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Controversies about iron in parkinsonian and control substantia nigra

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Abstract. According to the oxidative stress theory iron may play an important role in the pathogenesis of neurodegenerative diseases, as e.g. Parkinson's disease (PD). This review presents the results of studies, obtained by various methods, of iron in substantia nigra (SN) - a cerebral structure which degenerates in PD - and shows controversies concerning the amount of iron, its redox state, and the iron binding compounds. Taking into account all published experimental results, the increase in the concentration of iron in parkinsonian SN vs. control may be estimated as $(3 \pm 5)\%$. The presence of large amounts of divalent iron in *post mortem* SN can be unequivocally negated. It is, however, still possible that iron is involved in the pathogenesis of PD, as even minor changes in the amount and form of iron may initiate processes leading to cells death.

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INTRODUCTION

The possibility that iron could be a risk factor for human diseases was discussed by several authors (see Lauffer 1992 for a review). The possible role of iron in the etiology of various neurological diseases was presented in a book edited by P. Riederer and M.B.H Youdim (1993), and also in a recent review paper (Gelman 1995). Although a high concentration of iron in the brain was reported as early as 1922 (Spatz 1922), the first systematic study of the levels of iron in various parts of the human brain was performed by Hallgren and Sourander (1958). They found that the level of iron in human brain increases linearly with age, reaching a plateau in SN in the third decade.

It is generally believed that under normal conditions most of the iron in the brain is located in a safe form within an iron-core of ferritin. Loosely-bound iron and iron released from ferritin may participate in the Fenton reaction leading to the formation of highly reactive and damaging hydroxyl radicals (Halliwell 1992):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

These radicals may provoke lipid peroxidation in cells membranes leading to cells death (Dexter et al 1989a). This hypothesis is known as the oxidative stress hypothesis and is applied to some neurodegenerative diseases, e.g. Parkinson's disease (PD) (Fahn and Cohen 1992). The symptoms of PD are caused by a progressive degeneration of nervous cells located in substantia nigra (SN). SN is a small nervous structure of about 500 mg located bilaterally in mesencephalon. It produces a neurotransmitter - dopamine. The reason for the degeneration of neurons in SN in PD remains unknown at present. Large concentrations of iron, comparable to those in the liver, are found in SN. Based on findings in some experiments of an increase of iron in parkinsonian SN, Youdim et al. (1989) formulated a hypothesis that PD is a progressive siderosis of SN, which enhances the oxidative stress. These authors also reported a shift of the ratio Fe^{2+}/Fe^{3+} from 3:1 in control to 1:1 in parkinsonian SN. This ratio was considered by these authors as most favorable for the production of free radicals.

In this review we will present controversies concerning the increase of the amount of iron in SN in PD, the iron binding-sites, the redox state of iron and the correlation between the severity of the disease and the amount of iron in SN.

VARIOUS TECHNIQUES USED FOR THE INVESTIGATION OF NIGRAL IRON

Brain iron was investigated by different techniques like colorimetry (Hallgren and Sourander 1958, Loeffler et al. 1995), magnetic resonance -MRI- (Drayer et al. 1986, Rutledge et al. 1987, Antonini et al. 1993, Gorell et al. 1995, Ryvlin et al. 1995), spectrophotometry (Sofic et al. 1988, Sofic et al. 1991), histochemical methods - Perls' staining and Turnbull's staining- (Jellinger et al. 1990) and various spectroscopical methods: X-ray fluorescence -XRF- (Earle 1968), atomic absorption (Riederer et al. 1989, Griffiths and Crossman 1993), atomic absorption and emission (Uitti et al. 1989), inductively coupled plasma spectroscopy -ICP- (Dexter et al. 1989b, Mann et al. 1994), total X-ray fluorescence -TXRF- (Zecca and Swartz 1993) and Mössbauer spectroscopy -MS- (Bauminger et al. 1994, Gerlach et al. 1995, Gałązka-Friedman et al. 1996). Each of these techniques has advantages and disadvantages which will be discussed briefly.

