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Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain

Short Note

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Summary. Significant differences in the content of iron (III) and total iron were found in post mortem substantia nigra of Parkinson's disease. There was an increase of 176% in the levels of total iron and 255% of iron (III) in the substantia nigra of the parkinsonian patients compared to age matched controls. In the cortex (*Brodmann area 21*), hippocampus, putamen, and globus pallidus there was no significant difference in the levels of iron (III) and total iron. Thus the changes in total iron, iron (III) and the iron (II)/iron (III) ratio in the parkinsonian substantia nigra are likely to be involved in the pathophysiology and treatment of this disorder.

Keywords: Total iron, iron (III), iron (II), iron (II)/iron (III) ratio, Parkinson's disease, neurotoxicity.

Introduction

Nigrostriatal degeneration in Parkinson's disease (PD), neuronal cell death in the substantia nigra (SN) and loss of innervation in the corpus striatum is accompanied by a significant reduction especially of dopamine (DA) (Ehringer and Hornykiewicz, 1960) and catecholamine related enzymes (Birkmayer and Riederer, 1985). The activity of the rate limiting enzyme, tyrosine hydroxylase (TH) is considerably reduced in the striatum and SN of PD (Lloyd et al., 1975; Nagatsu et al., 1977; Riederer et al., 1978). Optimum function of TH requires

a sufficient amount of tyrosine, oxygen, tetrahydrobiopterin and activation by iron (II) (Carlsson, 1974; Kaufmann, 1977; Ikeda et al., 1965; Nagatsu et al., 1981; Rausch et al., 1988). Administration of the co-enzyme tetrahydrobiopterin to patients with PD as a trial for enzyme stimulation did not show any benefit (Birkmayer and Riederer, 1985). In contrast, intravenously applied iron in form of a special ferric-ferrous complex was reported to have considerably therapeutical effect in most Parkinsonian patients treated so far (Birkmayer and Birkmayer, 1986). However, at present the molecular mode of action of this iron preparation is unclear. It is known, however, that compared with other regions of the brain the basal ganglia, globus pallidus, and SN are especially enriched with iron deposits (Spatz, 1922). Owing to its large neuron density with high DA turnover and metabolic activity, SN is sensitive to toxic influences (Riederer et al., 1985; Halliwell and Gutteridge, 1985). Monoamine oxidase (MAO) has a high activity in this area and generates hydrogen peroxide (H_2O_2) directly during deamination (Youdim, 1988). H_2O_2 rapidly crosses cell membranes and in many cells it may be toxic. It is excessive H_2O_2 when in contact with reduced transitional metals Fe (II) and Cu (I) that liberates free radicals, such as the highly reactive cytotoxic hydroxyl ($^{\cdot}OH$) or superoxide ($O_2^{\cdot-}$) radicals which in turn enhance lipid peroxidation (Halliwell and Gutteridge, 1985).

Increased availability of non-reactive iron (Riederer et al., 1988a, b; Dexter et al., 1987; Drayer et al., 1986; Youdim, 1988) and a shift (oxidation) of iron (II) to iron (III) may be an additional factor of enhanced vulnerability of SN towards neurotoxic events. Therefore we have examined the concentration of total iron, iron (II), iron (III), and the iron (II)/iron (III) ratio in various brain areas of PD and controls.

