubiquinone reductase) complex II (succinate:ubiquinone reductase), complex III (ubiquinol-cytochrome c reductase), complex IV (cytochrome c oxidase), succinate dehydrogenase and citrate synthase were measured. Data were analysed using Mann Whitney U test and Wilcoxon signed rank test. Our results showed a significantly lower activity in complexes I, III and IV in PD patients as compared to age-matched controls. A low activity (residual activity less than 30% of controls) of complex I & III and I & IV was observed in 5 and 5 patients respectively. As compared to patients on L-Dopa treatment, de novo patients demonstrated no significant difference in the activity of complexes I, III and IV. Our results support the hypothesis of a generalized deficit in mitochondrial complexes deficiency in PD, not only related to the age or the treatment of the patients.

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Iron in pathogenesis of Parkinson's disease - Mössbauer spectroscopy studies

J. Gałązka-Friedman*[1], A. Friedman[2], E. Bauminger[3], M. Barcikowska-Litwin[4], J. Suwalski[5]

[1]Warsaw University of Technology - Institute of Physics, [2]Warsaw Medical Academy - Department of Neurology, [3]Hebrew University of Jerusalem - Racah Institute, [4]Polish Academy of Sciences, [5]Institute of Atomic Energy-Świerk, Poland

Samples of substantia nigra (SN) from parkinsonian and control brains were assessed by Mössbauer spectroscopy for identification of iron compounds.
In room temperature Mössbauer spectroscopy demonstrated ferric (Fe^{3+}) iron bound to ferritin. Measurements conducted in liquid helium temperature have shown another component biding ferric iron. The percentage amount of this compound was 25% in control SN and 50% in parkinsonian SN. In these formalin-fixed lyophylised samples we did not detect ferrous iron.
The importance of this finding for pathogenesis of Parkinson's disease will be presented.

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MICROPROBE ELEMENTAL MICROANALYSIS OF THE MPTP-TREATED VERVET MONKEY SHOWS INCREASED IRON IN THE SUBSTANTIA NIGRA

JA TEMLETT*, JP LANDSBERG, F WATT, GW GRIME
Neurology Unit, University of the Witwatersrand, Johannesburg, South Africa; and Nuclear Physics, Oxford, England.

Free radicals and trace metals may be responsible for the continued decline in dopaminergic cells of the substantia nigra (SNc).

African Green Monkeys were given intracarotid MPTP which produced *hemiparkinsonism* and ablation of the ipsilateral SNc dopamine cells. Sections of the midbrain were placed before the proton microprobe for elemental microanalysis (Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu and Zn). Adjacent sections confirmed cell location in the microprobes unstained sample. The lesioned and control sides were compared.

Multiple samples (grids 100 to 250um²) showed overall iron increased, but not other elements, on the MPTP-treated side alone. Iron levels of 531.5 (SEM 150.9; n=21) ppm differed to the normal sides 284.9 (SEM 58.5; n=26) ppm ($p<0.0002$).

To detect exactly where the excess iron was located multiple points were examined. Damaged dopamine cells were smaller, fewer in number and showed excess iron in, adjacent to the cell, as well as in the background. Cell free regions and other midbrain nuclei showed much lower iron concentration (228.3 ppm; n=6).

Since excess iron, but not other trace metals, was detected selectively in the SNc of MPTP-lesioned primates the possibility remains that free iron radicals induced MPTP dopamine cell death. This observation confirms what human postmortem iron analysis has shown in PD. Whether iron is directly responsible for or simply accompanies cell death remains to be demonstrated.

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Neurotoxicity of iron-melanin complex on dopaminergic neurons in nigro-striatal co-culture

