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Correlation between changes in CSF dopamine turnover and development of dyskinesia in Parkinson's disease

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ABSTRACT

To assess possible differences in dopamine metabolism that could parallel disease progression in Parkinson's disease (PD), we measured dopamine (DA) and its metabolites in the cerebrospinal fluid (CSF) in PD patients at different stages of disease: de novo (DEN), advanced not showing dyskinesias (ADV), and advanced with dyskinesias (DYS).

DA, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were significantly higher in DEN patients compared with other groups. A negative exponential correlation related DA level and disease duration. The HVA/DA ratio was significantly higher in the ADV and DYS group than that found in DEN group.

Our data show that disease progression produces an early large decay of DA levels, followed by a stabilization. On the contrary, a late change in DA turnover (increased HVA/DA ratio) is documented in patients with longer disease duration. Our results suggest that the appearance of dyskinesia may not be related to a further loss of DA terminals but to a different, abnormal, DA turnover.

1. Introduction

The content of dopamine (DA) and DA metabolites in the cerebrospinal fluid (CSF) of Parkinson's disease (PD) patients was extensively studied in the 1970s. First, a relationship was sought between CSF concentrations of DA, homovanillic acid (HVA) and the disease pathophysiology – i.e. if DA and HVA levels were lower in the CSF of PD patients than in normal subjects. Second, several studies tested whether DA levels could be used as a qualitative, all-or-none biomarker of the disease [1–5]. Third, it was explored whether DA levels correlated with disease severity [2,6,7]. While the results were quite unanimous on the first topic, a large variability was observed concerning the second and third issues [3,4]. Several factors, such as rostro-caudal spinal gradient for catecholamines or the time of CSF collection in relation to bed-rest, age and gender [8–10] may account for the inconsistency in the previous measurements of HVA levels, either in baseline conditions or following pharmacological stimulation. Moreover, the techniques utilized to measure DA and HVA varied considerably [11]. Therefore the question of whether DA content and metabolism changed along disease progression was not really addressed. Additionally, the use of levodopa to treat early diagnosed PD patients prevented further investigations at different stages of disease. Indeed, the last large study on CSF cathecolamine biochemistry [12] was performed on a group of very early patients which could not offer insights into changes in DA turnover related to disease progression.

Thus, the correlation between CSF DA content and its metabolism alongside disease progression is still debated. This issue could be investigated by measuring CSF DA and metabolites in an adequate cohort of PD patients drawn from all the stages of disease: early-de novo, advanced with wearing-off/fluctuations, and dyskinetic.

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2. Material and methods

2.1. Patients

Forty-five subjects with idiopathic PD (according to the UK Parkinson's Disease Society Brain Bank criteria [13]) were recruited for this study. In the de novo (DEN) patients, the diagnosis was confirmed by apomorphine and/or levodopa test by asymmetry of the dopaminergic deficit shown with [^{123}I]-FPCIT (DATSCAN, GE Healthcare – Amersham Health), by single photon emission computerised tomography, and by normal MRI imaging. In non-DEN patients, exclusion criteria were stable response to antiparkinsonian therapy; diphasic dyskinesias; evidence for other CNS disorder; cognitive and psychiatric impairment; diabetes and other metabolic disorders; use of any drug acting at CNS level, diuretics; and height of the patients not included in the criteria reported below.

Demographics and clinical characteristics are shown in Table 1. As expected, a statistically significant correlation was found between disease duration or Hoehn & Yahr (H&Y) stage and both rigidity and bradykinesia, as well as with the Unified Parkinson's Disease Rating Scale (UPDRS) score ($p < 0.001$ data not shown). This analysis ensured that the group was representative of a broad spectrum of disease stage. Patients were divided into: 18 DEN patients who had never received any medication for PD, except for testing their pharmacological response; 14 advanced fluctuating patients (ADV) not showing dyskinesias in response to dopaminergic pharmacotherapy but showing end-of-dose phenomenon or impaired bed motility during the night, or morning wearing-off; 13 advanced patients experiencing motor fluctuations plus peak-dose dyskinesias (DYS). Drug intake was withdrawn in ADV and DYS patients 20 days before CSF collection. A prolonged washout in PD patients in good general conditions has been already proposed and performed by other groups [14–16]. The UPDRS, motor section III score, was carried out directly by an expert neurologist. Patients gave their written informed consent to all the procedures, including drug withdrawal. All procedures were carried out with the appropriate understanding and written consent of the subjects, and were previously approved by the Local Ethics Committee.

