HHS Public Access

Author manuscript

J Neurochem. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

J Neurochem. 2021 July ; 158(2): 554–568. doi:10.1111/jnc.15371.

Differential abnormalities of cerebrospinal fluid dopaminergic vs. noradrenergic indices in synucleinopathies

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Abstract

The synucleinopathies Parkinson disease (PD), multiple system atrophy (MSA), and pure autonomic failure (PAF) are characterized by intra-cytoplasmic deposition of the protein alpha-synuclein and by catecholamine depletion. PAF, which manifests with neurogenic orthostatic hypotension (nOH) and no motor signs of central neurodegeneration, can evolve into PD+nOH. Cerebrospinal fluid (CSF) levels of catecholamine metabolites may indicate central catecholamine deficiency in these synucleinopathies, but the literature is inconsistent and incomplete. In this retrospective cohort study we reviewed data about CSF catecholamines, the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the norepinephrine metabolites 3,4-dihydroxyphenylglycol (DHPG) and 3-methoxy-4-hydroxyphenylglycol (MHPG). The compounds were measured in 36 PD, 37 MSA, and 19 PAF patients and in 38 controls. Compared to the control group, the PD, MSA, and PAF groups had decreased CSF MHPG ($p<0.0001$ each by Dunnett's post-hoc test), DHPG ($p=0.004$; $p<0.0001$; $p<0.0001$) and norepinephrine ($p=0.017$; $p=0.0003$; $p=0.044$). CSF HVA and DOPAC were decreased in PD ($p<0.0001$ each) and MSA ($p<0.0001$ each) but not in PAF. The three synucleinopathies therefore have in common in vivo evidence of central noradrenergic deficiency but differ in extents of central dopaminergic deficiency—prominent in PD and MSA, less apparent in PAF. Data from putamen ^{18}F-DOPA and cardiac ^{18}F-dopamine neuroimaging in the same patients, post-mortem tissue catecholamines in largely separate cohorts, and review of the neuropathology literature fit with these distinctions. The results suggest a "norepinephrine first" ascending pathogenetic sequence in synucleinopathies, with degeneration of pontine locus ceruleus noradrenergic neurons preceding loss of midbrain substantia nigra dopaminergic neurons.

Keywords

norepinephrine; Parkinson; multiple system atrophy; pure autonomic failure; MHPG; HVA

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CONFLICTS OF INTEREST: The authors have no conflicts of interest to disclose.

INTRODUCTION

The neurochemical hallmark of Parkinson's disease (PD) is depletion of the catecholamine dopamine in the nigrostriatal system—especially in the putamen (Kish *et al.* 1988). By the time the characteristic motor symptoms manifest clinically it is likely that a substantial proportion of striatal dopaminergic terminals have already been lost (DelleDonne *et al.* 2008). Comparable if not greater (Zarow *et al.* 2003) loss of neurons occurs in the pontine locus ceruleus (LC), possibly before the loss of substantia nigra (SN) neurons (Del Tredici & Braak 2013).

Multiple system atrophy (MSA) can be difficult to distinguish clinically from PD (Rajput *et al.* 1991). Both conditions are characterized by cytoplasmic deposition of the protein alpha-synuclein (α-syn)—in Lewy bodies in PD (Spillantini *et al.* 1997) and in glial cytoplasmic inclusions in MSA (Wakabayashi *et al.* 1998). PD and MSA are now considered to be forms of synucleinopathy (Bras *et al.* 2020; Yamasaki *et al.* 2019).

Pure autonomic failure (PAF) is a rare but scientifically important disease that manifests with neurogenic orthostatic hypotension (nOH) related to sympathetic noradrenergic deficiency (Ziegler *et al.* 1977), without motor signs of central neurodegeneration (Kaufmann 1996). Post-mortem studies of PAF patients have consistently reported Lewy body pathology (van Ingelghem *et al.* 1994; Bannister *et al.* 1967) or intra-neuronal α-syn deposition (Hague *et al.* 1997; Arai *et al.* 2000; Kaufmann *et al.* 2001) in the sympathetic nervous system or brainstem, justifying including PAF in the synucleinopathy family.

PAF can evolve into PD+nOH (Kaufmann *et al.* 2017), in line with the notion of a "body-first" pathogenetic process (Horsager *et al.* 2020) beginning in the autonomic nervous system and followed by ascending central catecholaminergic neurodegeneration in the brainstem (Del Tredici & Braak 2012; Van Den Berge *et al.* 2019).

There has long been interest in cerebrospinal fluid (CSF) levels of catecholamine metabolites as biomarkers of central catecholamine deficiency, and many studies over the past half century have compared PD, MSA, or PAF groups with controls (Table 1). Several publications have described reduced CSF levels of 3,4-dihydroxyphenylacetic acid (DOPAC), the main intra-neuronal metabolite of dopamine, in PD (Eldrup *et al.* 1995; Andersen *et al.* 2017; Engelborghs *et al.* 2003). We reported that PD, MSA, and PAF entail decreased CSF DOPAC (Goldstein *et al.* 2012b), with a lower DOPAC in PAF than in PD. More recently we found that in individuals with multiple PD risk factors, low CSF levels of DOPAC and 3,4-dihydroxyphenylalanine (DOPA), the precursor of the catecholamines, predict later development of the disease (Goldstein *et al.* 2018); however, DOPAC undergoes extensive extra-neuronal metabolism by catechol-O-methyltransferase (COMT) to form homovanillic acid (HVA), the main end-product of dopamine metabolism (see concept diagram in Figure 1), and individual differences in COMT activity could affect the utility of CSF DOPAC as a biomarker.

The literature about whether synucleinopathies entail CSF neurochemical evidence of central norepinephrine deficiency is inconsistent and incomplete. Eldrup et al. noted decreased CSF norepinephrine in PD (Eldrup *et al.* 1995), but Turkka et al. and Chia et al.

