Cerebrospinal fluid biomarkers of central catecholamine deficiency in Parkinson’s disease and other synucleinopathies

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Clinical catecholamine deficiency characterizes α-synucleinopathies such as Parkinson’s disease. We hypothesized that cerebrospinal fluid levels of neuronal metabolites of catecholamines provide neurochemical biomarkers of these disorders. To test this hypothesis we measured cerebrospinal fluid levels of catechols including dopamine, norepinephrine and their main respective neuronal metabolites dihydroxyphenylacetic acid and dihydroxyphenylglycol in Parkinson’s disease and two other synucleinopathies, multiple system atrophy and pure autonomic failure. Cerebrospinal fluid catechols were assayed in 146 subjects—108 synucleinopathy patients (34 Parkinson’s disease, 54 multiple system atrophy, 20 pure autonomic failure) and 38 controls. In 14 patients cerebrospinal fluid was obtained before or within 2 years after the onset of parkinsonism. The Parkinson’s disease, multiple system atrophy and pure autonomic failure groups all had lower cerebrospinal fluid dihydroxyphenylacetic acid [0.86 ± 0.09 (SEM), 1.00 ± 0.09, 1.32 ± 0.12 nmol/l] than controls (2.15 ± 0.18 nmol/l; P < 0.0001; P < 0.0001; P = 0.0002). Dihydroxyphenylglycol was also lower in the three synucleinopathies (8.82 ± 0.44, 7.75 ± 0.42, 5.82 ± 0.65 nmol/l) than controls (11.0 ± 0.62 nmol/l; P = 0.009, P < 0.0001, P < 0.0001). Dihydroxyphenylacetic acid was lower and dihydroxyphenylglycol higher in Parkinson’s disease than in pure autonomic failure. Dihydroxyphenylacetic acid was 100% sensitive at 89% specificity in separating patients with recent onset of parkinsonism from controls but was of no value in differentiating Parkinson’s disease from multiple system atrophy. Synucleinopathies feature cerebrospinal fluid neurochemical evidence for central dopamine and norepinephrine deficiency. Parkinson’s disease and pure autonomic failure involve differential dopaminergic versus noradrenergic lesions. Cerebrospinal fluid dihydroxyphenylacetic acid seems to provide a sensitive means to identify even early Parkinson’s disease.

Keywords: Parkinson’s; dopamine; norepinephrine; DHPG; DOPAC; biomarker

Abbreviations: DATATOP = deprenyl and tocopherol antioxidative treatment of parkinsonism; DHPG = dihydroxyphenylglycol; DOPAC = dihydroxyphenylacetic acid; L-DOPA = L-3,4-dihydroxyphenylalanine; MSA = multiple system atrophy; PAF = pure autonomic failure

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Introduction

Depletion of the catecholamine, dopamine, in the striatum (especially the putamen) is a defining neurochemical characteristic of Parkinson's disease. The classic description by Ehringer and Hornykiewicz (1960) of profoundly decreased striatal dopamine content in Parkinson's disease has been confirmed repeatedly and consistently for more than half a century (Goldstein et al., 2011) and led to the introduction of levodopa/carbidopa therapy, probably the first successful symptomatic treatment of a common neurodegenerative disease (Cotzias, 1971).

The same pivotal article also noted decreased content of another catecholamine, norepinephrine, in the hypothalamus. Post-mortem studies since then have shown that Parkinson’s disease involves decreased norepinephrine in the striatum and frontal cortex (Goldstein et al., 2011) and cerebellum (Kish et al., 1984) and at least some neuronal loss in the locus ceruleus (Halliday et al., 1990; Zweig et al., 1993; Zaro et al., 2003), the main source of norepinephrine in the brain. Outside the brain, patients with Parkinson’s disease (especially those with orthostatic hypotension) have decreased sympathetic noradrenergic innervation (Goldstein, 2007); however, there is scant in vivo evidence about whether Parkinson's disease entails central norepinephrine deficiency (Eldrup et al., 1995).

Currently the diagnosis of Parkinson’s disease is based on the neurological examination and responses to dopaminergic drugs. By the time midbrain substantia nigra pathological changes occur, however, neurodegeneration has been ongoing for some time (Braak et al., 2004; DelleDonne et al., 2008). Moreover, diagnosis by clinical assessment alone can be inaccurate in patients with mild symptoms of recent onset (Rajput et al., 1991). Researchers and clinicians therefore have long recognized the need for clinical diagnosis based on quantifiable measures—biomarkers—to refine qualitative assessments.

Given the marked central dopaminergic lesion, measurements of CSF levels of dopamine or its metabolites might provide a relatively straightforward, inexpensive, available diagnostic test. So far this expectation has not been realized. Although some reports have noted decreased levels of homovanillic acid, the end-product of dopamine metabolism (Zubenko et al., 1986; Chia et al., 1993; Loeffler et al., 1995), or of dihydroxyphenylacetic acid (DOPAC), the main neuronal metabolite of dopamine (Zubenko et al., 1986; Chia et al., 1993; Gonzalez-Quevedo et al., 1993; Eldrup et al., 1995; Kuhn et al., 1996; Engelborghs et al., 2003; Goldstein et al., 2008), an influential study based on assays of samples from the deprenyl and tocopherol antioxidative treatment of parkinsonism (DATATOP) trial reported negative results about both metabolites in Parkinson's disease (LeWitt et al., 1992). Current CSF biomarker research on Parkinson's disease emphasizes other biochemicals such as α-synuclein or proteomic patterns (El-Agnal et al., 2006; Zhang et al., 2008; Mollenhauer et al., 2011; Shi et al., 2011).

As explained later, the study from the DATATOP trial may have led to false negative inferences, due to questionable validity of the technique used to measure CSF levels of dopamine and DOPAC. Since then, no large study has evaluated the usefulness of CSF catecholamine neurochemistry in the diagnosis of Parkinson's disease. Revisiting this issue was a major purpose of the present study.

