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Elevated levels of Harman and Norharman in cerebrospinal fluid of Parkinsonian patients

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Summary. Death of dopaminergic neurons in Parkinson's disease (PD) may partially be caused by synthesis and accumulation of endogenous and exogenous toxins. Because of structural similarity to MPTP, β-carbolines, like norharman and harman, have been proposed as putative neurotoxins. In vivo they may easily be formed by cyclization of indoleamines with e.g. aldehydes. For further elucidation of the role of β-carbolines in neurodegenerative disorders harman and norharman levels in cerebrospinal fluid (CSF) were measured in 14 patients with PD and compared to an age- and sex-matched control group (n = 14). CSF levels of norharman and harman in PD were significantly higher compared to controls. These results may suggest a possible role of harman and norharman or its N-methylated carbolinium ions in the pathophysiological processes initiating PD. However the origin of increased levels of these β-carbolines remains unclear. On the one hand one may speculate, that unknown metabolic processes induce the increased synthesis of harman and norharman in PD. On the other hand a possible impact of exogenous sources may also be possible.

Keywords: Parkinson's disease, harman, norharman, cerebrospinal fluid

Introduction

The potent neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces cell death in dopaminergic neurons (Sayre et al., 1990). β-carbolines, like harman and norharman, show structural resemblance to MPTP and its ion MPP^+ (Neafsay et al., 1989; Sayre et al., 1990). In vivo they may easily be formed by cyclization of indoleamines with e.g. aldehydes (Neafsay et al., 1989; Sayre et al., 1990). Therefore several studies were initiated to elucidate the potential neurotoxicity of these compounds. After application of N-methylated β-carbolinium ions to rats by intracerebral infusion only a weak neurotoxicity was found because of limited uptake into

dopaminergic neurons (Sayre et al., 1990). In contrast N-methylated β-carbolinium ions, like 2-methyl-norharman, induce dopamine depletions, large lesions and gliosis after injection in the substantia nigra of rats (Neafsay et al., 1989). The best candidates for a MPPt like neurotoxicity of β-carbolinium ions are the 2,9-dimethylated forms (Collins et al., 1992). Therefore increased levels of toxic N-methylated carbolines may be one initiating factor in idiopathic parkinsonism (Matsubara et al., 1993). The underlying pathophysiological processes remain unclear up to now. 2-methylated β-carbolines may inhibit NADH-coenzyme Q reductase (complex 1) of the electron transport chain within mitochondria, thereby leading to a fall in ATP production and thus initiating cell death (Albores et al., 1990). Such a decrease in the activity of complex 1 has consistently been found in the brain, especially in homogenates of the substantia nigra, but also in platelets, muscle and lymphocytes of Parkinsonian patients (Schulz and Flint Beal, 1994). Aim of the present study was to determine the harman and norharman CSF levels in treated and de novo Parkinsonian patients and to compare them to age- and sex-matched controls.

Material and methods

CSF samples taken from 14 idiopathic Parkinsonian subjects (7 male, 7 female; mean age 60 ± 8.7, range 40–71; mean duration of PD 3.98 ± 4.15 (SD), range 0.3–15 years; n = 1 Hoehn Yahr Scale I, n = 4 Hoehn Yahr Scale II, n = 7 Hoehn Yahr Scale III, n = 1 Hoehn Yahr Scale IV) were analyzed. 7 patients received conventional Parkinsonian pharmacotherapy including l-dopa/benserazid or carbidopa preparations (n = 7), selegiline (n = 6), bromocriptine (n = 6), lisuride (n = 1) and 7 were previously untreated "de novo" Parkinson patients. The control group was age- and sex-matched and consisted of 14 patients without peripheral neurologic disorders (7 male, 7 female; mean age 60 ± 9.37, range 43–74). Patients with metabolic disturbances or other central neurological diseases beneath Parkinson's disease and subjects with clinical or biochemical signs of alcoholism, heroinism or depression were excluded.