(Turkka *et al.* 1987; Chia *et al.* 1993) did not observe decreases in either norepinephrine or 3-hydroxy-4-hydroxyphenylglycol (MHPG), the main end-product of central norepinephrine metabolism. Martignoni et al. reported low CSF norepinephrine in PD and MSA and reduced MHPG in MSA but not in PD (Martignoni *et al.* 1992). Herbert et al. also did not find decreased MHPG in PD (Herbert *et al.* 2013). Polinsky et al. found decreased CSF MHPG in MSA and in PAF (then called idiopathic orthostatic hypotension) (Polinsky *et al.* 1984). Our group reported decreased CSF levels of norepinephrine and 3,4-dihydroxyphenylglycol (DHPG), the main intra-neuronal metabolite of norepinephrine, in PAF and in a subgroup of MSA patients with orthostatic hypotension but not in MSA without orthostatic hypotension (Goldstein *et al.* 2003a). In our 2012 study we reported decreased CSF DHPG in PD, MSA, and PAF (Goldstein *et al.* 2012b); however, just as DOPAC is metabolized to HVA by COMT, DHPG is metabolized to MHPG, and our study was limited in that it did not include data about MHPG.

These considerations led us to revisit the issue of CSF biomarkers of central catecholamine deficiency in synucleinopathies, by comprehensively assaying CSF DOPA, DHPG, MHPG, DOPAC, HVA, and the catecholamines themselves in relatively large cohorts of PD, MSA, and PAF patients and controls. We assessed the efficacy of CSF levels of each of these neurochemicals for distinguishing the synucleinopathy group from the control group.

We were especially interested in measuring indices of central dopamine deficiency (CSF HVA and DOPAC) and norepinephrine deficiency (CSF MHPG, DHPG, and norepinephrine) in PAF. Since PAF does not entail clinical evidence of parkinsonism, we predicted that PAF patients would have normal CSF HVA and DOPAC levels. Meanwhile, noradrenergic deficiency, which in the periphery is a well established feature of PAF (Goldstein *et al.* 2003b; Ziegler *et al.* 1977), might extend to the brain, in which case CSF MHPG, DHPG, and norepinephrine would all be low.

To examine directly whether central noradrenergic deficiency is a common theme in synucleinopathies, in a mainly different cohort we assayed tissue catecholamine contents post-mortem in frontal cortex and putamen. Frontal cortex receives noradrenergic innervation exclusively from the pontine LC (Itoi *et al.* 2011), while putamen receives dopaminergic innervation from the mesencephalic SN. In PD and MSA we predicted decreases in both cortical norepinephrine and putamen dopamine. In PAF we predicted decreases in cortical norepinephrine and, based on post-mortem literature about SN neuronal loss (Table 2), variably decreased putamen dopamine.

METHODS

Subjects

The Intramural Research Board of the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health approved the protocols for this study (NIH Clinical Protocols 94N0186, 03N0004, and 18N0140). The participants gave informed written consent before any research procedures.

Data were reviewed for all subjects who had undergone lumbar puncture (LP) from 2004 to 2020 at the NIH Clinical Center and had CSF assayed for levels of catechols, MHPG, and HVA in the laboratory of the Autonomic Medicine Section (formerly Clinical Neurocardiology Section) in intramural NINDS.

The study was not pre-registered. No randomization was performed to allocate subjects in the study. No sample calculation was performed. The study was exploratory. The retrospective study included data for all subjects studied under the relevant protocols during the specified time period. There was no prospective estimation of the number of subjects that would be required. For all subjects the following comorbid conditions were exclusionary for the present analysis: symptomatic coronary artery disease, diabetes mellitus requiring drug treatment, history of stroke with residual symptoms, symptomatic cerebrovascular disease, and renal or hepatic parenchymal failure. Subject data were excluded in the retrospective analyses, rather than subjects being excluded according to predetermined criteria.

The patients in the synucleinopathy group had been referred based on signs of central neurodegeneration (parkinsonism or cerebellar ataxia) or OH. PD was diagnosed according to UK Parkinson's Disease Society Brain Bank Diagnostic Criteria (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/GetPdf.cgi?id=phd000042), with the exception that early severe autonomic involvement was not considered to be exclusionary (Kaufmann & Goldstein 2013; Goldstein 2006). Diagnostic categorization in terms of MSA and PAF was based on previously published consensus criteria (Kaufmann 1996). Data from MSA patients were included regardless of classification as parkinsonian or cerebellar types.

Control subjects were studied over about the same time period (2006–2019). The control subjects were either healthy volunteers or were patients who had been referred for autonomic testing, did not have clinical evidence of neurodegenerative parkinsonism or cerebellar ataxia, and were not diagnosed with a form of chronic autonomic failure. Patients were included in the control group based on retrospective analyses of their clinical and laboratory findings. The healthy volunteers were not recruited by advertisement but were self-referred by word of mouth or through the NIH Normal Volunteer Program.

CSF Neurochemicals

To obtain CSF for neurochemical assays, subjects at the NIH Clinical Center underwent LP under fluoroscopic guidance. In patients on levodopa, the drug was withheld for at least 72 hours before the LP while the patients were inpatients at the NIH Clinical Center. A total of 12 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 for catechols by personnel (C.H.) who were blinded as to clinical diagnosis, using batch alumina extraction followed by liquid chromatography with electrochemical detection (LCED) (Holmes *et al.* 1994; Goldstein *et al.* 2003a; Goldstein *et al.* 2008). The closest available stored aliquot was thawed and assayed for MHPG and HVA. The person carrying out the assay for MHPG and HVA (P.S.) was also blinded as to clinical diagnosis.