2.2. Collection and analysis of CSF samples

All of the subjects were hospitalised and underwent lumbar puncture on the same day of the clinical evaluation. All the procedures were performed according to LeWitt and Galloway [5]. To minimise the rostro-caudal gradient, lumbar puncture was performed at distinct interspaces according to patients' height (L4–L5, height 160–170 cm; L3–L4, height 170–180 cm; L2–L3, >180 cm). These procedures substantially eliminated the rostro-caudal gradient in our patients ($R^2 = 0.11$, $P = 0.021$, $n = 45$, compared to $R^2 = 0.90$ in Ref. [17]) (Fig. 1). Moreover, we included patients in the three groups selecting them in order to have homogeneous distribution of heights in the three groups (Fig. 1) and ANOVA analysis showed no significant height differences between the three groups.

The first 2 ml sample was used for routine analyses. Then a sample (200 μL) was collected, protected from light, acidified by adding 10 μL of perchloric acid 5 M and immediately frozen at −80 °C. Throughout this procedure all vital parameters were accurately monitored for 24 h.

The concentrations of DA, DOPAC and HVA in CSF samples were determined by HPLC analysis coupled with electrochemical detection (Model 5100A with a 5014B analytical cell, ESA, USA).

In the case of DOPAC and HVA, samples were loaded onto a C_{18} reverse-phase column (Atlantis dC_{18} 3 μm 4.6 × 150 mm, Waters, Ireland) and analysis was performed using the following conditions: mobile phase, 75 mM sodium phosphate, 50 mM EDTA, 0.5 mg/L sodium dodecyl sulfate, triethylamine 100 μL/L and CH_3CN 9%, final pH 3.0; flow rate 1.0 ml/min. Applied potentials were +0.30V and −0.30V at the first and second electrode, respectively. Signal to integrate was obtained by subtracting the output of detector II from that of detector I. Detector gain was adjusted to obtain a detection limit of 50 pg/mL for DOPAC, whose concentration in CSF samples was markedly lower than that of HVA. In the case of DA, analysis was performed using a shorter C_{18} column (symmetry C_{18} 3.5 μm 4.6 × 75 mm, Waters, Ireland) and increasing the percentage of CH_3CN to 12%. In comparison with the method described above, under these chromatographic conditions DA was eluted at a shorter retention time, thus resulting in narrowing of the peak shape, increased peak height and a better signal-to-noise ratio. Applied potentials were as above, but detector gain was adjusted to obtain a detection limit of 25 pg/mL.

2.3. Statistics

Biochemical data, given their non-homogeneous distribution of variances, were separately assessed by one factor non-parametric Kruskal–Wallis/median test for three groups. In case of significance, differences between pairs of groups were assessed by means of *post hoc* Mann–Whitney test. For each variable, both median and mean are reported in the tables. Ratios are reported only as median since parametric test cannot be utilized. Because values obtained by biochemical measurements were not normally distributed, the correlations between DA and its metabolites were assessed by Spearman's correlation. Clinical data were assessed by Kruskal–Wallis test and their correlations by means of Spearman's test. Multiple comparisons were corrected with Bonferroni test.