Although at first glance CSF dopamine, DOPAC, homovanillic acid or other dopamine metabolites might seem equally efficient in detecting central dopamine deficiency in Parkinson’s disease, levels of these compounds actually have different sources and meanings. CSF homovanillic acid is rather distantly related to neuronal dopamine stores and reflects several intervening processes. Since dopaminergic neurons do not contain catechol-O-methyltransferase, CSF homovanillic acid depends on uptake and intracellular O-methylation in extra-dopaminergic cells. Thus, in Parkinson’s disease the striatal content of homovanillic acid is not as severely decreased as that of dopamine (Lloyd et al., 1975).

CSF dopamine also may not provide an accurate reflection of central dopamine deficiency. Dopamine in extracellular fluid is derived mainly from exocytotic release in response to pathway traffic and escape of neuronal reuptake by the cell membrane dopamine transporter. Since it is likely that as dopaminergic neurons are lost, pathway traffic to the remaining terminals increases compensatorily, thereby augmenting dopamine delivery from those terminals to the extracellular fluid (Sossi et al., 2002). CSF dopamine may underestimate the extent of loss of neuronal dopamine stores. Moreover, CSF dopamine concentrations are infinitesimal (Scheinin et al., 1984), <10 pmol/l, sometimes below the detection limit of the assay method.

CSF DOPAC may be a superior index of central dopamine stores, compared to homovanillic acid or dopamine itself. DOPAC is formed from deamination of cytosolic dopamine catalysed by monoamine oxidase-A. Dopamine leaks continuously from vesicular stores into the cytosol. Therefore, the rate of DOPAC formation should be related to the amount of stored dopamine. Post-mortem putamen from patients with end-stage Parkinson's disease contains similarly drastically decreased tissue concentrations of dopamine and DOPAC (Goldstein et al., 2011), and CSF DOPAC is related directly to brain tissue content of this metabolite (Palfreyman et al., 1982).

Although several reports have noted low CSF DOPAC in Parkinson's disease, these were relatively small studies. Since CSF DOPAC has not yet been compared in Parkinson’s disease and other synucleinopathies, whether this measurement is specific has been unknown. Relationships among DOPAC, dopamine and endogenous L-3,4-dihydroxyphenylalanine (L-DOPA; a potential index of catecholamine biosynthesis) have also not been described in patients with synucleinopathies. Importantly, studies to date have not established whether patients with recent onset of parkinsonism have low CSF DOPAC.

In the rare disease, pure autonomic failure (PAF), the patients have Lewy body pathology (Kaufmann and Goldstein, 2010) and evidence for decreased numbers of substantia nigra dopaminergic neurons (Goldstein et al., 2008), but without parkinsonism. Instead, PAF features prominent orthostatic hypotension from sympathetic noradrenergic denervation (Ziegler et al., 1977; Orimo et al., 2002). Whether the very different clinical manifestations of the two Lewy body diseases are related to different patterns of loss of central dopaminergic versus noradrenergic neurons has been unknown.
Lewy bodies and Lewy neurites found in Parkinson’s disease and PAF contain abundant α-synuclein (Spillantini et al., 1997; Arai et al., 2000; Jellinger, 2003; Shishido et al., 2010), and abnormalities of the gene encoding α-synuclein are associated with Parkinson’s disease (Polymeropoulos et al., 1997; Edwards et al., 2010). Bases for the association between synucleinopathies and loss of catecholaminergic neurons remain incompletely understood. Multiple system atrophy (MSA) is a non-Lewy body synucleinopathy, which features cytoplasmic inclusions mainly in glial cells (Wakabayashi et al., 1998; Dickson et al., 1999). MSA can be very difficult to distinguish from Parkinson’s disease, either clinically (Rajput et al., 1991) or by striatal neuroimaging (Antonini et al., 1997). Unlike Parkinson’s disease with orthostatic hypotension and PAF, MSA entails normal sympathetic noradrenergic innervation in most patients (Orimo et al., 2001, 2002, 2007; Goldstein et al., 2003). MSA therefore constituted an important comparison group for the Lewy body diseases in this study.

Just as DOPAC is the main neuronal metabolite of dopamine, dihydroxyphenylglycol (DHPG) is the main neuronal metabolite of norepinephrine. A recent post-mortem study noted decreased putamen and cerebral cortex DHPG concentrations in Parkinson’s disease (Goldstein et al., 2011); however, whether Parkinson’s disease entails decreased CSF DHPG, norepinephrine or both is not known.

Here we report results of a study of CSF levels of catechols, including DOPAC and DHPG, in three forms of α-synucleinopathy: Parkinson’s disease, PAF and MSA. In general the study was designed to test whether CSF biomarkers of central catecholamine deficiency aid the diagnosis of Parkinson’s disease and other synucleinopathies. We assessed the sensitivity and specificity of CSF DOPAC for distinguishing Parkinson’s disease from control subjects and attempted to validate CSF DOPAC against two neuroimaging markers of the ability to retain dopamine in vesicular stores—the putamen:occipital cortex ratio of 18F-DOPA-derived radioactivity (Okinen et al., 2009) and the proportionate loss of radioactivity (‘washout’) between the peak value (at 30 min after tracer injection) and the value at 2 h after tracer injection (Goldstein et al., 2008). We separately analysed CSF neurochemical data from patients with recent onset of parkinsonism; examined relationships of levels of the deaminated metabolites to those of the parent catecholamines and of ı-DOPA, in order to evaluate the balance of synthesis and metabolism; and investigated whether different forms of synucleinopathy are associated with different patterns of catecholaminergic lesions as indicated by these neurochemical biomarkers.

## Materials and methods

### Subjects

The Intramural Research Board of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health approved the protocols for this study. All subjects gave informed written consent before the procedures.

Patients were referred to the NIH and carried an admission diagnosis of Parkinson’s disease, MSA or PAF. Clinical characteristics of the patients are shown in Table 1. The study as a whole included 146 subjects: 108 synucleinopathy patients (34 Parkinson’s disease, 54 MSA and 20 PAF) and 38 controls.

