

Somatostatin-like immunoreactivity in the cerebrospinal fluid of patients with Parkinson's disease and its relation to dementia

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SUMMARY Acetylcholinesterase, somatostatin-like immunoreactivity, and homovanillic acid levels were measured in the cerebrospinal fluid of 36 patients with early stages of Parkinson's disease and in 19 control patients. In patients with Parkinson's disease the levels of somatostatin-like immunoreactivity were lower than in the controls ($p < 0.01$); these values were lowest in the demented Parkinsonian patients. Concentrations of homovanillic acid were also significantly lower in Parkinsonian patients ($p < 0.05$). In contrast, no changes were observed in the acetylcholinesterase activity of patients with Parkinson's disease. The reduced somatostatin-like immunoreactivity in CSF agrees with previous post-mortem studies and indicates that Parkinson's disease and Alzheimer's disease may have some neurochemical features in common.

According to the literature about 20% of the patients with Parkinson's disease probably have dementia.¹ Neuropathological findings of the brain show that some demented Parkinsonian patients have histological changes characteristic of Alzheimer's disease.²⁻⁴ Upon neuropathological examination of the brain, however, some demented Parkinsonian patients are found to have no Alzheimer type changes.^{5,6} Reduced cell counts of the nucleus basalis of Meynert, which gives rise to cortical cholinergic innervation, have been shown in both Alzheimer's disease and Parkinson's disease.^{7,8} In numerous studies patients with Alzheimer's disease have been found to have reduced cortical choline acetyltransferase (ChAT), which is correlated with the severity of dementia and the number of senile plaques.^{9,10} Reduced cortical ChAT activities have been demonstrated in demented Parkinsonian patients with and without brain pathology of the Alzheimer type.^{6,11,12}

In addition to the cholinergic deficit in Alzheimer's disease and Parkinson's disease, recent studies have

shown disturbance of the somatostatin system.^{13,14} In Parkinson's disease reduced somatostatin levels have been found in the cortex and hippocampus and also in CSF.^{14,15} However, the somatostatin levels were also reduced in CSF of patients with Alzheimer's disease, Huntington's chorea, multiple sclerosis and those with depression.¹⁶⁻¹⁹

We recently demonstrated dementia-related decrease of cholinesterase activity and somatostatin-like immunoreactivity in CSF of patients with Alzheimer's disease.¹⁶ The purpose of this study was to measure these levels in CSF from patients with Parkinson's disease and to examine how they are related to dementia. In addition, we measured homovanillic acid as a marker of the dopaminergic system.

Patients and methods

Patients and controls

Thirty-six patients with early stages of classical Parkinson's disease (18 female, 18 male) aged 69 ± 9 years (mean \pm SD), range 44-81 years, participated after giving informed consent in this study. Duration of the disease ranged from 6 months to 10 years (mean 2.9 years). The disability stage of Parkinson's disease was recorded according to Hoehn and Yahr.²⁰ The severity of dementia was estimated using the dementia staging scale of Hughes *et al.*²¹ According to this scale, 10 patients had no signs of dementia, 19 had slight memory disorders (questionable dementia), and seven had mild to moderate dementia and met DSM III criteria. Symptoms of depression were assessed by Hamilton's rating

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scale.²² Eighteen of the patients were receiving anticholinergics, one patient was receiving levodopa combined with an inhibitor of dopa decarboxylase, and 17 patients were without drug treatment for Parkinson's disease. As controls we examined 19 patients (nine female, 10 male), aged 61 ± 10 years (mean \pm SD), range 38–78 years, who required diagnostic lumbar puncture for previous transient ischaemic attack, vertigo, headache, polyneuropathy or amyotrophic lateral sclerosis. According to clinical criteria none of these patients had signs of dementia, depression, multiple sclerosis or Parkinson's disease.

Samples

At 8–10 am a standard amount of lumbar CSF was obtained from the patients as they lay in a recumbent position. The samples were frozen immediately and stored at -80°C until assayed. The tubes for determinations of somatostatin-like immunoreactivity contained Aprotinin (700 KIU/ml) as enzyme inhibitor.