For assaying MHPG and HVA, 150 μL of thawed, centrifuged CSF was injected directly into the LCED system. For HVA, better reproducibility was obtained by quantifying peak areas than peak heights, and so for both metabolites the former method was used.

Putamen dopaminergic neuroimaging

The putamen/occipital ratio (PUT/OCC) of 18F-DOPA-derived radioactivity was used as a model-independent measure of striatal dopaminergic innervation (Goldstein *et al.* 2008; Hoshi *et al.* 1993; Jokinen *et al.* 2009). The proportionate loss of putamen radioactivity between the peak value (at about 30 minutes after tracer injection) and 2 hours after tracer injection ("washout") provided an inverse index of vesicular retention of 18F-dopamine derived from 18F-DOPA. The percent washout was calculated for the period between the peak value (at about 30 minutes after tracer injection) and the value at about 2 hours after tracer injection (Goldstein *et al.* 2008). 18F-DOPA scanning was done without carbidopa pre-treatment. 18F-DOPA was synthesized by the NIH PET Department and administered under Investigational New Drug (IND) #35,513. There is no Research Resource Identifier for this drug.

Cardiac noradrenergic neuroimaging

To evaluate peripheral noradrenergic innervation, cardiac 18F-dopamine PET scanning was done as described previously (Lamotte *et al.* 2019). PET scans were acquired on a GE Advance Tomograph (GE Healthcare) prior to January 2016 and on a Siemens PET/CT scanner after the GE Advance scanner was retired in January 2016. 18F-Dopamine was synthesized by the NIH PET Department and administered under IND #33,866. There is no Research Resource Identifier for this drug.

Post-mortem tissue catecholamines

Putamen and frontal cortex samples were from the University of Miami Brain Endowment Bank or the Pathology Department of the NIH Clinical Center. Brain tissue samples were obtained with a post-mortem interval less than 24 hours. Because of the direct injection used for measuring HVA and MHPG in CSF, these compounds were not measured in tissue samples.

Data Reduction, Analysis, and Statistics

Levodopa treatment would be expected to increase CSF HVA and DOPAC levels. To eliminate influences of outlying data from artifactual effects of treatment, we included data only from levodopa-treated patients who had CSF DOPA less than 6.79 pmol/mL (the upper limit of normal).

The graphics and statistical package was GraphPad Prism 9.0.0 (GraphPad Software LLC, San Diego, CA). Mean values for CSF levels of DOPA, dopamine, norepinephrine, DOPAC, DHPG, MHPG, and HVA in the synucleinopathy group were compared to those in the control group by independent-means t-tests. Mean values for across PD, MSA, PAF, and control groups were compared by analyses of variance with post-hoc group comparisons by Dunnett's test. For scatter plots of individual data, Pearson correlation coefficients were calculated. Receiver operating characteristic (ROC) curves were generated for distinguishing

the synucleinopathy from control groups were generated using GraphPad Prism for each neurochemical measure. An assessment of the normality of data was carried out as part of the testing using GraphPad Prism. Outlying data points were not excluded, but a few were not displayed in Figures. All clinical, neuroimaging, and neurochemical mean values were expressed ± 1 SEM. A p value less than 0.05 defined statistical significance.

RESULTS

Subject groups

CSF catechols, MHPG, and HVA data were analyzed from 130 subjects, including 92 synucleinopathy patients (36 PD, 37 MSA, 19 PAF). Mean (±SEM) ages and sex makeups were: PD 65±2 years, 22 men, 14 women; MSA 57±1 years, 25 men, 12 women; and PAF 65±2 years, 14 men, 5 women. Detailed clinical information about the 3 synucleinopathy groups, including treatment status for a variety of potentially interfering medications (e.g., a monoamine oxidase inhibitors (MAOI), catechol-O-methyltransferase inhibitor (COMTI), or selective serotonin reuptake inhibitor (SSRI)) are in a Supplementary Table.

There were 38 control subjects (mean age 52±2 years, 20 men, 18 women). Among the controls, 24 were healthy volunteers (46 ± 3 years old, 14 men, 10 women), and 14 were referred for autonomic testing and did not have parkinsonism or cerebellar ataxia or evidence of chronic autonomic failure (62 ± 3 years old, 6 men, 8 women).

CSF catecholamines and metabolites

Compared to the control group, the synucleinopathy group had lower mean CSF levels of DOPA (by 15%, $t=4.04$, $p<0.0001$), norepinephrine (by 35%, $t=3.99$, $p<0.0001$), DHPG (by 25%, $t=4.41$, $p<0.0001$), MHPG (by 21%, 4.39, $p<0.0001$), DOPAC (by 36%, $t=5.30$, $p<0.0001$), and HVA (by 36%, $t=4.62$, $p<0.0001$) (Figure 2). The groups did not differ in mean dopamine levels ($t=0.22$, $p=0.83$).

CSF concentrations of norepinephrine, DHPG, and MHPG were positively inter-correlated ($r=0.57$ for DHPG vs. NE, $r=0.59$ for MHPG vs. NE, $r=0.79$ for MHPG vs. DHPG, $p<0.0001$ each). In the scatter plots relating levels of MHPG or DHPG vs. norepinephrine, the y-intercept values for the lines of best fit were above the origin (Figure 3). The y-intercept value for MHPG, 28.4 pmol/mL, had a 95% confidence interval of 24.9–31.9 pmol/mL, and the y-intercept value for DHPG, 6.17 pmol/mL, had a 95% confidence interval of 5.20–7.15 pmol/mL. For the relationship between MHPG and DHPG, the y-intercept value for MHPG was 13.6 pmol/mL, with a 95% confidence interval of 9.66–17.6 pmol/mL.