Diagnostic categorization in terms of MSA and PAF was based on previously published consensus criteria (Kaufmann, 1996) supplemented by data about plasma levels of catechols and cardiac sympathetic neuroimaging, as follows. Both MSA and PAF entail neurogenic orthostatic hypotension; however, the two diseases differ clearly in the status of sympathetic noradrenergic innervation. In MSA, sympathetic noradrenergic innervation is usually intact, whereas in PAF there is substantial generalized noradrenergic denervation (Ziegler et al., 1977; Goldstein et al., 1989). In the present study, all patients with MSA had normal plasma DHPG levels during supine rest, and in 51 of 52 patients with MSA interventricular septal myocardial 18F-dopamine-derived radioactivity was within two standard deviations of the normal mean, indicating intact overall and cardiac sympathetic noradrenergic innervation (Goldstein et al., 2008). Most patients with PAF had low concentrations of both plasma DHPG and myocardial 18F-dopamine-derived radioactivity, indicating generalized sympathetic noradrenergic denervation. Patients with MSA were included regardless of classification in terms of parkinsonian, cerebellar or mixed subtypes.

Of the 34 patients in the Parkinson’s disease group, all 34 (100%) had bradykinesia, 32 (94%) had bradykinesia and cogwheel rigidity, 21 (62%) had bradykinesia and resting tremor and 20 (59%) had the triad of bradykinesia, cogwheel rigidity and resting tremor. Among 24 patients who had been treated with levodopa/carbidopa, 79% had noted improvement of the movement disorder. The mean score on the Unified Parkinson’s Disease Rating Scale (OFF levodopa) was 45 ± 5, corresponding to moderate parkinsonism. Thus, the patients with Parkinson’s disease had typical clinical findings.

Of the patients with Parkinson’s disease in this study, we were firmly confident in the diagnosis in 10, based on the following: (i) diagnostic post-mortem neuropathology (n = 2); (ii) typical symptoms and signs in the setting of familial Parkinson’s disease from autosomal dominant triplication of the α-synuclein gene (n = 1); (iii) typical symptoms and personal examination by the Director of the

### Table 1 Clinical characteristics of patient groups

|                | Parkinson’s disease | MSA | PAF |
|----------------|---------------------|-----|-----|
| Age years      | 63 ± 2              | 60 ± 1 | 61 ± 3 |
| Male/female    | 20/14               | 34/20 | 13/7 |
| Light skin %   | 100                  | 100  | 100  |
| Ashkenazi %    | 14                   | 7    | 5    |
| Age at motor onset (years) | 56 ± 2            | 55 ± 1  |       |
| Time to study (years)    | 6 ± 1               | 4 ± 1 |       |
| Bradykinesia % | 100                 | 70    |       |
| Rigidity %     | 94                   | 48    |       |
| Resting tremor % | 62****             | 9     |       |
| Levodopa response % | 79**              | 36    |       |
| UPDRS (off levodopa) | 45 ± 5           | 46 ± 7 |       |
| Slurred speech % | 15****            | 76    |       |
| Orthostatic intolerance % | 57               | 50    | 100  |
| Erectile failure % | 76                | 90    | 100  |
| Constipation % | 62                   | 78    | 100  |
| Decreased sweating % | 26                | 33    | 67    |
| Urinary symptoms % | 86                 | 93    | 67    |

Significant difference Parkinson’s disease versus MSA. **P < 0.01; ****P < 0.0001.
NINDS Parkinson’s disease Clinic, who confirmed the referral diagnosis and was blinded as to the results of the clinical laboratory testing (n = 2); (iv) typical symptoms and signs, referral by a Board-certified neurologist who had completed post-doctoral Fellowship in intramural NINDS, and documented low putamen 18F-DOPA-derived radioactivity (n = 1); (v) typical symptoms and signs with follow-up by a Board-certified neurologist over years after the patient underwent implantation of a brain stimulator that alleviated the Parkinsonian movement disorder (n = 1); or (vi) typical symptoms and signs with follow-up over years by neurologists on staff of the Experimental Therapeutics Branch of the NINDS (n = 3). One of the patients diagnosed with MSA and one with PAF died after participating in the study and had post-mortem neuropathological confirmation of their diagnosis.

In 14 subjects CSF was obtained before or within 2 years after the onset of parkinsonism (New Parkinson’s disease group). One of the New Parkinson’s disease group patients had triplcation of the α-synuclein gene (PARK4), one had statistical risk factors for Parkinson’s disease and 1.5 years later developed parkinsonism and was diagnosed with Parkinson’s disease, seven had prominent orthostatic hypotension and were thought initially to have MSA or PAF but later were diagnosed with Parkinson’s disease, one had an initial diagnosis of PAF but has developed parkinsonism (although not diagnosed with Parkinson’s disease at the time of writing), and the remainder were newly diagnosed with Parkinson’s disease based on typical findings upon examination by a board-certified neurologist.

Control data were from healthy volunteers at the NIH Clinical Centre (n = 9, 50 ± 2 years old), individuals without clinical evidence of chronic autonomic failure or central neurodegeneration (n = 9, 53 ± 3 years old), or elderly volunteers (70 years old or older, samples from E. Peskind, University of Washington, n = 20).

Cerebrospinal fluid and plasma catechols

To obtain CSF for neurochemical assays, subjects at the NIH Clinical Centre underwent lumbar puncture under fluoroscopic guidance. Six 1-ml aliquots of fluid were collected into chilled 1.5 ml plastic sample tubes, which were frozen immediately in dry ice and then stored at or below −70 C until the samples were assayed. The sixth aliquot was assayed by the same person (C.H.), who was blinded as to clinical diagnosis, using batch alumina extraction followed by liquid chromatography with electrochemical detection (Holmes et al., 1994; Goldstein et al., 2003, 2008). Limits of detection for catechols were about 10 pmol/l, or 10 fmol per assayed ml of CSF.