All techniques mentioned above determined the amount of iron either in absolute values, or only semi-quantitatively from relaxation rates in MRI or from Perl's staining; from the measurements the ratio of the iron content between parkinsonian and control SN was estimated. Only in three experiments (spectrophotometry (Sofic et al. 1989), Perls' and Turnbull's staining (Jellinger et al. 1990) and MS (Bauminger et al. 1994, Gałązka-Friedman et al. 1996) the authors tried to evaluate separately the amounts of ferric and ferrous iron in SN.

Two other techniques dealing with the determination of iron in SN were X-ray microanalysis (Hirsch et al. 1991, Jellinger et al. 1992) and laser microprobe analysis - LAMMA - (Good et al. 1992). Though these techniques are probably the most sensitive ones, they probe very localized areas and cannot be used for the determination of the total amount of iron in SN.

The size of investigated samples ranged from 10 mg in a study using ICP (Mann et al. 1994) to the whole substantia nigra (263-560 mg) in MS and MRI. The small size of samples needed for measurements by some techniques might be seen as an important advantage of the method, but taking into consideration the non-homogenous distribution of iron in SN - this small size of the samples may introduce an uncertainty in the evaluation of the amount of iron in whole SN per unit weight.

Most of the techniques applied in the investigation of nigral iron (all except MRI and MS) require special prep-

aration of the samples. These methods of preparation (e.g. homogenization in the presence of different chemicals, implantation into a matrix, etc.) could be a source of experimental errors. MS is the only technique which does not need any sample preparation, except freezing the sample, and which is able to distinguish unequivocally divalent and trivalent iron and is often able to identify the iron-containing compound. As this technique is not destructive, the same sample may be later investigated by other techniques. There is no oxidation or spin state of iron, which is Mössbauer silent. Though the technique is not the most sensitive one, any compound whose iron content exceeds 15 μg per sample will be detected.

MRI is the only technique which can be applied *in vivo* and may make it possible to follow the change in the amount of iron in SN in individual cases with time. This is a very promising technique, yet until now the correlation between the MRI signals and the concentration of iron in all its forms is not clear (Brooks et al. 1989, Bizzi et al. 1990, Schenck et al. 1990, Gorell et al. 1995). In most of the works it was assumed that R_2 ($1/T_2$) would be a good measure of the amount of iron, yet lately it has been suggested that this signal is determined by a combination of mechanisms, depending not only of iron concentration. Other parameters, like the echo time dependence (Ye et al. 1996) or R_2', the relaxation rate due to local magnetic field inhomogeneities (Gorell et al. 1995), have been suggested as better measures of iron concentrations.

IRON CONTENT IN THE WHOLE CONTROL SN AND DIFFERENCES IN THE AMOUNT OF IRON BETWEEN PARS COMPACTA AND PARS RETICULATA

The results obtained for the concentration of nigral iron in wet samples are summarized in Table I and the results in dried samples are summarized in Table II. For a better visualisation of the differences between the re-

TABLE I

Nigral iron as measured by various techniques in wet samples

Author	Method	Sample weight (mg)	Part of SN	No of CN samples	Amount of iron in CN (μg/g wet)	No of PD samples	Amount of iron in PD (μg/g wet)	PD/CN iron ratio
Hallgren and Sourander (1958)	col	?	SN	81	184.6 ± 65.2			
Sofic et al. (1988)	SPH	50-80	SN	8	48 ± 8	8	85 ± 11	1.77 ± 0.37
Sofic et al. (1991)	SPH	?	pc	9	62 ± 7	7	89 ± 9	1.44 ± 0.22
Sofic et al. (1991)	SPH	?	pr	9	91 ± 15	7	85 ± 9	0.92 ± 0.18
Zecca and Swartz (1993)	TXRF	?	SN	5	410 ± 223			
Griffiths and Crossman (1993)	AA	50	SN	6	140 ± 13	6	281 ± 22	2.01 ± 0.24
Gałązka-Friedman et al. (1996)	MS	263-560	SN	8	163 ± 12	6	159 ± 13	0.98 ± 0.11
Loeffler et al. (1995)	col	50	SN	8	60 ± 10 5600 ± 400 (ng/mg prot.)	14	50 ± 10 4600 ± 300 (ng/mg prot.)	0.82 ± 0.08
Mann et al. (1994)	ICP	10	SN	22	1159 ± 379 (ng/mg prot.)	18	1813 ± 846 (ng/mg prot.)	1.56 ± 0.89