Compared to the control group, the PD, MSA, and PAF groups had decreased mean CSF levels of MHPG ($p<0.0001$ each; Figure 4A), DHPG ($p=0.004$; $p<0.0001$; $p<0.0001$; Figure 4B), and norepinephrine ($p=0.017$; $p=0.0003$; $p=0.044$). With regard to central dopaminergic indices CSF HVA and DOPAC levels were decreased in the PD and MSA groups but not in the PAF group (Figure 4C, 4D). The PD, MSA, and PAF groups did not differ from the controls in CSF dopamine levels.

ROC curves were constructed to examine the efficiency of CSF DOPA, catecholamines, MHPG, and HVA for separating the synucleinopathy and control groups (Figure 5). The ROC area for DOPA was 0.70 ($p<0.0001$), dopamine 0.57 (not significant), DOPAC 0.76 ($p<0.0001$), HVA 0.74 ($p<0.0001$), norepinephrine 0.71 ($p<0.0001$), DHPG 0.70 ($p<0.0001$), and MHPG 0.83 ($p<0.0001$). Thus, the largest ROC area was for CSF MHPG. Based on the ROC curve for MHPG, at a specificity of 80% the sensitivity was 65% (blue dashed lines in Figure 5G).

CSF levels of the norepinephrine metabolite MHPG were positively correlated with those of the dopamine metabolite HVA ($r=0.45$, $p<0.0001$; Figure 6A). Similarly, CSF levels of deaminated norepinephrine metabolite DHPG were positively correlated with those of the deaminated dopamine metabolite DOPAC ($r=0.45$, $p<0.0001$). Across all subjects, CSF HVA was weakly positively correlated with CSF dopamine ($r=0.21$, $p=0.045$); however, within the PD, MSA, PAF, and control groups CSF HVA was unrelated to CSF dopamine.

Several synucleinopathy patients were on potentially interfering drugs. Among 6 patients on a MAO-B inhibitor, CSF MHPG and HVA did not differ from those in the remaining patients (40.1 ± 4.9 vs. 35.7 ± 1.0 pmol/mL, 95.3 ± 18.9 vs. 143.0 ± 9.4 pmol/mL); however, CSF DOPAC was lower in the subgroup on a MAOI (0.61 ± 0.14 vs. 1.18 ± 0.06 pmol/mL, $p=0.012$). Among 6 patients on a COMTI, none of the analyte levels were different from those in the remaining patients (data not shown). Among 22 patients on a SSRI, CSF MHPG and HVA did not differ from those in the remaining patients (33.3 ± 2.2 vs. 36.6 ± 1.2 pmol/mL, 116.5 ± 15.8 vs. 145.4 ± 10.7 pmol/mL); however, CSF DHPG was lower in the subgroup on a SSRI (7.2 ± 0.7 vs. 9.0 ± 0.4 pmol/mL, $p=0.017$).

Associations with other clinical laboratory data

A total of 110 subjects underwent CSF sampling and 18F-DOPA positron emission tomographic scanning. CSF HVA levels were positively correlated with PUT/OCC ratios of 18F-DOPA-derived radioactivity ($r=0.43$, $p<0.0001$; Figure 6B), as were CSF DOPAC levels ($r=0.32$, $p=0.0005$). Across 109 subjects CSF HVA levels were negatively correlated with washout percents of putamen 18F-DOPA-derived radioactivity ($r=-0.43$, $p<0.0001$; Figure 6C), as were DOPAC levels ($r=-0.33$, $p=0.0005$). Among subjects who had the combination of low CSF HVA levels, low PUT/OCC ratios, and increased washout of putamen 18F-DOPA-derived radioactivity, virtually all had PD or MSA, as indicated by the pink rectangles in Figures 6B and 6C.

PD and MSA patients had lower PUT/OCC ratios of 18F-DOPA-derived radioactivity (N=35, mean 2.03±0.06; N=37, mean 2.35±0.11) than did the control subjects (N=20, mean 3.01±0.13, $p<0.0001$ each). In contrast, PUT/OCC ratios were normal in the PAF group (N=18, mean 3.29±0.18). The mean washout percent of 18F-DOPA-derived radioactivity was higher in the PD (38±3%) and MSA (28±2%) groups than in the control group (18±2%; $p<0.0001$, $p=0.0002$) and was normal in the PAF group. Across the PD and MSA patients, CSF HVA levels were weakly positively correlated with PUT/OCC ratios (N=72, $r=0.27$, $p=0.025$) and tended to be negatively correlated with washout percents (N=71, $r=-0.22$, $p=0.068$). The PD and MSA groups did not differ in terms of either PUT/OCC ratios or washout percents (data not shown).

To address whether central noradrenergic deficiency is related to peripheral noradrenergic deficiency in synucleinopathies, we examined correlations of CSF MHPG with results of cardiac sympathetic neuroimaging in the same subjects. A total of 112 subjects had data for 18F-dopamine-derived radioactivity in the interventricular septal myocardium. 18F-Dopamine-derived radioactivity varied as a function of the type of synucleinopathy ($F=35.6$, $p<0.0001$), with radioactivity in the PD and PAF groups being lower ($p<0.0001$ each) than in the control group, while radioactivity in the MSA group did not differ from that in the control group.

CSF MHPG was also unrelated to cardiac 18F-dopamine-derived radioactivity (Figure 6D). Synucleinopathy patients with decreased CSF MHPG levels could be divided into two groups, those with PD or PAF (pink rectangle in Figure 6D) and those with MSA (blue rectangle).

CSF MHPG and HVA levels were unrelated to scores on the University of Pennsylvania Smell Identification Test (UPSIT, $N=93$, $r=0.04$; $N=94$, $r=-0.06$). CSF MHPG and HVA levels were also unrelated to Montreal Cognitive Assessment scores ($N=32$, $r=0.23$, $p=0.21$; $N=33$, $r=0.00$).