Methods

Acetylcholinesterase (AChE) activity was determined according to a modification of the method developed by Ellman *et al.*²³ The reaction mixture (3.0 ml) in the total cholinesterase measurements consisted of final concentrations of 0.09 M phosphate buffer (final pH 7.6), 0.84 mM 5.5-dithiobis-(2-nitrobenzoic acid), 0.5 mM acetylthiocholine iodide as substrate, and 0.5 ml diluted CSF (1 + 4 with H_2O). A specific acetylcholinesterase inhibitor, BW284C51 (5.6 μM), was used to inhibit true AChE and the residual activity was subtracted from the cholinesterase activity to obtain acetylcholinesterase activity. The mixtures were incubated at $+37^\circ\text{C}$ for 20 min, and the yellow colour formed was read at 412 nm. Colour formation was linear for at least 30 min.

The somatostatin-like immunoreactivity (SLI) was determined using a commercial RIA kit (Immunonuclear Corporation, Minnesota, USA).¹⁶ CSF samples (600 μl) were extracted with acetone-petroleum ether. After evaporation, the residue was redissolved in 200 μl assay buffer for radioimmunoassay. For increased sensitivity, the assay was run disequibrated; 200 μl standard or sample and 100 μl antiserum were incubated for 20 h at $+4^\circ\text{C}$, after which 100 μl tracer was added and incubation was continued for 24 h.

Homovanillic acid was measured by high performance liquid chromatography (HPLC) using electrochemical detection. CSF (300 μl) was mixed with 100 μl of the internal standard mixture, 200 nM 3-methoxy-4-hydroxy-phenylethanol in 1 M HClO_4 containing 0.1 M $\text{Na}_2\text{S}_2\text{O}_5$ and 0.01 M Na_2 EDTA. The samples were centrifuged, and 25 μl of the supernatant was injected into a HPLC column. For separation of HVA, the method described by Mefford was used; the mixture contained 0.1 M acetic acid with 0.1 M citric acid and 20% methanol (pH 4.1) and had a flow rate of 1.2 ml/min.²⁴ Homovanillic acid was detected at a flow cell potential of 0.85 V. The liquid chromatography system consisted of an Altex 110A pump, an Ultrasphere ODS column (250 \times 4.6 mm, 5 μm particle size), and an LC-4B electrochemical detector by BAS equipped with a glassy carbon electrode.

Table 1 Cerebrospinal fluid acetylcholinesterase (AChE), somatostatin-like immunoreactivity (SLI) and homovanillic acid (HVA) in patients with Parkinson's disease and in controls

	Parkinsonians	Controls	<i>p</i> *
AChE (nmol/ml/min)	27.9 \pm 1.3 (36)	24.6 \pm 1.9 (17)	NS
SLI (fmol/ml)	9.7 \pm 0.4 (35)	12.5 \pm 0.7 (19)	0.01
HVA (pmol/ml)	173.7 \pm 13.8 (34)	223.7 \pm 16.0 (16)	0.05

*Significance of difference between groups; ANCOVA, adjusted for age. Values are expressed as mean \pm SEM with number of patients in parentheses. NS = not significant.

Statistical methods

The difference between the groups of Parkinsonian patients and controls was assessed by Student's two-tailed *t* test for independent samples and analysis of covariance (ANCOVA) adjusted for age. The adjustment for age was done because there was a statistically significant difference in age between the Parkinsonians and controls.

Results

Mean levels of somatostatin-like immunoreactivity and homovanillic acid in CSF were significantly reduced in patients with Parkinson's disease. Acetylcholinesterase activity did not differ from that of the control patients (table 1). The reduction in SLI levels was most marked in the CSF of demented Parkinsonian patients (table 2). Also the values for patients with Parkinson's disease who showed no signs of dementia seemed to be lower than those for the controls. However, there were no statistically significant differences in SLI levels when the groups of Parkinsonian patients with different degree of dementia were compared with the controls or with each other (ANCOVA, adjusted for age). Similar results were obtained concerning the relation of SLI values and severity of Parkinson's disease (table 3). No difference in levels of somatostatin-like immunoreactivity, acetylcholinesterase, or homovanillic acid was found

Table 2 Somatostatin-like immunoreactivity (SLI) in CSF of patients with Parkinson's disease. Relation to severity of dementia.