PD, Parkinson's disease; CN, control; col, colorimetry; SPH, spectrophotometry; TXRF, total reflection X-ray fluorescence; AA, atomic absorption; ICP, inductively coupled plasma spectroscopy; MS, Mössbauer spectroscopy; SN, whole substantia nigra; pc, pars compacta; pr, pars reticulata. In the experiments by Sofic et al.(1988, 1991), Griffiths and Crossman(1993), and Gałązka-Friedman et al. (1996) - the errors of the concentration of iron are cited as SEM, in the others - as SD

TABLE II

Nigral iron as measured by various techniques in dried samples

Author	Method	Sample weight (mg)	Part of SN	No. of CN samples	Amount of iron in CN (μg/g wet)	No. of PD samples	Amount of iron in PD (μg/g wet)	PD/CN iron ratio
Riederer et al. (1989)	AA	50-80	oral	4	340 ± 100	11	430 ± 100	1.26 ± 0.47
Riederer et al. (1989)	AA	50-80	caudal	4	230 ± 100	11	280 ± 60	1.22 ± 0.59
Dexter et al. (1989b)	ICP	100-250	SN	9	580 ± 60	7	780 ± 60	1.34 ± 0.17
Dexter et al. (1989b)	ICP	100-250	pc	3	740 ± 60	8	960 ± 60	1.30 ± 0.13
Uitti et al. (1989)	AA&E	50-500	SN	12	613 ± 56	9	653 ± 55	1.07 ± 0.13
Gałązka-Friedman et al. (1996)	MS	90-150	SN	1	890 ± 70	1	644 ± 60	0.93 ± 0.18

PD, Parkinson's disease; CN, control; XRF, X-ray fluorescence; AA, atomic absorption; ICP, inductively coupled plasma spectroscopy; AAandE, atomic absorption and emission; MS, Mössbauer spectroscopy; SN, whole substantia nigra; pc, pars compacta. The results are given for the experiments 1 - 5 as mean ±SEM, and for exp. 6 - as mean ± instrumental error. For experiments 1 - 5 the weight of the sample is given for before drying, and for the last one - after lyophilization.

sults, they are also presented in Figs. 1 and 2. Different amounts of iron are, of course, found per unit mass of wet or dried tissues.

As seen from Table I and Fig. 1, the results obtained for wet control samples with the use of colorimetry (Hallgren and Sourander 1958) - (185 ± 65 μg/g of wet tissue), atomic absorption (Griffiths and Crossman 1993) - (140 ± 13 μg/g) and MS (Gałązka-Friedman et al. 1996) - (163 ± 12 μg/g), belong to one group of quite consistent results. The results of spectrophotometry on homogenized samples (Sofic et al. 1988) (48 ± 8 μg/g) and those obtained by TXRF (Zecca and Swartz 1993) - (410 ± 223 μg/g) are significantly different. The high standard deviation of the mean in the TXRF measurements was due to the wide range of the results, related, according to these authors, to the wide range of the age