On the other hand, CSF MHPG was related to the magnitude of OH. CSF MHPG was lower in 64 subjects with an orthostatic fall in systolic blood pressure ≥ 20 mmHg than in 39 subjects with a fall in blood pressure <20 mm Hg ($t=4.08$, $p<0.0001$). Across all subjects, the magnitude of orthostatic decrease in blood pressure was correlated with CSF MHPG ($r=0.37$, $p<0.0001$). Across 36 PD patients, those with OH ($N=17$) had lower CSF MHPG than did those without OH ($N=19$, $t=2.25$, $p=0.031$). Too few MSA and PAF patients had no OH ($N=6$, $N=3$) to carry out meaningful statistics.

Post-mortem tissue catecholamines and their metabolites

Autopsy samples of putamen were assayed from 73 subjects (24 PD, 22 MSA, 4 PAF, 23 controls). The synucleinopathy group had decreased putamen norepinephrine concentrations compared to the control group (by 78%, $t=6.33$, $p<0.0001$). The PD and MSA groups had lower mean putamen norepinephrine concentrations than did the controls ($p<0.0001$; Figure 7C), whereas among the 4 PAF patients putamen norepinephrine was variable, with 2 having low levels. Putamen dopamine was lower in the synucleinopathy than control group (by 89%, $p<0.0001$). Putamen dopamine levels in the PD and MSA groups were lower than in the controls ($p<0.0001$ each, Figure 7D). As for putamen norepinephrine, putamen dopamine was variable among the 4 PAF patients, with 2 having low levels.

Frontal cortical samples were assayed from 55 subjects (19 PD, 12 MSA, 2 PAF, 22 controls). The synucleinopathy group had decreased cortical norepinephrine concentrations compared to the control group (by 65%, $t=3.05$, $p=0.0036$; Figure 7). The PD and MSA groups had lower mean norepinephrine concentrations than did the controls (by 79% in PD, $p<0.0001$ and 80% in MSA, $p<0.0001$; Figure 7A). There were only 2 PAF patients with cortex norepinephrine data. Cortical dopamine levels in the 3 synucleinopathy groups did not differ from those in the controls (Figure 7B).

DISCUSSION

The concept that CSF levels of catecholamines or their metabolites provide biomarkers of central catecholamine deficiency is important but not novel. On the contrary, there is abundant relevant literature over more than a half century, as documented by the contents of Table 1; however, the literature has been inconsistent, incomplete, and unpersuasive. We believe the present results are consistent and tell a convincing story in terms of differential abnormalities of CSF dopaminergic vs. noradrenergic indices across synucleinopathies.

Novel aspects of this study include the comparisons of 3 synucleinopathy groups in terms of CSF levels of the deaminated and O-methylated deaminated metabolites of dopamine and norepinephrine and levels of the parent catecholamines in the same participants; relationships between levels of the deaminated and O-methylated deaminated metabolites; cross-correlations of CSF HVA with central ^{18}F-DOPA- and of CSF MHPG with cardiac ^{18}F-dopamine-derived radioactivity; post-mortem tissue concentrations of dopamine and norepinephrine in cortex and putamen in synucleinopathy and control groups; and, most importantly, in vivo and post-mortem neurochemical evidence for central noradrenergic deficiency across synucleinopathy groups, in contrast with evidence for central dopaminergic deficiency in PD and MSA but not in PAF.

Central noradrenergic deficiency: A common theme in synucleinopathies

Two general types of findings from the present study support the view that PD, MSA, and PAF have in common central noradrenergic deficiency. First, in all three synucleinopathy groups CSF levels of MHPG (the main end-product of norepinephrine metabolism in the brain), DHPG (the main intra-neuronal metabolite of norepinephrine), and norepinephrine itself were decreased compared to controls. Second, in a largely different cohort, post-mortem assays of tissue catecholamines revealed decreased norepinephrine contents in the frontal cortex and putamen in the synucleinopathy group.

Validation of CSF indices

Strongly positive inter-correlations among norepinephrine, DHPG, and MHPG levels and between DOPAC and HVA levels cross-validated these neurochemical indices. Moreover, virtually all the PD and MSA patients also underwent brain 18F-DOPA positron emission tomographic scanning and had decreased PUT/OCC ratios of 18F-DOPA-derived radioactivity, accelerated loss of putamen radioactivity, and low CSF HVA (Figure 6B, 6C), confirming these neuroimaging and neurochemical modalities as in vivo biomarkers of central dopamine deficiency.

Differential abnormalities of CSF dopaminergic vs. noradrenergic indices

In the PAF group, mean CSF DOPAC and HVA levels, PUT/OCC ratios of 18F-DOPA-derived radioactivity, and washout percents of putamen 18F-DOPA-derived radioactivity all were not decreased from those in controls. These results contrasted strikingly with those in the PD and MSA groups, in which values for these dopaminergic indices clearly were decreased. The results therefore indicate differential abnormalities of central dopaminergic vs. noradrenergic indices across these synucleinopathies.

Axons emanating from the LC arborize widely and are the main source of norepinephrine in the brain—in particular, they are the sole source of norepinephrine in the cerebral cortex (Itoi *et al.* 2011). A LC lesion might contribute to early non-motor manifestations such as cognitive or olfactory dysfunction (Zweig *et al.* 1993; Ross *et al.* 2006; Cash *et al.* 1987; Del Tredici & Braak 2013) even before locomotor abnormalities become evident. Considering that PAF can evolve into PD+nOH (Kaufmann *et al.* 2017), PAF might represent not only a "body-first" (Goldstein *et al.* 2012a; Van Den Berge *et al.* 2019; Horsager *et al.* 2020) but also a "norepinephrine-first" process. Thus, in this study all but one of the PAF patients had neuroimaging evidence of cardiac noradrenergic deficiency, and the sole exception developed low 18F-dopamine-derived radioactivity during follow-up testing. As of this writing he has not developed motor signs of central neurodegeneration.