	SLI fmol/ml
Controls	12.5 \pm 0.7 (19)
Parkinsonians	
0	10.4 \pm 1.0 (9)
0.5	9.7 \pm 0.5 (19)
1 + 2	9.0 \pm 0.9 (7)

No significant differences between the groups; ANCOVA adjusted for age. The severity of dementia was estimated using the rating scale of Hughes *et al.*²¹; 0 = no signs of dementia, 0.5 = slight memory disorders (questionable dementia), 1 + 2 = mild or moderate dementia (patients met DMS III criteria). Values are expressed as mean \pm SEM with number of patients in parentheses.

Table 3 Somatostatin-like immunoreactivity (SLI) in CSF of patients with Parkinson's disease. Relation to stage of disability.

	SLI fmol/ml
Controls	12.5 ± 0.7 (19)
Parkinsonians	
I	11.4 ± 1.3 (8)
II	9.4 ± 0.4 (22)
III + IV	8.4 ± 0.7 (5)

No significant differences between the groups; ANCOVA adjusted for age. The disability stage of Parkinson's disease (I-IV) was estimated according to Hoehn and Yahr.²⁰ Values are expressed as mean ± SEM with number of patients in parentheses.

between patients with Parkinson's disease who were receiving cholinergic medication and those who did not. The levels of SLI, AChE and HVA were also similar in depressed (depression scores according to Hamilton's scale ≤ 12) and in non-depressed Parkinsonian patients (depression scores > 12).

Discussion

The relationships between dementia and derangements in neurotransmitter systems have been poorly determined in Parkinson's disease.²⁵ Previous reports have indicated cholinergic dysfunction in patients with Alzheimer's disease as well as in Parkinsonian patients with cognitive impairment.^{2, 5, 6} Although low acetylcholinesterase activities have been reported in CSF of Alzheimer patients, we could not demonstrate a significant change in this cholinergic enzyme activity in Parkinsonian patients with mild or moderate dementia compared with the controls or patients without cognitive impairment.^{16, 26} The unaffected AChE levels of demented patients with Parkinson's disease may be explained by the fact that patients in the present study were in the early stages of the disease and the cognitive impairment observed was at most moderate. AChE activity in CSF should be measured in Parkinsonian patients with more advanced disease and more severe dementia. On the other hand, the cognitive dysfunction in Parkinson's disease may be based on the disturbance of other transmitter systems than the cholinergic one.

In patients with Parkinson's disease the mean somatostatin-like immunoreactivity was reduced compared with the values for controls. These values seemed to be related also to the severity of dementia and stage of disability, although statistical significances were not found. The lowest levels of SLI were observed in patients with the most severe cognitive impairment, a finding which agrees with the post mortem data of Epelbaum *et al.*¹⁴ In CSF studies without neuropathological verification of diagno-

sis, however, we cannot exclude the possibility that Parkinson's disease and Alzheimer's disease co-exist. Therefore the CSF results must be interpreted cautiously. Dupont *et al* also found low levels of somatostatin in CSF of patients with Parkinson's disease, but the relation of these values to the severity of dementia was not studied.¹⁵ However, levels of CSF somatostatin also seem to be reduced in several other degenerative diseases.¹⁶⁻¹⁸ Thus further studies are needed to determine the specificity and significance of somatostatin changes in CSF.

In addition to the low homovanillic acid concentrations, the affected somatostatin, methionine-enkephalin and substance P values in CSF strengthen the view that extensive peptidergic disorder is present in Parkinson's disease.^{15, 27} According to the CSF findings concerning somatostatin, patients with Parkinson's disease may have some neurochemical features associated with dementia that are similar to the changes found in Alzheimer's disease. Furthermore, the present findings suggest that the cholinergic system may remain more intact in Parkinson's disease than in Alzheimer's disease. This supports the hypothesis that cognitive dysfunction in Parkinson's disease and Alzheimer's disease may have different mechanisms.

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