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Trace amine metabolism in Parkinson's disease: Low circulating levels of octopamine in early disease stages

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ABSTRACT

Recent evidence suggests that trace amines such as tyramine and octopamine, alternative products of tyrosine metabolism (an aminoacid parent of dopamine and noradrenaline), play a role in the homeostasis of the extrapyramidal system. However, the relevance of these trace amines in the pathogenesis of Parkinson's disease is still largely unknown. Here, we assessed the plasma levels of octopamine and noradrenaline in three sub-groups of PD patients, namely *de novo*, non-fluctuating and fluctuating patients, versus age-matched control subjects. We show that octopamine is detectable in plasma of all subjects, the mean levels of which are significantly lower in PD patients, including *de novo* patients, when compared to controls ($p < 0.001$). Unlike this, no changes in plasmatic noradrenaline levels were found in the *de novo* patients, but only in plasma of fluctuating and non-fluctuating PD patients. These findings raise the possibility that Parkinson's disease is firstly characterized by abnormalities of tyrosine decarboxylase, rather than tyrosine hydroxylase, enzyme activity. Given the role of this enzyme in the production of trace amines, circulating octopamine levels may hold promise as a biomarker of early Parkinson's disease.

Parkinson's disease (PD) is a progressive neurological disorder characterized by the symptomatological triad of rigidity, bradykinesia and resting tremor. Much evidence suggests that these features are due to degeneration of dopaminergic nigrostriatal neurons. However, symptoms as hyposmia, sleep disorders, depression, and autonomic failure that accompany or precede the extrapyramidal signs indicate that the disease may initiate in other centers of the central nervous system (CNS) [6,7,20].

Elusive amines, such as tyramine, octopamine and synephrine, are products of tyrosine metabolism arising from the activation of dopamine decarboxylase [2]. For decades, these amines have been considered functionally unimportant in the CNS, because of their low physiological concentrations (thereby, the term "elusive" or "trace" amines) and, more important, the absence of specific receptors. New evidence, however, suggests that elusive amines may play an important role in the physiology of the human brain. Two new G protein-coupled receptors, nominated trace amine associated receptors (TAARs), have been discovered in mammalian brain [4]. These receptors are localized in many brain areas, e.g. amygdala, hypothalamus, thalamus, cerebellum, locus coeruleus, raphe magnum. [5]. Although the physiological ligands for these and other TAAR receptor subtypes remain unknown, it is possible that one or more of these receptors are activated by octopamine or other trace amines. Intriguingly, octopamine and other trace amines are detectable in both plasma and platelets of human subjects, the levels of which may vary in different physiopathological conditions [11–13]. Despite increasing evidence suggesting that tyramine and octopamine modulate the function of the extrapyramidal system [14–16], no information is available with regard to the possible role of elusive amines in the pathogenesis of PD. We here approached this unexplored topic by investigating the plasma levels of noradrenaline along with octopamine, products of tyrosine hydroxylase and tyrosine decarboxylase enzymes, respectively, in PD patients.

PD patients were stratified in three sub-groups: "*de novo*" (DN) PD patients, non-fluctuating (NF) PD patients and fluctuating (F) PD patients. The DN group comprised 16 patients displaying PD symptoms since one year or less. Symptoms affecting these patients consisted mainly of unilateral hand tremor at rest and increased extrapyramidal tone, with UPDRS (Unified Parkinson's Disease Rating Scale) not exceeding 12 points. All DN patients were not treated with any drug employed for PD treatment. The NF group, the most numerous, consisted of 47 PD patients characterized by bradykinesia, bilateral tremor at rest and increased bilateral extrapyramidal tone, with UPDRS ranging from 18 to 36. NF patients were treated

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with L-DOPA supplemented with carbidopa; therapy was effective in reducing or abolishing PD symptoms. The F PD group comprised 21 patients with fluctuating parkinsonian symptoms such as bradykinesia, festination, tremor, on–off phenomena, motor block and dyskinesia and, less frequently, orthostatic hypotension, with UPDRS score greater than 38. These patients, treated with L-DOPA and dopamine agonists (800–1200 mg/day), showed incomplete response to therapy. The majority of PD patients did not show comorbidity with hypertension or metabolic disease. PD diagnosis was made accordingly to currently accepted criteria [19]. Control group consisted of twenty-eight age-matched healthy subjects displaying no symptoms related to PD or other neurological and metabolic diseases.

Following informed consent, all subjects underwent blood sampling (20 ml) for assessment of plasma levels of noradrenaline and octopamine. Peripheral venous blood was drawn from subjects from the antecubital vein at 9 AM, after 5 min of resting in the supine position and before the daily assumption of the morning therapy. Blood was collected in a tube containing citric acid/citrate dextrose as anticoagulant. The plasma poor platelet (PPP) fraction was obtained after centrifugation of whole blood and fast speed centrifugation ($2100 \times g$ for 15 min) of the plasma rich platelet (PRP) fraction, previously obtained by preliminary low centrifugation of whole blood ($165 \times g$, 10 min). PPP aliquots were stored at −80 °C until assay.

The chromatographic system employed for noradrenaline assessments in plasma consisted of a model 307 pump, an autosampler model 234 with 100 μl loop (Gilson, Villiers-le-Bel, France) and a Sunfire stainless-steel column measuring 250 mm × 4.6 mm i.d. (Waters Co., Milford, MA). The mobile phase consisted in formic acid 0.1 mM, EDTA 0.5 mM, citric acid 1 mM, diethylamine 34 mM, acetonitrile 22% (v/v), sodium azide 7.7 mM, pH 3.2. The detection was performed with an electrochemical coulometric detector (Coulochem 5100, ESA Inc., Belford, MA, USA) coupled with an analytical high sensitivity cell model 5011 (provided with two electrodes set at +0.20 and +0.55 V). The signal generated was integrated by a computing integrator Gilson 712 software. The intra- and inter-assay relative standard deviations were 1.6% and 3.4%, and lower limit of detection was 1.2 pg for all catecholamines. All analytical standards and reagents used were purchased from Sigma–Aldrich (Milan, Italy) [17].