For plasma catechol assays, arm venous blood was drawn through an indwelling intravenous catheter after the subject was at rest supine for at least 15 min. The plasma was separated by refrigerated centrifugation and frozen and stored at −80 C until assayed by the same method of batch alumina extraction and liquid chromatography with electrochemical detection (Holmes et al., 1994).

Dopaminergic neuroimaging

A total of 54 subjects had both lumbar punctures for CSF catechols and 18F-DOPA PET scanning for assessment of striatal dopaminergic innervation (Goldstein et al., 2008). The putamen:occipital ratio of 18F-DOPA-derived radioactivity was used as a model-independent measure of striatal dopaminergic innervation (Hoshi et al., 1993; Goldstein et al., 2008; Jokinen et al., 2009). In the same patients, the fractional loss (‘washout’) of radioactivity, an inverse measure of the ability to retain catecholamines, was calculated for the period between the peak value (at ~30 min after tracer injection) and the value at ~2 h after tracer injection.

Data reduction, analysis and statistics

Levodopa treatment increases CSF DOPAC levels (Baraczka et al., 1983). In our experience this effect can be noted in some patients even after discontinuation of levodopa/carbidopa treatment for 72 h before lumbar puncture. To eliminate influences of outlying data from artefactual effects of treatment, we conducted statistical testing after including data about CSF dopamine and DOPAC only from subjects with CSF l-DOPA ≥5 nmol/l or plasma l-DOPA ≥10 nmol/l at the time of CSF sampling, regardless of diagnostic group. By these stringent criteria, CSF DOPAC and dopamine data were excluded from 17 patients with Parkinson’s disease, 22 patients with MSA, one patient with PAF and three controls, leaving a total of 103 data points for DOPAC (17 Parkinson’s disease, 32 MSA, 19 PAF and 35 controls). CSF DOPAC data were included for all 10 patients in whom we were firmly confident of the diagnosis of Parkinson’s disease.

Levodopa treatment does not affect plasma or CSF levels of norepinephrine or of methoxyhydroxyphenylglycol, an end-product of norepinephrine metabolism (Chia et al., 1993), and in our study, across all subjects CSF DHPG and norepinephrine were unrelated to CSF or plasma l-DOPA. Therefore, statistical testing about CSF DHPG or norepinephrine was done without any data exclusion.

Table 2 shows the top 10 medications (separate from levodopa/carbidopa) taken by patients in the Parkinson’s disease, MSA and PAF groups. The main medications were dopamine receptor agonists, selective serotonin reuptake inhibitors, drugs for orthostatic hypotension, aspirin and an inhibitor of catechol-O-methyltransferase.

Treatment with a monoamine oxidase-B inhibitor (e.g. selegiline, rasagiline) might not be expected to decrease CSF levels of DOPAC or DHPG, since catecholaminergic neurons do not express monoamine oxidase-B; however, we previously noted decreased levels of these deaminated metabolites in plasma of humans treated with selegiline (Eisenhofer et al., 1986). Therefore, we analysed separately the CSF neurochemical data from the three patients who were being treated with a monoamine oxidase-B inhibitor prior to CSF sampling. We also analysed separately the results for the 12 patients with Parkinson’s disease who were treated with a dopamine receptor agonist.

Table 2 Medications taken at the time of testing in patient groups with Parkinson’s disease, MSA or PAF

| Drug          | Parkinson’s disease (%) | MSA (%) | PAF (%) |
|---------------|-------------------------|---------|---------|
| Dopamine agonist | 26                      | 11      | 0       |
| SSRI          | 24                      | 9       | 7       |
| Midodrine     | 24                      | 22      | 11      |
| Fludrocortisone | 21                     | 26      | 17      |
| Aspirin       | 18                      | 20      | 4       |
| COMTI         | 12                      | 11      | 0       |
| Beta-blocker  | 12                      | 0       | 0       |
| Rasagline     | 9                       | 0       | 0       |
| Amantadine    | 9                       | 6       | 0       |
| Selegline     | 0                       | 4       | 0       |

COMTI = catechol-O-methyltransferase inhibitor; SSRI = selective serotonin reuptake inhibitor.
Mean values for CSF levels of DOPAC, DHPG, dopamine, norepinephrine and L-DOPA were compared across groups by analyses of variance, with post hoc group comparisons using the Fisher’s Protected Least Significant Difference test. The graphics and statistical package was KaleidaGraph 4.01 (Synergy Software). For scatter plots of individual data (e.g. relating DHPG to DOPAC) linear regression was used with calculation of Pearson correlation coefficients. For comparisons of frequencies between groups in 2 × 2 tables, chi-squared (χ²) was calculated. All clinical, neuroimaging and neurochemical mean values were expressed ± 1 SEM. A P-value of <0.05 defined statistical significance.

Results

The main catechols identified in CSF were, in descending order of concentrations, DHPG, L-DOPA, DOPAC, norepinephrine and dopamine (Table 3). We could not convincingly identify DOPAL in CSF from our subjects, despite a diligent search for the unusually shaped and characteristic chromatographic peak (Goldstein et al., 2011). Epinephrine was also below the detection limit (~10 nmol/l) in most samples. As expected, levels of the deaminated metabolites exceeded by far those of the parent catecholamines.

Across all subjects, mean CSF DOPAC and dopamine were much higher in subjects with CSF L-DOPA metabolites exceeded by far those of the parent catecholamines.

The synucleinopathy patients differed highly significantly from controls overall (Table 4). Table 4 also shows CSF neurochemical data for patients on a monoamine oxidase-B inhibitor (n = 3) or a dopamine receptor agonist (n = 12). In these subgroups, CSF DOPAC was decreased from the mean value in the controls.

Cerebrospinal fluid dopaminergic abnormalities in synucleinopathies

The Parkinson’s disease, MSA and PAF groups all had decreased mean CSF DOPAC compared with the control group (P < 0.0001; P < 0.0001; P = 0.0002, respectively; Fig. 1). The Parkinson’s disease group tended to have lower CSF DOPAC than did the PAF group (P = 0.07), while the Parkinson’s disease and MSA groups did not differ (Figs 1–3).