H. Mochizuki, K. Nishi, Y. Furukawa, Y. Mizuno
Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

In Parkinson's disease, biochemical and nuclear magnetic resonance analysis have shown a selective and highly significant elevation of iron in melaninized substantia nigra dopamine neurons. Several evidences indicate that iron-melanin interaction could be crucial in the initiation of neurodegeneration of dopaminergic neurons in Parkinson's disease. The cytotoxic nature of iron and melanin on dopaminergic neuron was investigated in rat nigro-striatal co-cultures. Ventral mesencephalon and neostriatum from embryonic rat brain on the 14th day of gestation were mechanically dissociated and plated into 24 well culture dishes pretreated with polyethylenimine. Cell density in each well was 2.4×10^5/ cm² and number of cell ratio between ventral mesencephalon and neostriatum was 1:2. Experiments were performed during the days of 7 to 14 in vitro. Immunohistochemical studies using anti-tyrosine hydroxylase (TH) antibodies revealed many TH positive cells. Fe^{3+} and dopamine-melanin was added to the wells at different concentrations with or without 1.5 mM deferoxamine mesylate. After incubation with Fe^{3+} and dopamine-melanin, the number of TH positive cells decreased remarkably. There was no significant reduction in the number of TH positive cells in culture pretreated with deferoxamine mesylate. The result suggests neurotoxicity of iron-melanin complex on dopaminergic neurons in vitro.

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Free dopamine and iron contribute to the hydroxyl radical generation in the methamphetamine-induced experimental parkinsonism

Kondo, T., Sugita, Y., Kanazawa, A., Ito, T., Mizuno, Y.
Dept. of Neurol., Juntendo Univ. School of Med., Tokyo, Japan

Methamphetamine (MA) is known to be a potent releaser of amines from the terminals of respective neurons and an inhibitor of MAO. DA content in the striatum is thought to be an important factor in the degeneration of the DA terminals in the MA toxicity. We thought extracellular free DA might contribute to the hydroxyl radical formation. This question prompted us to evaluate the hydroxyl radical formation in MA-treated animals. Salicylate (SA) reacts with hydroxyl radicals to form 2,3- and 2,5-dihydroxybenzoic acids (2,3- and 2,5-DHBA). Therefore, they are good indicators of the hydroxyl radical formation in vivo. We measured DHBAs in the striata using HPLC-ECD in animals (C57BL/6 male mice) treated with MA. The TH activity in the striatum was diminished after intraperitoneal injection of MA. The pretreatment with dimethylsulfoxide (DMSO), a hydroxyl radical scavenger, partially alleviated this decrease in the TH activity. Amounts of 2,3-DHBA in the striata in mice treated with MA elevated significantly ($P<0.01$) compared to those of the controls. The 2,3-DHBA (n moles/g wwt) level in DA depleted animals (0.18±0.06) was even lower than the control mice (0.30±0.09). The 2,3-DHBA level in mice pretreated with DMSO (0.09±0.04) was significantly lower than that of the animals with the MA-treatment alone (0.60±0.18). The administration of deferoxamine, an iron chelator, suppressed the increase of 2,3-DHBA in the MA-treated mice. Our results suggest free DA and iron contribution to the hydroxyl radical generation in MA-induced experimental parkinsonism, leading to the degeneration of DA terminals.

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Differential vulnerability of pigmented and non pigmented catecholaminergic neurons in MPTP-treated monkeys

Herrero M.T., Hirsch E.C., Luquin M.R., Javoy-Agid F., Obeso J.A., Agid Y.
INSERM U289. Hôpital de la Salpêtrière, Paris. Francia. Neurología Experimental. Universidad de Navarra. España.

MPTP and its metabolite (MPP+) bind to neuromelanin (NM) raising the possibility that the presence of NM in dopaminergic neurons may directly influence their vulnerability to MPTP. We directly tested this hypothesis by a quantitative analysis of melanized neurons in the midbrain of 6 MPTP-treated cynomolgus monkeys (2 severe cases and 4 mild cases) and 2 matched control monkeys. Animals were sacrified 90 days after the last intravenous injection of MPTP. Catecholaminergic neurons were identified by tyrosine hydroxylase (TH) immunohistochemistry and neuromelanin-pigmented cells by Masson silver impregnation.
A severe loss of TH+ neurons was observed in the mesencephalon both in the severe and mild MPTP cases. In MPTP-treated monkeys, the loss of TH+ neurons was severe in the SN, 88%, intermediate in catecholaminergic region A8 and A10, 50 and 56% respectively, and minimal in the central gray substance and the locus coeruleus 10 and 13% respectively. However, numerous TH+ Masson-negative neurons also degenerate in the ventral mesencephalon. The data indicate that the loss of TH+ neurons is severe in regions with a high proportion of melanized neurons. This result suggest that NM may exacerbate the loss of TH+ neurons but, since non-melanized neurons also degenerate, NM do not seem to be the only factor provoking cell death.