Sample collection

After 10 hours patients' fasting and resting in bed lumbar puncture was performed between 8 a.m. and 9 a.m with the patient in the lateral decubitus position and before arising from bed. Patients gave informed consent. CSF was collected directly from the needle in three different test tubes for (1) cell count and cell morphology, glucose, chloride, total proteins, isoelectric focusing and electrophoresis (8 ml) and (2) measurement of norharman and harman. Sample 2 was immediately frozen and stored at −80°C. Blood-tinged CSF was discarded, samples with a cell count greater than 5 WBC/ml, or with abnormal protein, glucose or chloride content were not used. Time period between freezing and work up of CSF samples for estimation of β-carbolines was no longer than three months. Measurement of norharman and harman levels in CSF was performed by high-performance liquid chromatography (HPLC) (Rommelspacher et al., 1991a,b).

Statistical analysis

Comparison of norharman and harman CSF levels of Parkinsonian patients and age- and sex matched controls were performed by the two tailed Mann-Whitney U-test. For correlation linear regression was used.

Results

CSF levels of norharman from subjects with Parkinson's disease compared to controls were significantly increased ($p = 0.02$). CSF levels of norharman in Parkinsonian patients were 22.5 ± 17.9 (mean ± SD), range 3.4–71.1 pg/ml, while in the controls norharman levels amounted to 10.76 ± 9.6, range 0.6–31.1 pg/ml (Fig. 1). Mean values of harman in the Parkinsonian group were also significantly ($p = 0.01$) elevated (15.1 ± 11.7, range 0–39.1 pg/ml) compared to controls (4.9 ± 5.3, range 0–12.8 pg/ml) (Fig. 2).

To exclude influence of age, CSF levels of β-carbolines were correlated with age of Parkinsonian patients (harman: $p = 0.44$, $r^2 = 0.04$; norharman $p = 0.38$, $r^2 = 0.05$) and controls (harman: $p = 0.42$, $r^2 = 0.06$; norharman $p = 0.13$, $r^2 = 0.18$). No significant differences between harman ($p = 0.53$) and norharman ($p = 0.62$) levels were found, comparing de novo (norharman 16.8 ± 8.1, range 3.4–26.9 pg/ml; harman 13.3 ± 12.2, range 0–32.4 pg/ml) with treated (norharman 28.1 ± 23.6, range 7.5–71.1 pg/ml, harman 16.8 ± 11.9, range 1–39.1 pg/ml) Parkinsonian patients.

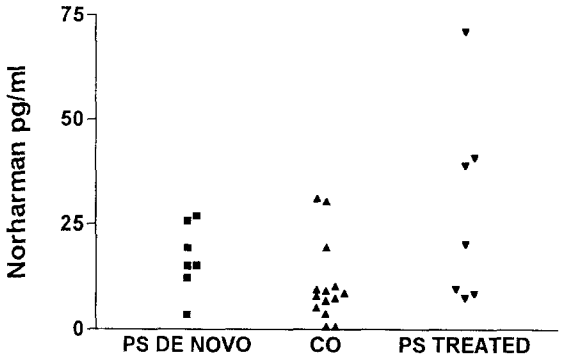

Fig. 1. Values of norharman in untreated "de novo" Parkinsonian patients (PS DE NOVO), controls (CO) and treated patients with idiopathic Parkinson's disease (PS TREATED)

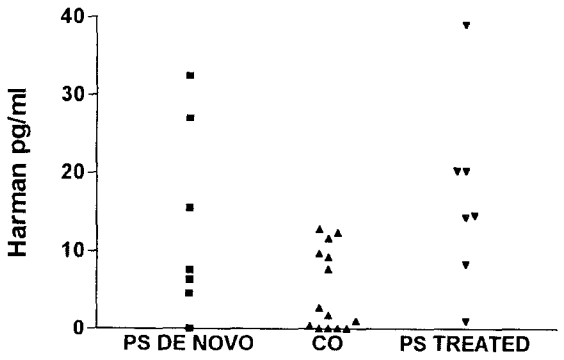

Fig. 2. Values of harman in untreated "de novo" Parkinsonian patients (PS DE NOVO), controls (CO) and treated patients with idiopathic Parkinson's disease (PS TREATED)