In the subgroup of patients with a confident diagnosis of Parkinson’s disease, CSF DOPAC and DHPG were both significantly decreased compared with controls, as in the Parkinson’s disease group overall (Table 4). Table 4 also shows CSF neurochemical data for patients on a monoamine oxidase-B inhibitor (n = 3) or a dopamine receptor agonist (n = 12). In these subgroups, CSF DOPAC was decreased from the mean value in the controls.

Table 3 CSF and plasma levels of catechols in control subjects and patients with synucleinopathies

| Group          | DOPA nmol/l | DA nmol/l | DOPAC nmol/l | NE nmol/l | DHPG nmol/l |
|----------------|-------------|-----------|--------------|-----------|-------------|
| CSF Controls   | 3.68 ± 0.12 | 0.09 ± 0.01 | 2.15 ± 0.18 | 0.98 ± 0.09 | 11.02 ± 0.62 |
| Synucleinopathy| 2.67 ± 0.08 | 0.06 ± 0.01 | 1.05 ± 0.06 | 0.72 ± 0.06 | 7.76 ± 0.29 |
| Parkinson’s disease | 2.87 ± 0.16 | 0.07 ± 0.02 | 0.87 ± 0.09 | 0.76 ± 0.07 | 8.82 ± 0.44 |
| New PD         | 2.68 ± 0.16 | 0.06 ± 0.02 | 0.81 ± 0.08 | 0.62 ± 0.08 | 8.26 ± 0.70 |
| MSA            | 2.84 ± 0.12 | 0.07 ± 0.01 | 1.00 ± 0.09 | 0.72 ± 0.16 | 7.75 ± 0.42 |
| PAF            | 2.53 ± 0.17 | 0.05 ± 0.01 | 1.32 ± 0.12 | 0.58 ± 0.12 | 5.82 ± 0.65 |
| PARK4          | 2.73        | 0.40       | 0.74         | 0.31       | 7.65        |
| PLASMA Controls| 7.36 ± 0.27 | 0.09 ± 0.01 | 6.42 ± 0.38 | 1.69 ± 0.22 | 5.05 ± 0.29 |
| Synucleinopathy| 6.40 ± 0.17 | 0.08 ± 0.01 | 6.38 ± 0.32 | 1.27 ± 0.12 | 4.14 ± 0.22 |
| Parkinson’s disease | 4.38 ± 0.39 | 0.11 ± 0.02 | 6.62 ± 0.78 | 1.30 ± 0.15 | 4.38 ± 0.39 |
| New PD         | 6.56 ± 0.46 | 0.12 ± 0.02 | 7.28 ± 0.92 | 1.23 ± 0.26 | 4.45 ± 0.45 |
| MSA            | 6.78 ± 0.25 | 0.08 ± 0.01 | 6.56 ± 0.45 | 1.67 ± 0.21 | 5.06 ± 0.26 |
| PAF            | 5.90 ± 0.29 | 0.05 ± 0.01 | 5.88 ± 0.53 | 0.57 ± 0.12 | 2.41 ± 0.25 |
| PARK4          | 8.40        | (coelute)  | 5.47         | 1.77       | 4.31        |

Different from controls: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
Coelute = chromatographic dopamine peak obscured by a nearby peak; DA = dopamine; NE = norepinephrine; ‘New PD’ = recent onset of parkinsonism, since one patient had not yet been diagnosed formally. Data for L-DOPA, dopamine and DOPAC were excluded if plasma L-DOPA were >10 nmol/l or CSF L-DOPA were >5 nmol/l.
A receiver operating characteristic curve was constructed to examine the efficiency of CSF DOPAC in separating Parkinson’s disease from control subjects (Fig. 4A). At a specificity of 80%, the sensitivity of CSF DOPAC was 89%. The receiver operating characteristic curve for the New Parkinson’s disease group showed that CSF DOPAC was highly efficient in separating New Parkinson’s disease from control subjects (Fig. 4B). At a specificity of 89%, the sensitivity of DOPAC was 100%.

Consistent with a precursor-product relationship between dopamine and DOPAC, individual values for CSF DOPAC were positively correlated with those for dopamine in all the groups (Fig. 2). For a given CSF level of dopamine, patients with synucleinopathies had lower CSF levels of DOPAC than control subjects (Fig. 2A). From the lines of best fit for the scatter plots, the y-intercept value and slope were lower in the Parkinson’s disease group than the controls (y = 0.653 + 2.54x versus y = 1.61 + 6.18x). In particular, 15 of 16 (94%) patients with Parkinson’s disease had a CSF DOPAC level below the line of best fit for the entire subject group.

Cerebrospinal fluid noradrenergic abnormalities in synucleinopathies

The Parkinson’s disease, MSA and PAF groups all had decreased CSF levels of DHPG, the deaminated metabolite of norepinephrine, compared with the control group (P=0.004; P<0.0001; P<0.0001; Figs 3 and 5A). The Parkinson’s disease group had higher CSF DHPG than did the PAF group (P=0.001). The Parkinson’s disease and MSA groups did not differ in CSF DHPG. CSF levels of DHPG were positively correlated with those of DOPAC (r=0.45, P<0.0001; Fig. 5). As highlighted by the shaded areas in Fig. 5B, the distributions of CSF DHPG and...
DOPAC differed clearly between the Parkinson’s disease and PAF groups ($\chi^2 = 11.9, \ P = 0.0006$).