Results of post-mortem assessments of PAF cases also fit with the norepinephrine-first interpretation. All of 14 PAF patients in whom data have been reported for sympathetic ganglion tissue have had Lewy bodies (or eosinophilic or hyaline bodies) in this tissue (Table 2). Of the 14, 8 had SN neuronal loss, and of these all 8 also had LC neuronal loss. One patient had no detectable TH activity in LC and normal TH activity in SN (Black & Petito 1976). Of 5 PAF patients with normal SN neurons, 4 also had normal LC neurons, and in 1 there was no comment about the LC neuron number. In the present study, although the number of data points for PAF patients was small, frontal cortical norepinephrine content was significantly decreased from control, while putamen dopamine content was variable. Our findings in PAF seem to fit with Braak's stage 2 (pontine LC lesion) and in PD with stage 3 (mesencephalic SN lesion) in the spatiotemporal progression of Lewy body forms of synucleinopathy (Braak *et al.* 2004). A prospective study of LC and SN melanin neuroimaging in PAF patients could test the norepinephrine-first idea (Knudsen *et al.* 2018b; Sommerauer *et al.* 2018).

Clinical significance of central noradrenergic deficiency

The clinical significance of decreased central noradrenergic innervation in synucleinopathies is unknown. Extensive animal research has indicated a variety of roles of norepinephrine derived from the LC in neurobehavioral phenomena such as vigilance, sleep, olfaction, memory of distressing events, emotional eating, nociception, mood, and social appropriateness; and it is suspected that loss of LC neurons may be causally related to non-motor manifestations such as cognitive dysfunction, anxiety/depression, inattention, and pain (Del Tredici & Braak 2013). The advent of central noradrenergic neuroimaging (Belfort-DeAguiar *et al.* 2018; Pietrzak *et al.* 2013; Knudsen *et al.* 2018a) offers an opportunity to determine whether abnormalities in these neuropsychological realms are related to loss of central noradrenergic innervation and if so in which brain regions.

Across all subjects, CSF levels of MHPG were associated with the magnitude of OH. In particular, within the PD group the subgroup with nOH had lower CSF levels than did the subgroup without nOH. There were too few MSA patients without nOH in the present study to conduct meaningful statistics; however, it has been reported that LC 18F-DOPA-derived radioactivity is lower in MSA with than without OH (Lewis *et al.* 2012), and we reported previously that patients with MSA+OH have decreased CSF DHPG (Goldstein *et al.* 2003a).

It is possible that central noradrenergic deficiency, reflecting a lesion of the LC, contributes to baroreflex-sympathoneural failure that in turn is a determinant of nOH. The LC is known to project to the nucleus of the solitary tract, where all baroreceptor afferents initially synapse in the brain, and to the rostral ventrolateral medulla (RVLM), a major source of descending projections to sympathetic pre-ganglionic neurons. In PD there are decreases in LC neurons (Zarow *et al.* 2003) and variable decreases in RVLM (Halliday *et al.* 1990; Benarroch *et al.* 2000) neurons. MSA is associated with decreases in LC neurons (Benarroch 2003; Benarroch *et al.* 2002) as well as in RVLM and A5 noradrenergic neurons (Benarroch *et al.* 2008; Benarroch *et al.* 2000). Detailed brainstem immunohistochemistry in these regions has not been described in PAF.

Determinants of CSF MHPG

Lines of best fit for scatter plots relating MHPG to norepinephrine and to DHPG were above the origin. These findings suggest that even if there were no norepinephrine release there would still be intra-neuronal metabolism to DHPG, due to passive leakage of norepinephrine from vesicular stores, followed by extra-neuronal metabolism of DHPG to MHPG (see concept diagram in Figure 1). In the periphery, ongoing norepinephrine metabolism reflects net leakage from vesicular stores, a process that occurs independently of pathway traffic-induced exocytosis (Eisenhofer *et al.* 2004). Based on the present results the same may hold true for norepinephrine metabolism in the brain. We propose that CSF MHPG has two main sources—norepinephrine that is released by exocytosis in response to nerve pathway traffic and norepinephrine that leaks passively into the cytoplasm from vesicular stores independently of pathway traffic. Other likely determinants of CSF MHPG levels are circulating MHPG (Kopin *et al.* 1983) and sulfoconjugation (Tyce *et al.* 1989).

Utility as diagnostic biomarkers

Areas under ROC curves were significantly above 0.5 for most of the CSF neurochemical measures in this study. The largest area was for CSF MHPG (area=0.83), with sensitivity 65% at a specificity of 80%. These results indicate moderate efficiency in separating synucleinopathy from control groups but do not support the application of these measures in individual diagnosis.

STUDY LIMITATIONS

Steps in intra-neuronal catecholamine synthesis, storage, release, reuptake, and metabolism are complex (Figure 1), and the catecholamine metabolites that were assayed in CSF provide only indirect indices of central dopaminergic and noradrenergic innervation and function. The combined measurements of norepinephrine, DHPG, and MHPG, with strongly positive inter-correlations and highly significant differences between the synucleinopathy and control groups for all three analytes, help buttress the inferences drawn; however, even with careful attention to the conditions at the time of sampling there was substantial inter-individual variability in the data in all the subject groups. Possibly this variability reflects genetic differences for several enzymes and transporters.

CSF HVA and DOPAC were only weakly correlated with neuroimaging indices of putamen dopamine deficiency. This can be explained by the indirectness of both types of measures in evaluating loss and dysfunctions of catecholaminergic neurons.

Since the CSF and post-mortem neurochemical data were obtained in largely separate cohorts, the CSF neurochemical indices could not be validated by comparison with tissue catecholamine contents.