Octopamine assay was performed following acid deproteinization of PPP and strong cationic exchange (SCX) extraction, pre-column o-phthalaldehyde (OPA) derivatization and reverse-phase HPLC with electrochemical detection. A total of 1 ml of plasma was first deproteinized by addition of 250 μl of sulphosalicilic acid 15% (v/v) followed by centrifugation ($2100 \times g$ for 10 min at +4 °C). The deproteinized plasma samples and octopamine standard solutions were then injected into solid phase extraction (SPE) columns (SCX, IST 200 mg, Biotage Uppsala, Sweden). After two washing steps with 1 ml methanol 50.0% (v/v) and 1 ml of water, the samples were eluted with 1 ml of $Na_2B_4O_7$ 0.4 M and methanol 80:20 (v/v). Before HPLC analysis, derivatizing solution (obtained mixing 25 μl of OPA 0.3 M and 25 μl of sodium sulphite 1.0 M in 1 ml of $Na_2B_4O_7$ 0.1 M) was added to each sample (10 μl of solution in 250 μl of SPE extracts). The samples were then incubated for 30 min at room temperature. A total of 50 μl of each sample was injected into the HPLC system (equipped by two Gilson pumps model 305 and 307, and a Gilson 50 ml loop model 234 autosampler); the separation was performed on a stainless-steel 5 mm column X-Terra 250 mm × 4.6 mm i.d. (Waters Corporation, Milford, MA). The gradient of the mobile phase was obtained with Na_2HPO_4 20 mM pH 8.0 and CH_3CN 78:22 (v/v) (pump model 305) and Na_2HPO_4 20 mM pH 8.0 and CH_3CN 40:60 (v/v) (pump model 307). The detector and the high sensitivity cell (+0.20 and +0.60 V at the first and second electrode, respectively) are the same as that utilized for catecholamines. The intra- and inter-assay relative standards deviations were below 5.3% and lower limit of detection was 10 pg for both amines.

Table 1
Demographic characteristics of the subjects included in the study.

Groups	Age (yrs) ± SD	Male	Female	Total	Years of disease
Ctr	60.01 ± 8.84	9	19	28	
DN	61.94 ± 8.87	11	5	16	<1
NF	68.15 ± 9.90	31	16	47	>3
F	68.86 ± 10.54	12	9	21	>5

Statistical analysis was conducted employing SPSS software, version 13. Value distributions for all parameters tested were evaluated by the Kolmogorov–Smirnov test. Parameters with Gaussian distribution were compared by Welch's *t*-test or Student's *t*-test for non-equal and equal variance assumption, respectively (Lavene's test for variance). Values displaying non-parametric distribution were compared employing the Mann–Whitney *U*-test.

The demographic characteristics of the control and PD subgroups are shown in Table 1. The NF and F PD patient groups were slightly older with respect to control and DN groups. Mean duration of parkinsonian symptoms increased from the DN to the NF and the F patient groups. Although patient sub-groups and control group were not sex-matched, mean levels of octopamine and noradrenaline, here measured in male and female subjects within the groups, were at all times comparable (data not shown).

In comparison to controls, the mean plasma level of octopamine of all PD patients included in the study was significantly lower ($p < 0001$, Mann–Whitney test) (Table 2). Upon comparison of the octopamine plasma levels among the three sub-groups of PD patients, the mean octopamine level of the *de novo* patient group was significantly lower than that of the non-fluctuating patients displaying good L-DOPA response ($p < 0.001$, Mann–Whitney test) as well as that of the fluctuating patients displaying on–off phenomena ($p < 0.001$, Mann–Whitney test) (Fig. 1).

Noradrenaline mean plasma levels were significantly lower in PD patients, when compared with the control group ($p < 0.001$, Mann–Whitney test) (Table 2). However, in contrast to octopamine, mean noradrenaline levels in the *de novo* PD patient group were similar to that of the control group, whilst that in the other two PD patient sub-groups (NF and F) was significantly lower ($p < 0.001$ and $p = 0.003$, respectively). Noradrenaline plasma levels were also significantly ($p = 0.02$) reduced in the NF patient group with respect to *de novo* PD patients (Fig. 2).

The above results show that plasma levels of noradrenaline and octopamine, products of tyrosine hydroxylase and tyrosine decarboxylase, respectively, are significantly lower in PD patients with respect to control subjects, suggesting that PD may be phenotypically characterized by alterations in tyrosine metabolism. Also, octopamine was below control levels in all PD patient groups, including patients in the early stage of the disease (i.e. *de novo*

Table 2
Noradrenaline and octopamine plasma levels in PD sub-groups and controls.

Groups	Octopamine (ng/ml)			Noradrenaline (pg/ml)		
	Mean	SE	*p*-Value	Mean	SE	*p*-Value
Ctr	4.28	0.28		227.33	28.30	
PD	1.80	0.16	<0.001*	109.15	13.51	<0.001*
DN	0.65	0.22	<0.001**	151.01	25.89	0.02§
NF	2.00	0.17	<0.001*	86.26	19.44	<0.001*
F	2.27	0.38	<0.001*	121.55	24.78	0.003*

* *p* value referred to the comparison with Ctr groups.
** *p* value referred to the comparison between DN and F or NF groups.
§ *p* value referred to the comparison between DN and NF groups.