When the Parkinson’s disease group was stratified in terms of orthostatic hypotension, the subgroup with orthostatic hypotension had lower CSF and plasma DHPG levels ($8.17 \pm 0.41$ and $4.53 \pm 0.53 \text{nmol/l}$) than the subgroup without orthostatic hypotension ($10.22 \pm 0.91$ and $8.27 \pm 1.71 \text{nmol/l}, \ P = 0.02$ and $P = 0.002$). Across patients with Parkinson’s disease CSF

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**Table 4** CSF levels of catechols in patients with Parkinson’s disease and confident diagnosis or on dopaminergic drugs

|                  | DOPA nmol/l | DA nmol/l | DOPAC nmol/l | NE nmol/l | DHPG nmol/l |
|------------------|-------------|-----------|--------------|-----------|-------------|
| Confident diagnosis (n = 10) | 3.76 ± 0.60 | 0.11 ± 0.04 | 0.84 ± 0.12*** | 0.78 ± 0.15 | 8.23 ± 0.96* |
| Drug             |             |           |              |           |             |
| MAO-B Inhibitor  | 3.66        | 0.38      | 1.23         | 0.89      | 10.74       |
| MAO-B Inhibitor  | 3.45        | 0.03      | 0.50         | 1.08      | 14.25       |
| MAO-B Inhibitor  | 2.50        | 0.13      | 0.52         | 0.56      | 5.26        |
| DA Agonist (n = 12) | 3.73 ± 0.56 | 0.10 ± 0.03 | 0.97 ± 0.13 | 0.70 ± 0.09*** | 9.82 ± 0.99 |

DA = dopamine; NE = norepinephrine. Significantly below control, *P < 0.05, ***P < 0.001.

Among patients on a monoamine oxidase (MAO)-B inhibitor, mean CSF DOPAC ($0.75 \pm 0.24 \text{nmol/l}$) was below control ($P < 0.05$). See ‘Materials and methods’ section for definition of confident diagnosis.

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**Figure 3** Histograms comparing mean (± SEM) CSF concentrations of (A) DOPAC and (B) DHPG in patient groups with synucleinopathy and control subjects. Numbers in each group are shown in white squares. Different from controls: ***$P < 0.01$; ****$P < 0.0001$. Note decreased DOPAC and DHPG in all three synucleinopathy groups, with group differences in the pattern of abnormalities.

**Figure 4** Receiver operating characteristic curve showing high efficiency of CSF DOPAC in separating patients with Parkinson’s disease from control subjects. (A) Parkinson’s disease overall; and (B) parkinsonism of recent onset.
DHPG was positively correlated with plasma DHPG ($r = 0.51$, $P = 0.003$).

The MSA and PAF groups had decreased mean CSF norepinephrine compared to the control group ($P = 0.05$; $P = 0.01$), but the Parkinson’s disease group did not. For a given norepinephrine level, subjects with low DHPG levels all had either MSA or PAF (Fig. 2B). Several patients with PAF had especially low DHPG for given norepinephrine levels.

**Low cerebrospinal fluid L-DOPA in synucleinopathies**

Since L-DOPA is the immediate product of the rate-limiting enzymatic step in catecholamine biosynthesis, CSF L-DOPA may provide a biomarker of this key aspect of central catecholaminergic neuronal function. The Parkinson’s disease, MSA and PAF groups all had low mean CSF L-DOPA compared to the control group ($P = 0.0003$; $P < 0.0001$; $P < 0.0001$). The groups did not differ among themselves in mean CSF L-DOPA.

Individual values for CSF levels of L-DOPA correlated positively with those of both DOPAC and DHPG ($P < 0.0001$ each, Fig. 6). Consistent with dependence of production of both metabolites on tyrosine hydroxylation, the $y$-intercept values for the scatter plots relating DOPAC to L-DOPA and DHPG to L-DOPA were close to the origin.

If there was decreased dopamine metabolism for a given rate of synthesis, then the CSF DOPAC concentration would be low for a given L-DOPA concentration. Analogously, if there was decreased norepinephrine metabolism the CSF DHPG concentration would be low for a given L-DOPA concentration. As indicated by the scatter plots in Fig. 6, the Parkinson’s disease group had lower DOPAC than expected and the PAF group lower DHPG than expected for L-DOPA.

**Neurochemical-neuroimaging cross-validation of central dopaminergic lesion**

A total of 54 subjects underwent both lumbar puncture for CSF catechols and ${}^{18}$F-DOPA PET scanning for striatal dopaminergic neuroimaging. Patients with Parkinson’s disease had lower putamen:occipital ratios (1.84 ± 0.09) of ${}^{18}$F-DOPA-derived radioactivity than control subjects (3.09 ± 0.15, $P < 0.0001$).

The proportionate loss of putamen radioactivity between the peak value (at ~30 min after tracer injection) and 2 h after tracer injection (‘washout’) provided an inverse index of vesicular retention of ${}^{18}$F-dopamine derived from ${}^{18}$F-DOPA. Washout of ${}^{18}$F-DOPA-derived radioactivity was accelerated in the Parkinson’s disease (38 ± 3%) and MSA (28 ± 2%) groups compared to the control group (18 ± 2%, $P < 0.0001$, $P = 0.0002$; Fig. 7). All 10 patients with Parkinson’s disease had washout exceeding 20% and CSF DOPAC <1.5 nmol/l, whereas 0 of 10 control subjects had this combination ($\chi^2 = 16.4$, $P < 0.0001$).

**Catecholaminergic abnormalities outside the brain in synucleinopathies**

CSF levels of norepinephrine, DHPG and L-DOPA were positively correlated with plasma levels of these catechols (Supplementary Figs 8 and 9); however, CSF DOPAC and dopamine were not (data not shown). Patients with PAF stood out in terms of low CSF and plasma levels of DHPG and norepinephrine and disproportionately low DHPG for corresponding norepinephrine levels (Supplementary Fig. 8). All subjects with low CSF and plasma L-DOPA had a synucleinopathy.
Discussion

Here we report that Parkinson’s disease as well as two other forms of α-synucleinopathy show several clear abnormalities in CSF levels of catechols. As discussed below, these abnormalities likely reflect loss or dysfunction of central dopaminergic and noradrenergic neurons.