Postmortem tissue and methods

Brain tissue (SN, putamen, hippocampus, globus pallidus, and cortex (*Brodmann area 21; BA 21*) from 8 patients with PD [4 male, 4 female; mean age 75.3 years; range 66 to 86 years, postmortem time, 40.7 ± 26.6 (range 11–78) hours; duration of PD 7.5 ± 3.4 (range: 2–12) years] and from neurologically normal control subjects [4 male, 4 female; mean age 71.3 ± 12.5 years; range 51–91 years, postmortem time, 26.1 ± 23.3 (range 5–79) hours] were obtained at autopsy and dissected according to a standard protocol by a neuroanatomist. The brain areas were quickly frozen at —80 °C until analysis. Diagnosis was confirmed in all cases by pathological and neuropathological examination. In PD drug therapy consisted of combined L-DOPA therapy (1-DOPA plus the peripherally acting decarboxylase inhibitor benserazide, amantadine sulfate, and anticholinergics). Controls had died without any evidence of neurological or psychiatric disease. All brains were examined histologically by routine staining methods and were diagnosed by a neuropathologist. Drug treatment consisted of cardiovascular active drugs and antibiotics. In six cases of either controls or PD the examination of DA in the striatum showed a severe depletion of the amine ranging between 90.4% (CN) and 97% (putamen) indicating a near total denervation. The cause of death (PD group) was bronchopneumonia (n = 6), pulmonary embolism after leg vein thrombosis and hypertensive heart disease (n = 1), and cardic arrest after colon carcinoma (n = 1). In controls the cause of death was bronchopneumonia (n = 2), myocardial infarction (n = 1), pulmonary thromboembolism and arteriosclerotic cerebro-vascular dis-

ease (n = 1), coronary arteriosclerosis and old infarction (n = 1), coronary thrombosis (n = 1), cor pulmonale, chronic bronchitis and emphysema (n = 1), and pleural mesothelioma (n = 1).

The determination of total iron, and iron (II) in brain tissue was done using a modification of the spectrophotometric method by Siedel et al. (1984). Iron (II) was then determined by using the iron (II) chelator ferrozine® (commercial kit obtained from Boehringer Mannheim GmbH, Federal Republic of Germany). Tissue samples (50–80 mg) were homogenized in 1.0 ml hydrochloric acid, pH 2.5, containing pepsine and 50 µl 80 mmol/l ferrozine®. The homogenates were divided in two portions. In the first portion Fe (II) was determined. In the second portion 10 mg of granulated ascorbic acid was added to reduce Fe (III) to Fe (II). These homogenates were incubated for 20 min at 37 °C, and centrifuged at 10,000 **g**, 4 °C, for 15 min. Then absorbances of the supernatants and iron standards were read against sample blank at 578 nm within 30 min. Data were given as µg/g fresh weight. All results were analysed using Student's t-test and Wilcoxon's Rank Sum test.

Results

We have compared the total content of iron, iron (II), iron (III), and the iron (II)/iron (III) ratio in corresponding samples of Parkinson's disease and matched controls (Table 1). In putamen, cortex (BA 21), hippocampus, and globus pallidus there was no significant difference in the levels of total iron, iron (II), iron (III), and of the iron (II)/iron (III) ratio.

Table 1. Total ferrous and ferric iron in parkinsonian brain

		Fe^{++}	Fe^{+++}	Total iron	$\frac{Fe^{++}}{Fe^{+++}}$
Substantia nigra					
Control	(8)	32 ± 7.0	16 ± 4.2	48 ± 8.2	2.45 ± 0.54
P.D.	(8)	43 ± 8.0	42 ± 4.8**	85 ± 11.1*	1.06 ± 0.17**
Putamen					
Control	(8)	65 ± 14	31 ± 5.6	96 ± 19	2.44 ± 0.56
P.D.	(8)	47 ± 10	31 ± 8.5	78 ± 17	1.88 ± 0.45
Gl. Pallidus					
Control	(6)	27 ± 6.3	53 ± 12	81 ± 18	0.5 ± 0.04
P.D.	(6)	29 ± 7.7	67 ± 16	97 ± 24	0.4 ± 0.05
Hippocampus					
Control	(6)	10 ± 0.8	15 ± 2.7	24 ± 3.3	0.8 ± 0.14
P.D.	(6)	11 ± 1.9	13 ± 2.4	25 ± 3.9	0.9 ± 0.10
Cortex (BA 21)					
Control	(6)	15 ± 2.6	14 ± 2.2	28 ± 5.4	0.9 ± 0.16
P.D.	(6)	16 ± 2.7	13 ± 2.5	28 ± 5.1	1.1 ± 0.24

Data as µg/g fresh weight; means ± seem; number of regions in ( )
Wilcoxon Rank Sum Test; * $p < 0.040$; ** $p < 0.0019$
In six cases putamen showed a near total depletion of dopamine (mean loss 97%) indicating a severe grade of degeneration, equivalent to grade 4^+ in neuropathological examinations

However, a significant increase in the concentration of total iron and iron (III) and a significant decrease of the iron (II)/iron (III) ratio was found in SN of PD.