We did not assay HVA or MHPG in brain tissue. Our assay method involved direct injection of CSF into the LCED system. Liquid chromatography with tandem mass spectroscopy offers an opportunity in the future to obtain data about catecholamines, HVA, and MHPG in the same brain tissue samples, but so far there are no published methods demonstrating adequate validity and reliability for assaying levels of HVA, and MHPG in human brain tissue.

There were relatively few data points about brain tissue catecholamines in PAF patients. PAF is a rare disease, and to our knowledge there is no availability of banked tissue from PAF patients.

In LCED chromatographs, dopamine has a relatively long retention time and therefore appears as a short, wide peak. In CSF samples there often are contaminating small peaks that can make it difficult to quantify dopamine. These factors, combined with low dopamine concentrations in CSF, question the validity and reliability of CSF dopamine in our study. In contrast, norepinephrine has a much shorter chromatographic retention time, the peak is far more spikey, and CSF norepinephrine levels are normally higher than dopamine levels.

Because of the unusual referral pattern of patients to the NIH, our research focus on autonomic disorders, and the comprehensive inpatient testing that was done, the synucleinopathy groups in this study may not reflect those in the general population. In particular, PD+nOH probably was over-represented.

CONCLUSIONS AND PERSPECTIVE

The results of this study indicate that three synucleinopathies—PD, MSA, and PAF—entail central norepinephrine deficiency. We report clear distinctions among these diseases, in that by both CSF neurochemical and PET neuroimaging approaches there is more extensive central dopamine deficiency in PD and MSA than in PAF. The retrospective results of this study should be confirmed in a more formally designed cohort study, especially to assess clinical correlates of the CSF neurochemical abnormalities reported here. Prospective studies of brainstem neuroimaging by PET or MRI in PAF patients could test the “norepinephrine-first” concept.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The research reported here was supported (in part) by the research Division of Intramural Research, National Institutes of Health (NINDS).

Tandees Najimi assisted with culling relevant literature.

FINANCIAL SUPPORT:

Division of Intramural Research, NINDS, NIH.

ABBREVIATIONS

α-syn	alpha-synuclein
ALDH	aldehyde dehydrogenase
AR	aldehyde/aldose reductase
COMT	catechol-O-methyltransferase
COMTI	catechol-O-methyltransferase inhibitor
CSF	cerebrospinal fluid
CTX	cerebral cortex
Cys-DOPA	5-S-cysteinyldopa
Cys-DA	5-S-cysteinyldopamine
DA	dopamine
DHPG	3,4-dihydroxyphenylglycol
DOPA	3,4-dihydroxyphenylalanine
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPEGAL	3,4-dihydroxyphenyglycolaldehyde
HVA	homovanillic acid
LAAAD	L-aromatic-amino-acid decarboxylase
IND	Investigational New Drug
LCED	liquid chromatography with electrochemical detection
LP	lumbar puncture
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MHPG	3-methoxy-4-hydroxyphenylglycol

MSA	multiple system atrophy
NE	norepinephrine
nOH	neurogenic orthostatic hypotension
OCC	occipital cortex
OH	orthostatic hypotension
Ox.	spontaneous oxidation
PAF	pure autonomic failure
PD	Parkinson disease
PMID	PubMed ID number
PUT	putamen
PUT/OCC	putamen/occipital cortex ratio
ROC	receiver operating characteristic
RVLM	rostral ventrolateral medulla
SN	substantia nigra
SSRI	selective serotonin reuptake inhibitor
TH	tyrosine hydroxylase
VMAT	vesicular monoamine transporter

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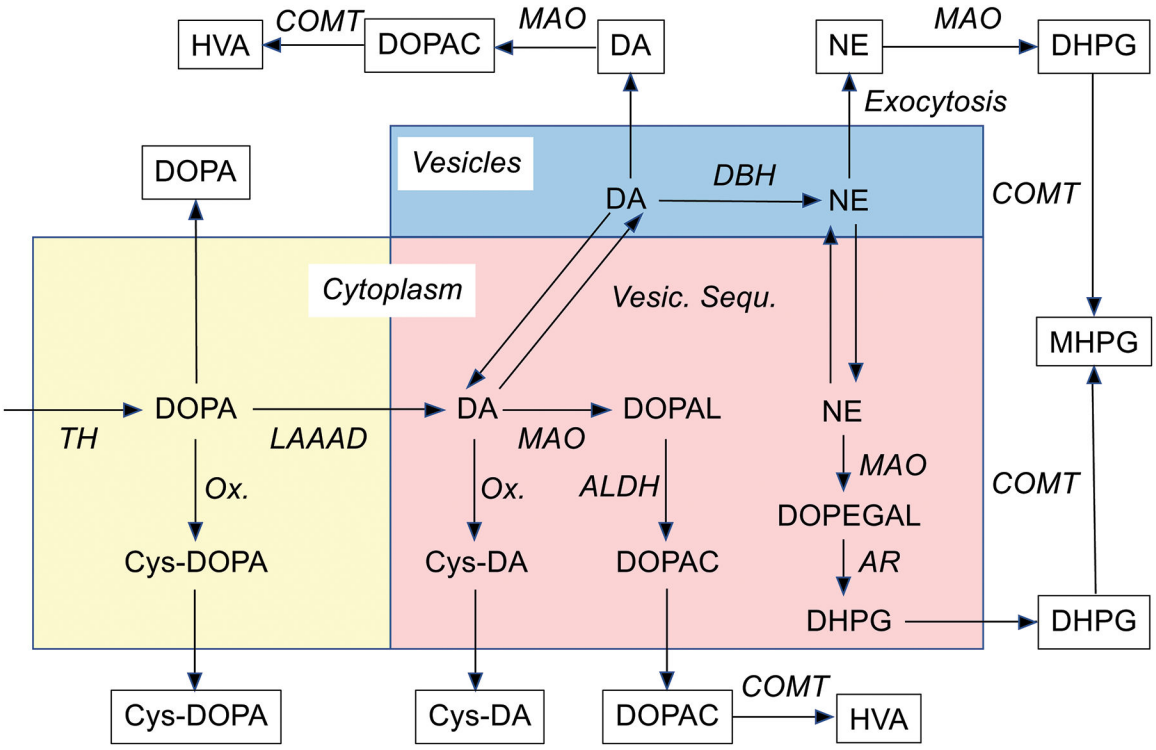

Figure 1: Concept diagram depicting sources of levels of catecholamines and their metabolites in cerebrospinal fluid.

