Increased plasma neurotensin concentrations in patients with Parkinson’s disease

R-M Schimpff, C Avard, G Fénelon, A-M Lhiaubet, L Tennezé, M Vidailhet, W Rostène

Abstract
Plasma neurotensin (NT) was measured by radioimmunoassay in propanol extracted and unextracted plasma from 16 parkinsonian patients (four before treatment) and 16 age and sex matched controls. Mean plasma NT concentrations were consistently higher in parkinsonian patients than in controls and higher in the four untreated patients than in levodopa treated patients suggesting that plasma NT measurement may represent an easy detectable additional index in diagnosing parkinsonism and provides a novel approach to research in this field. (J Neurol Neurosurg Psychiatry 2001; 70:784–786)

Keywords: Parkinson’s disease; plasma neurotensin; dopamine

The tridecapeptide neurotensin (NT), is closely linked to dopaminergic systems in the brain, which are damaged in Parkinson’s disease. Because NT was easily measurable in human plasma and changes in CSF concentrations of NT were shown to be associated with physiopathological situations involving central dopaminergic perturbations, this pilot study aimed to investigate the possibility that plasma NT concentrations may be altered in parkinsonian patients. If so, plasma NT could serve as a trait marker in diagnosis, irrespective of the severity or duration of the disorder, or as a state marker detectable at the onset of parkinsonism.

Subjects and methods
SUBJECTS
The 32 subjects comprising this study were informed of its purpose before blood sampling and all provided written consent. Each patient with Parkinson’s disease was matched with an age and sex matched control. The number of subjects was determined statistically to obtain possible significant data according to the French law (Huret’s law) for a preliminary protocol. The study received the approval of the local ethics committee (CCPPRB Tenon 40–96, Amdt 1) and was promoted by INSERM (Convention No 97 C 77 305).

PATIENTS WITH PARKINSON’S DISEASE
Sixteen patients (seven women, nine men aged between 50 and 70 years (mean (SEM) 58.8 (2.1)) were selected on the basis of at least three of the usual five diagnostic criteria for Parkinson’s disease: akinesia, muscular rigidity, tremor in relaxed muscles, asymmetric onset, marked remission (>50%) after levodopa treatment. Patients with further neurological symptoms, or under neuroleptic treatment, or with a medical history of severe conditions (vascular brain damage, encephalitis, poisoning) were excluded. Four patients (mean age, 65.7 years, early in the disease stage I, disease duration range 3–24 months) were studied before levodopa treatment. The 12 other patients (mean age, 63.1 years, 10 at disease stage II, and 2 at stage I, disease duration range 8 months-12 years) were taking levodopa (300–900 mg/day), with a good treatment response.

CONTROLS
Sixteen control subjects (seven women, nine men aged 50–70 years (mean (SEM) 60.5 (2.1)) were matched to the age and sex of the patients. None of the control subjects had clinical or laboratory evidence of any major disease or were taking any medication.

BLOOD SAMPLES
Blood (5 ml) was collected between 0 900 and 10 00 hours after overnight fasting, in tubes containing EDTA, K3, and aprotinin (Becton-Dickinson Systems Europe, Meylan, France). Plasma samples were stored at −20°C. All samples were tested within 2 months of storage.

PLASMA EXTRACTION
Plasma was extracted using disposable ODS-silica columns (C-18 columns, SEP-PAK, Waters, Cartridge, Millipore, St. Quentin en Yvelines, France) and eluted with propanol as previously described. The technique selectively extracts low molecular weight forms of NT-like immunoreactive material.

MEASUREMENT OF NT-LIKE IMMUNOREACTIVITY
The NT radioimmunoassay (RIA) and its validation have been described elsewhere. Briefly, NT was iodinated using lactoperoxidase. The rabbit NT antibody used cross reacted with
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Results

The level of significance chosen was p<0.05. Results are expressed as mean (SEM). Using Student’s t test to compare NT values between groups, the difference between controls and patients was significant in assays of whole plasma samples than in assays of plasma extracts. *p<0.05; **p<0.01; ***p<0.001 versus controls.

NT and NT 1–11, but not with neuromedin N or NT 8–13. It was incubated at 1/15000 final concentration with NT standards ranging from 1 to 1000 fmol/tube. Sampleswere assayed in triplicate in a total volume of 700 µl for 72 hours at 4°C. Assay sensitivity was 1 fmol/ml and reproducibility was <6% within assays and <10% between assays. All results are expressed in fmol NT/ml plasma.

FAST PROTEIN LIQUID CHROMATOGRAPHY (FPLC) OF PLASMA

Values of NT for extracts and crude plasma proved to be different. To test possible differences in the distribution of different molecular weight forms of NT, control samples, and samples from patients with Parkinson’s disease were subjected to FPLC on prepacked glass columns of Superose 12 HR 10/30 (Pharmacia Biotech, Orsay, France). Distribution of the NT forms in the different fractions was expressed as fmol NT/fraction.