Discussion

After the discovery, that the street drug contaminant MPTP and especially its ion MPP^+ are selective toxins for the nigrostriatal dopaminergic system, it has been postulated, that accumulation and production of endogenous and exogenous environmental substances or abnormal metabolism of those compounds may be one of the initiating factors of PD. Mammalian central nervous system indole metabolites, like e.g. the β-carbolines norharman and harman, have been proposed to be such potential endogenous neurotoxins due to their structural similarity to MPP^+, the active oxidized product of MPTP. Structurally, 2-methyl-norharman differs from MPP^+ by an indol nitrogen bridge (Fields et al., 1992). These N-methylated β-carbolines can arise in vivo from condensation of an indoleamine with an intermediary metabolite, e.g. glyoxyl acid or aldehydes, to form the 1,2,3,4-tetrahydro-β-carboline, followed by oxidation/decarboxylation and 2(N)-methylation (Matsubara et al., 1992). The physiological role of β-carbolines is still unclear. In the case of norharman autoradiographic studies revealed enriched high affinity binding sites in hypothalamic, thalamic accumbens and amygdaloid nuclei as well as in hippocampal, neocortical and olfactory-related structures (Pawlik et al., 1990).

Biochemically norharman acts as a natural endogenous sedative but not hallucinatory agent in a certain range of concentration (Fekkes et al., 1992; Fekkes and Bode, 1993). Moreover norharman may stimulate a specific beta-carboline receptor, different from the benzodiazepine-GABA receptor complex (Pawlik et al., 1990; Rommelspacher et al., 1991a). In the case of harman it has been demonstrated, that this substance is a strong endogenous inhibitor of monoaminooxidase A (MAO-A) in contrast to norharman. Therefore harman may represent an endogenous antidepressive agent (Rommelspacher et al., 1994).

In PD an enhanced synthesis of N-methylated compounds was observed probably due to the failure of further catabolisation and detoxification of those N-methylated compounds (Green et al., 1991). It was suggested, that dysfunction of hydroxylation is mediated by an isoenzyme of cytochrome P-450 or perhaps xanthine- or aldehyde oxidases leading to accumulation of N-methylated substances (Green et al., 1991). Interestingly precursors of those N-methylated β-carbolines, like norharman and harman, were found significantly higher in the substantia nigra than in the cortex in samples from brains without degeneration of substantia nigra (Matsubara et al., 1993). So especially in the substantia nigra of patients with PD increased 2-N-methylation of β-carbolines may take place. These 2-methylated β-carbolines may stimulate or even induce neuronal degeneration due to their toxic effects on mitochondrial respiration in the substantia nigra of Parkinsonian patients, where complex 1 deficiency was reported in tissue homogenates (Albores et al., 1990; Schulz and Flint Beal, 1994). On the basis of these studies detection of significantly increased CSF concentrations of norharman and harman in Parkinsonian patients compared to controls may give another hint for the hypothesis of a possible initiating and/or sustaining effect of β-carbolines on

the degenerative process in PD. However it remains unclear, whether enhanced CSF levels of β-carbolines in PD are induced by endogenous processes or caused by unknown exogenous factors. It seems unlikely, that our results are related to Parkinsonian pharmacotherapy, because no significant differences of β-carboline CSF levels appeared between treated and de novo subjects with PD. Moreover no statistical significant influence of age on CSF-levels of β-carbolines were found. Due to the fact, that plasma levels of β-carbolines do not significantly change over a period of time under physiological conditions, one may assume, that the occurence of increased CSF levels of norharman and harman reflects a longstanding metabolic alteration leading to increased formation and/or decomposition in PD (Rommelspacher et al., 1991a,b; Fekkes et al., 1992b). Former studies revealed increased plasma levels of norharman in alcoholics and of harman and norharman in heroin addicts (Rommelspacher et al., 1991b; Stohler et al., 1993). Therefore we cannot exclude, that endogenous biosynthesis of norharman and harman is compensatory enhanced to relieve psychopathological states like anxiety or depression, occuring frequently in PD, alcohol- or heroine-addiction. It seems possible, that those patients with elevated levels of harman and norharman may represent a subgroup within PD with lower risk of developing psychopathological disturbances due to the potential antidepressive qualities of β-carbolines.

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