Low cerebrospinal fluid DOPAC indicates central dopamine deficiency in synucleinopathies

CSF levels of DOPAC, the main neuronal metabolite of dopamine, were decreased in all three synucleinopathies and especially in Parkinson’s disease. Low CSF DOPAC seems to be quite efficient...
in distinguishing Parkinson’s disease from healthy control subjects. Receiver operating curve analysis showed that at a specificity of 89% the sensitivity of CSF DOPAC was 100% for distinguishing patients with recent onset of parkinsonism from control subjects. Values were equally low in patients with relatively recent onset of parkinsonism and in patients with established Parkinson’s disease, consistent with major loss of dopaminergic neurons early in the disease process.

The robust findings in the receiver operating characteristic curves support the view that CSF DOPAC provides a sensitive and reliable diagnostic biomarker of the central dopaminergic lesion that characterizes Parkinson’s disease. Measurements of CSF catechols are reliable, if the CSF is frozen immediately upon sampling to prevent degradation of the catechol contents; the assays are done in a laboratory competent in assaying CSF catechols by batch alumina extraction followed by liquid chromatography with series electrochemical detection; and for clinical purposes the assays are done in a laboratory certified for reporting diagnostic data about levels of catechols.

Negative findings in an influential report based on the DATATOP trial (LeWitt et al., 1992) may have contributed to a shift of attention from CSF catecholamine neurochemistry to other potential CSF biomarkers of Parkinson’s disease (El-Agnaf et al., 2006; Zhang et al., 2008). In retrospect, questionable sensitivity and specificity of the assay method used for CSF DOPAC and dopamine could have led to false negative results from the DATATOP samples. CSF dopamine was reported to average more than CSF DOPAC, yet as noted previously (Scheinin et al., 1984) and confirmed here, CSF dopamine levels actually are infinitesimally low—far lower than CSF DOPAC. Since in the study by LeWitt et al. (1992) only 50 μl of CSF was assayed (20 times less than in the present study), the detection method may have been insufficiently sensitive to quantify validly the low CSF DOPAC and dopamine levels seen in patients with synucleinopathy. Moreover, because the CSF was injected directly without purifying the catechols first, such as by batch alumina extraction as in the present study (Holmes et al., 1994; Goldstein et al., 2003, 2008), the chromatographic results in the LeWitt et al. (1992) study could have been compromised by co-chromatographing contaminating substances.

Among the Parkinson’s disease and other synucleinopathy groups the decrease in CSF DOPAC was much more apparent than in dopamine. Why would there not be equivalent proportionate decreases in levels of the parent catecholamine and its main neuronal metabolite? We offer two potential explanations, which may differentially augment the rate of entry into extracellular fluid of dopamine with respect to DOPAC (Loeffler et al., 1995). Second, there may be decreased activity of aldehyde dehydrogenase, the enzyme that catalyzes conversion of the immediate product of dopamine deamination to DOPAC. A recent post-mortem study reported neurochemical evidence for decreased putamen aldehyde dehydrogenase activity in Parkinson’s disease (Goldstein et al., 2011), and others have noted decreased substantia nigra aldehyde dehydrogenase 1A1 gene expression (Galter et al., 2003; Mandel et al., 2005) and protein content (Werner et al., 2008). Lipid peroxidation products potently inhibit aldehyde dehydrogenase (Florang et al., 2007; Jinsmaa et al., 2009), providing a potential link between oxidative injury and decreased DOPAC production from dopamine.

Theoretically, even if there were no dopamine release the catecholamine would still undergo intra-neuronal metabolism to DOPAC. Thus, y-intercept values for lines of best fit for scatter plots relating CSF DOPAC to dopamine were clearly above the origin in all groups. Ongoing dopamine metabolism to DOPAC probably reflects net leakage from vesicular stores, a process that occurs independently of pathway traffic-induced exocytosis (Eisenhofer et al., 2004). The lower y-intercept value in the Parkinson’s disease group than in the controls might therefore reflect decreased central dopamine stores, decreased aldehyde dehydrogenase activity or both.

CSF DOPAC was decreased substantially in patients with MSA, as noted previously (Goldstein et al., 2008). The parkinsonian form of MSA can be very difficult to distinguish from Parkinson’s disease with orthostatic hypotension, especially in patients with relatively recent onset of signs of central neurodegeneration. Although CSF DOPAC seems to be a sensitive biomarker to identify loss of central dopaminergic neurons, our results indicate that low CSF DOPAC is not specific for Parkinson’s disease and does not separate MSA from Parkinson’s disease. We suggest that in patients with parkinsonism, orthostatic hypotension and low CSF DOPAC, in whom the differential diagnosis of Parkinson’s disease with orthostatic hypotension versus MSA is an issue, cardiac sympathetic neuroimaging should be done, because patients with Parkinson’s disease with orthostatic hypotension invariably have neuroimaging evidence of cardiac sympathetic denervation, whereas patients with MSA typically do not (Orimo et al., 2001, 2007; Goldstein, 2003).

All patients with Parkinson’s disease who underwent brain 18F-DOPA PET scanning as well as lumbar puncture had decreased putamen:occipital cortex ratios of 18F-DOPA-derived radioactivity, accelerated loss of putamen radioactivity, and low CSF DOPAC, cross-validating the neuroimaging and neurochemical modalities. Decreased vesicular retention of neuronal catecholamines can produce all three abnormalities, and we recently obtained evidence for decreased vesicular uptake of neuronal catecholamines in patients with Lewy body diseases (Goldstein et al., 2011). 18F-DOPA scanning is quite expensive, involves radioactivity exposure, and is available at relatively few centres. The present results indicate that the CSF neurochemical approach provides a cost-effective, valid alternative.

**Low cerebrospinal fluid DHPG indicates central norepinephrine deficiency in synucleinopathies**

The CSF DHPG findings provide in vivo neurochemical support for a central noradrenergic lesion in Parkinson’s disease. Post-mortem studies have established that Parkinson’s disease entails loss of neurons of the locus ceruleus (Freed, 1990; Zarow et al., 2003), the main source of norepinephrine in the brain, although studies
low cerebrospinal fluid L-DOPA indicates decreased catecholamine biosynthesis in synucleinopathies

Since L-DOPA is the immediate product of the rate-limiting enzymatic step in catecholamine biosynthesis, the results about CSF L-DOPA fit with decreased central catecholamine synthesis from loss of catecholaminergic terminals. Positive correlations of both CSF DOPAC and DHPG with L-DOPA suggest a balance of synthesis with loss of dopamine and norepinephrine in the CNS.