The FPLC was performed at neutral and acidic pH and the ability of acidified fractions to bind125I-NT was studied.

STATISTICS

Results are given as mean (SEM). Dunnett’s test was used for comparison between groups. The level of significance chosen was p<0.05.

Results

PLASMA NT CONCENTRATIONS

In plasma extracts, the mean NT concentration of controls was 7.7 (0.9) fmol/ml plasma, whereas for patients with Parkinson’s disease it was significantly higher (14.6 (2.6)) fmol/ml, p<0.01; fig 1). The mean for the four untreated patients was 17.8 (3.8) (p<0.001).

For unextracted samples, the mean concentration for controls was 45.3 (5.5) fmol/ml plasma. In patients, the mean was significantly higher (38.6 (8.7) fmol/ml, p<0.05; fig 1). The difference between controls and the mean for the four untreated patients (72.4 (6.9)) was even greater and more significant (p<0.01).

ELUTION PROFILES OF NT-LIKE IMMUNOREACTIVITY AFTER SUPEROSE 12 CHROMATOGRAPHY

In view of the apparent discrepancies between NT values for unextracted plasma and extracts, the distribution of the different molecular weight forms of NT was investigated with FPLC at neutral pH. Radiolabelled NT in buffer or after incubation with plasma consistently eluted in fractions corresponding to 2.2–1.4 kDa. For all samples studied, there were three peaks of NT-like immunoreactivity corresponding to three molecular weight ranges, 101–52, 21–14, and 2.2–1.4 kDa, respectively. Minimal amounts of immunoreactivity eluted with material below 1 kDa.

In the controls, the amounts of NT eluting in the three major peaks corresponded to 37.3 (3.0)%, 23.7 (4.9)%, and 24.8 (3.9)% respectively of total NT recovered. In the patients, these percentages were similar: 35.9 (4.0)%, 27.5 (7.9)%, and 23.4 (4.0)% respectively. In both groups there was significantly more NT-like material in the high molecular weight fractions than in the low fractions (p<0.01).

Discussion

Complex mechanisms determine the course of Parkinson’s disease and, to date, no biological markers have been identified to distinguish patients with the disease from healthy subjects. Neurotensin is a neurotransmitter involved in the regulation of dopaminergic systems and may play a part in Parkinson’s disease. It is readily measurable in the bloodstream and the aim of this pilot study was to test the hypothesis that plasma NT may be used as marker for Parkinson’s disease.

Mean plasma NT concentrations in patients were consistently higher in both extracted and unextracted plasma than in controls. Furthermore, untreated patients had higher NT concentrations than treated patients. This may be related to the finding that treatments aimed to restore cerebral dopamine concentrations and release alter NT concentrations. Our results indicate that measurement of extracted plasma NT concentrations in patients with Parkinson’s disease may prove useful as an index in diagnosis. However, the mechanisms involved in this increase in plasma NT are still unknown. It is known that 90% of NT is located in the periphery, mainly in the gut and lymphocytes and that central modifications in neurotransmitter release as in Parkinson’s disease may result in alterations of peripheral NT release. Accordingly FPLC analyses show several peaks of NT activity. Large forms of NT are known to be released from intestinal storage and are found in blood samples from the hepatic portal vein. Interestingly our data indicate that the distribution of different forms
of circulating NT is similar in controls and patients, suggesting that these different forms do not interfere with the assay.

In conclusion, the present clinical findings are consistent with data from experimental studies in rats and postmortem studies of human brain, which have shown increased NT in Parkinson’s disease.10 Our findings also demonstrate the presence of several forms of NT in the bloodstream, which may account for the differences between amounts of NT in extracts and in crude plasma samples.

From this pilot study, measurement of plasma NT concentrations seems to be a usable and accessible marker for studying the pathogenesis of the disease. In the near future, determination of putative plasma markers such as neuropeptides may be added to the diagnostic criteria for Parkinson’s disease. The present work opens a new avenue for this.

This study was supported by a grant from the Assistance Publique-Hôpitaux de Paris to the Clinical Investigation Center of Saint-Antoine University Hospital. We are grateful to P Jaillon, C Funk-Brentano, and to the nursing staff and secretariat of the Clinical Investigation Centre at Saint-Antoine University Hospital. We are indebted to D Pélaprat (INSERM U.339) for his critical reading and constructive comments. M Groussard was invaluable in the preparation of bibliography, S Carvajal-Gonzales in computer support, and Dr A Dunn in linguistic and critical review of the manuscript.


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*J Neurol Neurosurg Psychiatry* 2001 70: 784-786
doi: 10.1136/jnnp.70.6.784

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