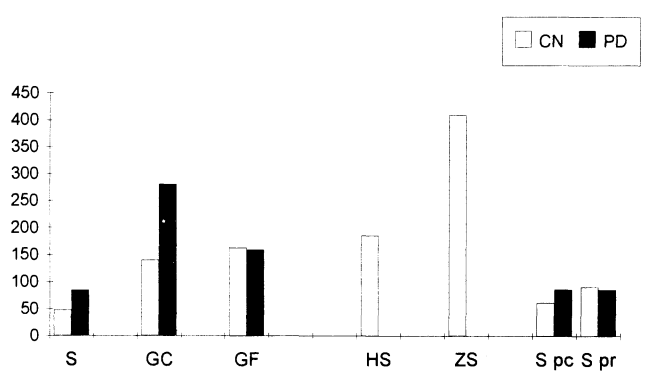

Fig. 1. Concentration of total iron in wet samples of substantia nigra according to various authors. S, Sofic et al. (1988) - spectrophotometry; GC, Griffiths and Crossman (1993) - atomic absorption; GF, Gałązka-Friedman et al. (1996) - Mössbauer spectroscopy; HS, Hallgren and Sourander (1958) - colorimetry; ZS, Zecca and Swartz (1993) - total reflection X-ray fluorescence; S, Sofic et al. (1991) - spectrophotometry; pc, pars compacta; pr, pars reticulata. Values are represented as μg/g wet tissu

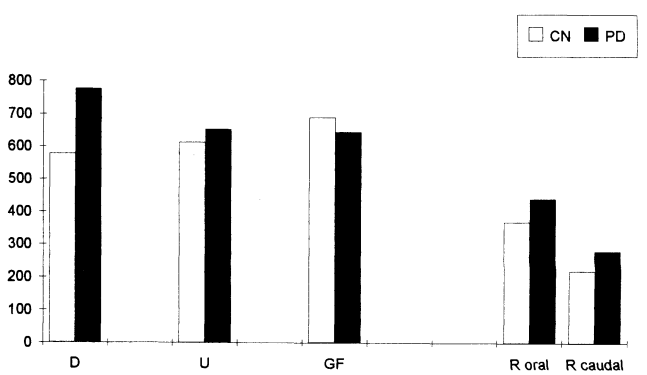

Fig. 2. Concentration of total iron in dried samples of substantia nigra according to various authors. D, Dexter et al. (1989b) - inductively coupled plasma spectroscopy; U, Uitti et al. (1989) - atomic absorption and emission; GF, Gałązka-Friedman et al. (1996) - Mössbauer spectroscopy; R, Riederer et al. (1989) - atomic absorption, oral and caudal part of SN. Values are represented as μg/g dry tissue

of the patients (40 - 70 years). The results obtained by spectrophotometry are significantly lower than those obtained by other methods.

Three experiments gave similar results for the concentration of iron in dried control samples (Table II and Fig. 2): ICP (Dexter et al. 1989b) - (578 ± 62 μg/g), atomic absorption and emission (Uitti et al. 1989) - (613 ± 56 μg/g), and MS (Gałązka-Friedman et al. 1996) - (690 ± 70 μg/g). The concentrations of iron as measured by Riederer et al. (1989), using atomic absorption, in the oral and caudal part of SN (370 ± 100 μg/g and 220 ± 100 μg/g respectively) are significantly lower.

In two experiments (Mann et al. 1994, Loeffler et al. 1995) the amount of iron was cited in ng/mg of protein, which is difficult to compare with other results expressed as μg of Fe/g wet tissue. It should, however, be noted that these two experiments gave results inconsistent with each other (1,160 and 5,600 ng iron/mg protein respectively). Loeffler et al. (1995) cite also the amount of protein per wet weight. From this one may calculate the amount of iron as ~60 μg/g wet tissue. This concentration is similar to that found by Sofic et al. (1988) and much smaller than that found by other methods. For the measurements by Loeffler et al. (1995) and those by Sofic et al. (1988), the tissues were homogenized and it seems therefore inevitable to conclude that much of the tissue iron is lost during homogenization.