It is, however, interesting to note that in controls the concentrations of iron in hippocampus and cortex (BA 21) is much lower than those found in other brain regions (Table 1). Furthermore, the ratio between iron (II) and iron (III) is shifted towards iron (II) in the nigro-striatal system, while it is around the ratio of 1 in the other three brain areas.

Discussion

Metals, especially iron (II) have always been suspected to act in a bimodal manner either as important cofactors for enzymes like TH (Kaufman, 1977) or to be involved in processes leading to cell death (Halliwell and Gutteridge, 1985). For these reasons we examined the distribution of iron, iron (II) and iron (III) in brains from subjects with PD. The results of the present study clearly show that there were profound differences of total iron and the iron (II)/iron (III) ratio in SN of PD. However, this finding is to some extent at variance with recent findings by Dexter et al. (1987a, b) who reported increased iron in Brodmann's area 10 and SN. Furthermore, these data (Table 1; Dexter et al., 1987a, b) contrast in part with earlier reports by Earle (1968) stating that iron was above the control values in caudate nucleus and globus pallidus. The reason for this discrepancy is not known, but may be related to the methods used for determination of iron and the severity of PD. The latter aspect seems to be most relevant, as recent findings by Drayer et al. (1986), Rutledge et al. (1987), and Riederer et al. (1988a) do show a dependence of stages of severity as determined by the percentage of nerve cell loss or by clinical observations ("Parkinson plus", i.e. PD combined with dementia or other complicating factors; Fischer et al., 1983) and the increase of iron in SN and other brains areas. Although copper, zinc and calcium and possibly magnesium show rather uniform regional concentrations (Greiner et al., 1975; Ule et al., 1974; Riederer et al., 1988a), that of iron has a marked characteristic distribution. Thus, the highest iron contents are present in globus pallidus > putamen > substantia nigra > hippocampus and BA 21 confirming earlier reports (Spatz, 1922; Völkl and Ule, 1972). While this distribution per se might contribute to increase the vulnerability of the nigrostriatal system to endogenous or exogenous neurotoxins, it is suggested that excess iron (II) itself in SN and putamen may additionally provoke a process of degeneration expecially when the membrane integrity breaks down. The significant increase in the iron (II)/iron (III) turnover in SN of PD may be an indirect indication of enhanced oxidative processes.

The processes by which iron is transported across the blood brain barrier (BBB) and deposited in such high concentrations in a rather circumscribed topography is not known. There are three possible explanations; a) either iron uptake is increased via the breakdown of BBB in some individuals prone to

PD; b) the brain iron turnover, which in any case is extremely slow as compared with liver (see Ben-Shachar et al., 1986), is further decreased resulting in the accumulation in substantia nigra, c) the transport across to CSF is decreased and d) it could not be excluded, that the increased iron content was due to the medication rather than to the disease. This possibility does not appear to be remote since DOPA, dopamine and to a smaller extent, metabolites like HVA are capable of chelating iron and could conceivably lead to increased tissue retention of iron. However, an increase in nigral iron concentration was also found in formalin-fixed Parkinsonian brains long before the clinical use of L-DOPA (Earle, 1968). Furthermore, and in agreement with the findings by Dexter et al. (1987a, b) the decrease of copper (Riederer et al., 1988a) argues against an influence of L-DOPA, which significantly increases copper content in experimental studies (Donaldson et al., 1974). Whatever the mechanisms are, it is now apparent that increased availability of iron may contribute to the neurodegenerative aspect of this disease (Crichton, 1979; Switzer, 1982; Halliwell and Gutteridge, 1985). However, it cannot be decided by now whether this is of primary or secondary importance.

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