Differential loss of noradrenergic versus dopaminergic neurons in two Lewy body diseases

Distinctive clinical manifestations of Lewy body diseases may relate to different patterns of abnormalities of central catecholaminergic innervation. In Parkinson’s disease, DOPAC was more consistently decreased than DHPG, whereas in PAF, DHPG was more consistently decreased than DOPAC. These results support the notion that in Parkinson’s disease the pathogenetic process leads to relatively greater loss of dopaminergic than noradrenergic neurons, resulting in parkinsonism, whereas in PAF the process leads to relatively greater loss of noradrenergic than dopaminergic neurons, resulting in orthostatic hypotension from sympathetic noradrenergic denervation (Goldstein et al., 2002). Consistent with this view, putamen:occipital ratios of 18F-DOPA-derived radioactivity and rates of loss of putamen radioactivity are normal in patients with PAF and decreased in Parkinson’s disease (Goldstein et al., 2008). Low norepinephrine and DHPG concentrations in both CSF and plasma in PAF indicate central as well as peripheral noradrenergic lesions in this disease. Scatter plots for CSF DOPAC and DHPG versus L-DOPA supported the proposal that in synucleinopathies parkinsonism is associated with a dopaminergic and orthostatic hypotension with a noradrenergic lesion, because plots relating DOPAC to L-DOPA showed that most patients with low DOPAC for given L-DOPA levels had Parkinson’s disease or MSA (Fig. 6A), whereas most patients with low DHPG for given L-DOPA levels had PAF or MSA (Fig. 6B). Bases for differential loss of dopaminergic versus noradrenergic neurons in Parkinson’s disease versus PAF remain unknown.

Study limitations

The main limitation of this study is the indirectness of CSF DOPAC and DHPG as indices of central dopaminergic and noradrenergic innervation. Several processes intervene, including vesicular reuptake, neuronal and non-neuronal uptake, monoamine oxidase, aldehyde/aldose reductase, aldehyde dehydrogenase, catechol-O-methyltransferase and axoplasmic transport of vesicles or vesicle-related proteins. On the other hand, measuring levels of the deaminated metabolites should provide a better indication of neuronal catecholamine stores and intraneuronal metabolism than levels of either the parent amines or of their metabolic end-products.

We doubt that CSF levels of catechols in this study were influenced by circulating levels. Because of the efficient blood–brain barrier for catecholamines (Weil-Malherbe et al., 1959) and active secretion of acidic catecholamine metabolites into extracellular fluid (Palfreyman et al., 1982; Emanuelsson et al., 1987), CSF dopamine, norepinephrine and DOPAC should not be affected by circulating levels of these compounds. DHPG, a glycol, does penetrate cell membranes; however, catechol-O-methyltransferase in cells constituting the blood–brain barrier probably efficiently metabolizes DHPG to methoxyhydroxyphenylglycol. Moreover, DHPG concentrations in CSF normally substantially exceed those in plasma (Raskind et al., 1999; Goldstein et al., 2003).

In patients already on levodopa/carbidopa, prolonged withdrawal may be required before lumbar puncture, to avoid artefactual effects of the treatment on CSF levels of L-DOPA, dopamine and DOPAC. In this study we included dopamine and DOPAC data only from subjects with plasma and CSF L-DOPA well within the normal range.

Although plasma L-DOPA levels are related to catecholamine synthesis in sympathetic nerves (Goldstein et al., 1987; Kvetansky et al., 1992a, b), whether CSF L-DOPA is related to central catecholamine synthesis has not yet been directly tested.

Few laboratories have the capability to measure CSF dopamine, DOPAC, norepinephrine, DHPG and L-DOPA simultaneously or
have adequately sensitive and specific assays to quantify the very low levels of dopamine seen in synucleinopathies. An effort is under way to render the Catecholamine Resource of the Clinical Neurocardiology Section in intramural NINDS more available for clinicians and for collaborative research.

Conclusion

This study shows that low CSF DOPAC, DHPG and L-DOPA levels indicate central dopaminergic and noradrenergic lesions in synucleinopathies. CSF DOPAC is especially low in Parkinson’s disease and DHPG low in PAF, consistent with greater loss of dopaminergic than noradrenergic neurons in the former and greater loss of noradrenergic than dopaminergic neurons in the latter. CSF DOPAC seems to be a sensitive diagnostic biomarker of Parkinson’s disease, even in patients with recent onset of parkinsonism. Low CSF DOPAC, however, is not specific for Parkinson’s disease, and measurement of CSF catechols is of no value in distinguishing MSA from Parkinson’s disease. Neither the clinical presentation nor striatal dopaminergic neuroimaging efficiently distinguishes among different forms of synucleinopathy, although a combined approach involving striatal dopaminergic and cardiac noradrenergic neuroimaging may do so (Goldstein et al., 2008). The goal of the study was not to diagnose Parkinson’s disease differentially from other causes of neurodegeneration but to apply a neurochemical approach that is relatively inexpensive and available, yet valid, to detect the central catecholamine deficiency that characterizes synucleinopathies such as Parkinson’s disease. In both MSA and Parkinson’s disease, tracking CSF catechols might enable a means to test treatments that could retard the neurodegeneration; however, from the results of the present study one cannot draw inferences about the validity or reliability of using catecholamine metabolite levels in blood or CSF to monitor clinical and neuroprotective effects of medication. The present results support the value of measuring CSF levels of catecholamines and their metabolites to identify central catecholamine deficiency, which in our view is a fundamental neurochemical characteristic of Parkinson’s disease and other synucleinopathies.

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Supplementary material

Supplementary material is available at Brain online.

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