The problem of the amount of iron in different parts of SN remains one of the most controversials, and various laboratories give various results of measurements. To the above presented problem of the correct evaluation of the amount of iron in brain tissue, new difficulties in precise macroscopic separation of pars compacta and pars reticulata are added. Pars compacta is the part of SN containing mostly melanized dopaminergic neurons. Pars reticulata contains much more glial cells. The precise separation of these two parts is possible only with the use of a microscope, and was performed in an experiment dealing with measurements of iron only by Jellinger et al. (1990). The amount of iron was assessed in this experiment semi-quantitatively with the use of Perls' staining. Based on the intensity of staining, the total amount of iron is arbitrarily determined (0 - no iron, 1+ low, 2+ medium, 3+ high concentration of iron). These authors claim that in control SN they found more iron in pars reticulata than in pars compacta, and that the increase of iron in SN in PD occurred only in pars compacta. Similar results were presented by Sofic et al. (1991) in homogenized control samples, where they found higher amounts of iron in pars reticulata than in pars compacta (91 ± 16 vs. 62 ± 8 μg/g), yet the overall amount was much lower than that found by other methods. On the other hand, in measurements performed by Dexter et al. (1989b) on dried samples, a lower concentration of iron was found for the whole SN (578 ± 61 μg/g) than for pars compacta (746 ± 61 μg/g), suggesting that pars compacta has higher concentration of iron than pars reticulata, both in PD and control. MS on one dried sample found a two-fold higher concentration of iron in pars compacta as compared to that in the surrounding tissue (Gałązka-Friedman et al. 1996).

Drayer et al. (1986), found from T_2 weighted images in MRI, comparable concentrations of iron in pars compacta and pars reticulata in 4 out of 6 cases of PD, but the results of Ryvlin et al. (1995), using the same method in 45 PD patients and 45 control, suggested higher amounts of iron in pars reticulata. Antonini et al. (1993) doubt the possibility of a precise separation of these two parts of SN by MRI.

NIGRAL IRON IN PARKINSON'S DISEASE - COMPARISON WITH CONTROL

The first comparison between the iron concentration in parkinsonian and control brains was done by Earle (1968), who used the XRF (X-ray fluorescence) method. He found a 2-fold increase of iron in parkinsonian brains as compared to control. These measurements were done without calibration, so absolute values for the amount of iron are not available. The errors of the calculations of the increase of iron are not cited. It is also not completely clear which areas of the brains were compared. Some of the control brains from that study had been stored in formalin for over seventy years prior to the analysis and one cannot exclude the possibility of a leak of iron to formalin during years of storage. In this experiment the PD brains were stored for much shorter periods.

Another study where a 2-fold increase of iron in parkinsonian SN, but only in pars compacta, was found, was by Jellinger et al. (1990). This experiment was discussed above. The precise separation of pars compacta and pars reticulata was, combined with only a semi-quantitative method of evaluation of the amount of iron. No increase of iron was found in this experiment in pars reticulata.

A significant increase in the amount of iron in parkinsonian vs. control SN (the ratio being 2.01 ± 0.16) was also noticed by Griffiths and Crosssman (1993), who

used atomic absorption. The concentration of iron in parkinsonian SN found by these authors (281 ± 22 μg/g wet tissue) is 3.3 times higher than that found by Sofic et al. (1991) (85 ± 11 μg/g wet tissue), and 1.8 times higher than the concentration of iron found by Gałązka-Friedman et al. (1996) (159 ± 13 μg/g wet tissue), who used Mössbauer spectroscopy. The concentration found by these authors in control is consistent with that found by others (Hallgren and Sourander 1958, Gałązka-Friedman et al. 1996), but the amount found in PD is considerably higher.

In the two experiments where the amount of iron in SN was calculated per mg of protein, the results were inconsistent with each other. Mann et al. (1994) show an increase of nigral iron in PD, while Loeffler et al. (1995) - show a decrease of iron in parkinsonian SN vs. elderly control.

In experiments performed with dried samples the discrepancy between the various results is smaller. Measurements by AA and ICP showed an increase of about 30% in the concentration of iron in PD compared to control SN. Uitti et al. (1989) found only a small and non significant increase, and Gałązka-Friedman et al. (1996) did not find any.