Fitting with non-linear models for the relationship between DA, HVA and DOPAC and between those compounds and disease duration was estimated by regression analysis in Prism 3.0 (Graphpad Software Inc, San Diego, CA, USA). Convergence was reached when two consecutive iterations changed the sum-of-squares by less than 0.01%. Coefficient of determination (R^2) and absolute sum-of-squares $S_{y.x}$ were calculated to select the best-fit model. To compare two different fittings, F test was used after demonstration of normal (normality test and runs test) residual distribution. The simpler equation was chosen unless the more complicated fits significantly better with $P < 0.05$. If the confidence intervals of curve fittings were very wide, the model was rejected disregarding the results of the F test. Five

Table 1
Demographic and clinical data of the three populations.

De Novo (DEN)					Advanced (ADV)						Dyskinetic (DYS)					
Patients	Age (years)	Disease duration (months)	UPDRS	H&Y	Patients	Age (years)	Disease duration (months)	UPDRS	H&Y	Previous levodopa equivalent (mg)	Patients	Age (years)	Disease duration (months)	UPDRS	H&Y	Previous levodopa equivalent (mg)
1	75.0	8.0	24.0	2.0	1	68.0	48.0	39.0	3.0	68.0	1	62.0	140.0	64.0	4.0	62.0
2	74.0	6.0	31.0	2.0	2	53.0	72.0	33.0	2.5	53.0	2	60.0	120.0	66.0	4.0	60.0
3	66.0	12.0	9.0	1.0	3	73.0	60.0	26.0	2.5	73.0	3	51.0	50.0	62.0	4.0	51.0
4	71.0	24.0	24.0	1.5	4	79.0	60.0	38.0	3.0	79.0	4	66.0	132.0	61.0	4.0	66.0
5	71.0	30.0	24.0	2.0	5	63.0	96.0	44.0	3.0	63.0	5	66.0	96.0	56.0	3.0	66.0
6	58.0	32.0	37.0	2.5	6	68.0	60.0	50.0	4.0	68.0	6	64.0	120.0	68.0	5.0	64.0
7	68.0	11.0	15.0	1.5	7	73.0	60.0	40.0	3.0	73.0	7	56.0	146.0	67.0	5.0	56.0
8	69.0	6.0	24.0	2.0	8	78.0	30.0	63.0	5.0	78.0	8	66.0	70.0	65.0	4.0	66.0
9	69.0	28.0	43.0	3.0	9	67.0	120.0	36.0	2.5	67.0	9	64.0	84.0	67.0	4.0	64.0
10	62.0	12.0	14.0	1.5	10	65.0	36.0	45.0	3.0	65.0	10	63.0	156.0	72.0	5.0	63.0
11	51.0	12.0	23.0	2.0	11	71.0	66.0	32.0	2.5	71.0	11	66.0	160.0	61.0	4.0	66.0
12	64.0	24.0	35.0	2.5	12	78.0	36.0	25.0	2.5	78.0	12	65.0	108.0	62.0	4.0	65.0
13	65.0	12.0	18.0	1.5	13	70.0	12.0	66.0	4.0	70.0	13	68.0	216.0	71.0	5.0	68.0
14	63.0	36.0	18.0	1.0	14	73.0	48.0	38.0	2.5	73.0						
15	67.0	24.0	16.0	1.0												
16	68.0	6.0	10.0	1.0												
17	69.0	12.0	9.0	1.0												
18	66.0	24.0	30.0	1.5												
Mean	66.4	17.7	22.4	1.7	Mean	69.9	57.4	41.1	3.1	69.9	Mean	62.8	122.9	64.8	4.2	62.8
SD	5.7	9.9	9.9	0.6	SD	6.9	27.2	12.1	0.8	6.9	SD	4.7	43.4	4.4	0.6	4.7
Median	67.5	12.0	23.5	1.5	Median	70.5	60.0	38.5	3.0	70.5	Median	64.0	120.0	65.0	4.0	64.0
Lower quartile	64.3	11.3	15.3	1.1	Lower quartile	67.3	39.0	33.8	2.5	67.3	Lower quartile	62.0	96.0	62.0	4.0	62.0
Upper quartile	69.0	24.0	28.5	2.0	Upper quartile	73.0	64.5	44.8	3.0	73.0	Upper quartile	66.0	146.0	67.0	5.0	66.0