The changes in the concentration of iron in parkinsonian SN vs. control were estimated also *in vivo* with the use of MRI. Rutledge et al. (1987) found a decrease of the amount of iron in PD, but in another paper (Antonini et al. 1993) a small increase was obtained. Also in two recent papers using MRI an increase of iron in PD was reported (Gorell et al. 1995, Ryvlin et al. 1995), in one of them (Gorell et al. 1995) of almost 30%. Most of the authors (Rutledge et al. 1987, Antonini et al. 1993, Ryvlin et al. 1995) based their conclusions on T_2 values. The T_2 values measured by Gorell et al. (1995) are significantly higher for parkinsonian SN than for control, indicating, according to the interpretations given by others (Rutledge et al. 1987, Antonini et al. 1993, Ryvlin et al. 1995), a considerable decrease of the amount of iron in PD. By introducing corrections based on additional measurements of the transverse relaxation time in the presence of local field inhomogeneities, marked by these authors as T_2*, they finally obtained results, which point to an increase of iron in SN in PD.

The errors quoted in Tab. I and II for the concentration of iron obtained for PD and control SN are those given in the original papers. The results obtained with the use of Perls' staining and MRI are not included in the tables, as they are only semi-quantitative. In the last column of the tables the ratio of the concentrations of iron in PD vs. control is given. The error given for this ratio is equal to one standard deviation, calculated from the results and errors given by the authors. As can be seen from these tables, this ratio changes between 2.0 and 0.82 in wet samples and between 1.34 and 0.93 in dried samples.

According to Riederer et al. (1989) the concentration of iron in SN is related to the severity of the disease, as an increase of iron was found only in SN of patients with severe PD. Gorell et al. (1995) found a correlation between the concentration of iron in SN, as assessed by their method of evaluation of MRI measurements, and motor performance of PD patients. On the other hand no correlation between the staging of the disease and the amount of iron was found by Antonini et al. (1993) with MRI nor by the MS technique (Gałązka-Friedman et al. 1996).

REDOX STATE OF IRON IN SN

As far as we are aware, there is only one experiment in which a high concentration of divalent iron in both parkinsonian and control SN was found (Sofic et al. 1988), yet these results are cited in many papers (Ben-Shachar and Youdim 1992, Youdim et al. 1993, Gerlach et al. 1994, Youdim 1994). In this one experiment (Sofic et al. 1988) the spectrophotometric method was used. Neither Turnbull's staining (Jellinger et al. 1990), nor MS (Bauminger et al. 1994, Gałązka-Friedman et al. 1996) were able to confirm these results. The disagreement between the former data and the others may be due to an artifact related to sample preparation for spectrophotometric evaluation. Homogenization of the samples with pepsin and hydrochloric acid, used in this procedure, may lead to release of iron from ferritin, and to its reduction to divalent iron. The presence of ascorbic acid within SN cells (Riederer et al. 1989) makes it even more plausible. Samples used for MS do not need any physical nor chemical pretreatment, and MS is probably the most reliable technique to distinguish between divalent and trivalent iron. The redox state determined in all these papers is, of course, obtained from post mortem samples. It may therefore well be that *in vivo* the oxidation state of iron is different, but no method can so far determine the redox state *in vivo*.

IDENTIFICATION OF IRON CONTAINING COMPOUNDS IN SN

The techniques used in all these experiments, except MS, did not allow the identification of the iron contain-

ing compounds. In the discussion in the literature concerning this problem Hallgren and Sourander (1958) suggested that nigral iron may be bound to ferritin. Ben-Shachar and Youdim (1992) thought that iron could be chelated or bound to another protein or to a small soluble molecule. They hypothesized that neuromelanin (NM) could be a candidate for binding the iron.

Two experiments tried to determine the presence of iron in neuromelanin in SN: energy dispersive X-ray microanalysis (Hirsch et al. 1991, Jellinger et al. 1992) and laser microprobe analysis -LAMMA- (Good et al. 1992).The results of these experiments are not consistent with each other, as Hirsch et al. (1991) did not find iron in neuromelanin, while the others did. Both these techniques are very sensitive and can detect tiny amounts of iron, yet they probe very localized regions, and this may be the reason for the discrepancy between the results.

MS showed that most of the iron in SN (between 85 and 100%), both in PD and control, was ferritin-like, and suggested that iron bound to NM, or other monomeric iron, could be no more than 15% of the total amount of iron in SN (Gałązka-Friedman et al. 1996). In a recently published paper, Gerlach et al. (1995) showed, also using MS, that small iron clusters were present in NM extracted from SN. Zecca and Swartz (1993), using TXRF, estimated the concentration of iron in NM extracted from SN, as being 22-times higher than the concentration of iron in the whole SN. It remains an open question, however, whether the iron seen in extracted NM is bound to NM in SN or gets attached to it during the isolation procedure. This later possibility was recently supported by an experiment by Zecca et al. (1996) who found that when synthetic melanins, void of iron, was incubated in homogenated nervous tissue (putamen), it had also, after isolation from the homogenate, an iron content comparable to that found in native NM after its isolation from homogenates.

CONCLUSIONS

We tried to compare the results dealing with the quantitative evaluation of iron in SN. There are several recent review articles dealing with the problem of the amount of iron in SN and its role in the etiology of PD (Jellinger and Kienzl 1993, Youdim et al. 1993, Gerlach et al. 1994, Youdim 1994). Most controversies concern the increase of the total amount of iron in parkinsonian SN compared to control. The large discrepancies between the results obtained in various laboratories need an explanation. In this review we have shown that even the absolute amount of iron in human SN, both in PD and control, is still controversial. The concentration of iron found in control SN ranges between 48 and 410 μg/g wet tissue - almost a factor of 10! With this large spread, it is not too surprising that there is also a large inconsistency in the results concerning the increase of iron in SN of PD patients compared to control. As seen from Table I, whereas 4 works found an increase of iron in parkinsonian SN by a factor between 1.3 and 2.0, two investigations could not find any increase. In dried samples (Table II) the maximal increase of iron concentration in SN in PD compared to control was smaller than in non-dried samples and was about 25% (Dexter et al. 1989b, Riederer et al. 1989), and only a marginal or no increase was found by others (Uitti et al. 1989, Gałązka-Friedman et al. 1996).

Possible explanations for the discrepancies between the results obtained in various laboratories may concern:

1. Techniques used for the determination of the amount of iron. Techniques using destructive procedures like homogenization seem to induce a leak of iron from SN, as well as a change of its redox state.

2. The size of the samples studied may play a role, as iron is probably not homogeneously distributed even within pars compacta of SN.

3. There is a very large diversity in the concentration of iron present in SN in different individuals, also in controls.

Though different techniques might introduce systematic errors in the evaluation of the concentration of iron in SN, it is quite reasonable to assume that the systematic errors may be similar for control and PD cases, and thus the techniques used should influence less the results obtained for the ratio between iron concentration in SN of PD and control (disregarding the possibility that the preparation of the samples may cause a different loss of iron from its binding sites in PD and control tissues). The ratios given in the last columns of the tables were calculated using mean values and their standard error of the mean (SEM). We calculated then the weighted mean (Barford 1987) for this ratio, taking into account all results of measurements of dried and wet samples of whole SN and their cited errors. We did not include in these calculations the results of MRI, the measurements where the iron content was assessed separately for different parts of SN (experiment 3., 4. of Table I., experiment 1., 2., 4. of Table II) and the measurements by MS only on one dried sample. The value obtained in this way for the

weighted mean of the ratio between iron concentration in SN of PD and control is 1.03 ± 0.05.

The average concentration of iron in control SN, taking into account most measurements in wet samples, seems to be about 160 μg/g tissue. Most of this iron is stored in ferritin. If an increase of the amount of iron does play a role in the pathogenesis of PD, it is surely not due to an increase of ferritin iron. There might, however, be some increase in NM iron (or loosely bound iron), which could be involved in the etiology of PD. Even if the difference in the concentration of iron between PD and control SN is marginal, biological processes are not linear and small changes in the properties or amount of iron may initiate new processes leading to cells death. A small shift in the Fenton reaction toward a temporary increase of the amount of divalent iron may lead to an increase of production of free radicals. Therefore, there is no need for a substantial increase of the total amount of iron in SN to provoke an increase of the amount of free radicals and to cause the degeneration of nervous cells. Further studies are needed for a better understanding of this problem.

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