Biochemicals are in plain text and processes in italics. The colored rectangles represent (blue) vesicles and two cytoplasmic compartments, one involving processes proximal to cytoplasmic dopamine (banana color) and the other distal to cytoplasmic dopamine production (pink). Abbreviations: ALDH=aldehyde dehydrogenase; AR=aldehyde/aldose reductase; COMT=catechol-O-methyltransferase; Cys-DOPA=5-S-cysteinyldopa; Cys-DA=5-S-cysteinyldopamine; DA=dopamine; DHPG=3,4-dihydroxyphenylglycol; DOPA=3,4-dihydroxyphenylalanine; DOPAL=3,4-dihydroxyphenylacetaldehyde; DOPEGAL=3,4-dihydroxyphenylglycolaldehyde; HVA=homovanillic acid; LAAAD=L-aromatic-amino-acid decarboxylase; MAO=monoamine oxidase; MHPG=3-methoxy-4-hydroxyphenylglycol; NE=norepinephrine; Ox.=spontaneous oxidation; TH=tyrosine hydroxylase; VMAT=vesicular monoamine transporter.

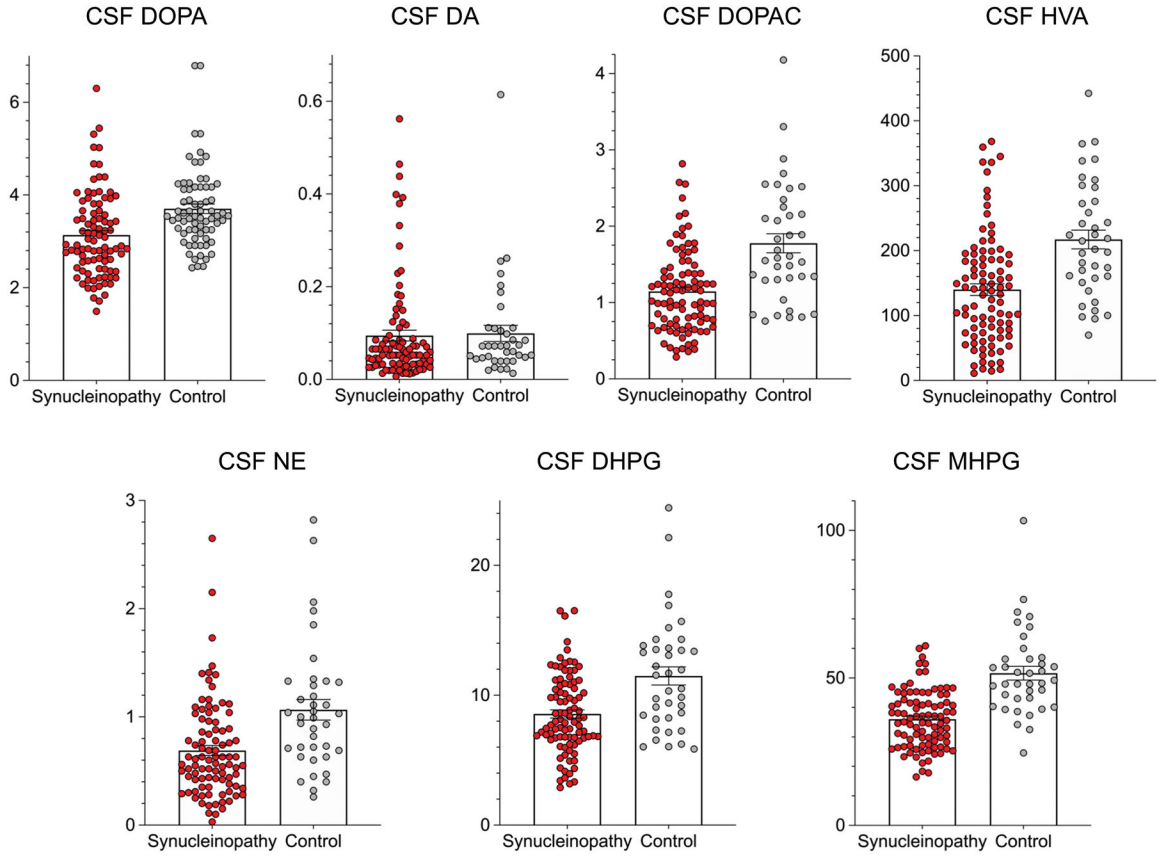

Figure 2: Individual values for cerebrospinal fluid (CSF) concentrations of DOPA, DA, DOPAC, HVA, NE, DHPG, and MHPG in synucleinopathy patients (N=98, red) and controls (N=32, gray). Rectangles show group mean values. Error bars are SEMs. Abbreviations: DA=dopamine; DHPG=3,4-dihydroxyphenylglycol; DOPA=3,4-dihydroxyphenylalanine; DOPAC=3,4-dihydroxyphenylacetic acid; HVA=homovanillic acid; MHPG=3-methoxy-4-hydroxyphenylglycol; NE=norepinephrine. The population distributions in the synucleinopathy group (with the except of DA) are shifted downward with respect to those in the controls.

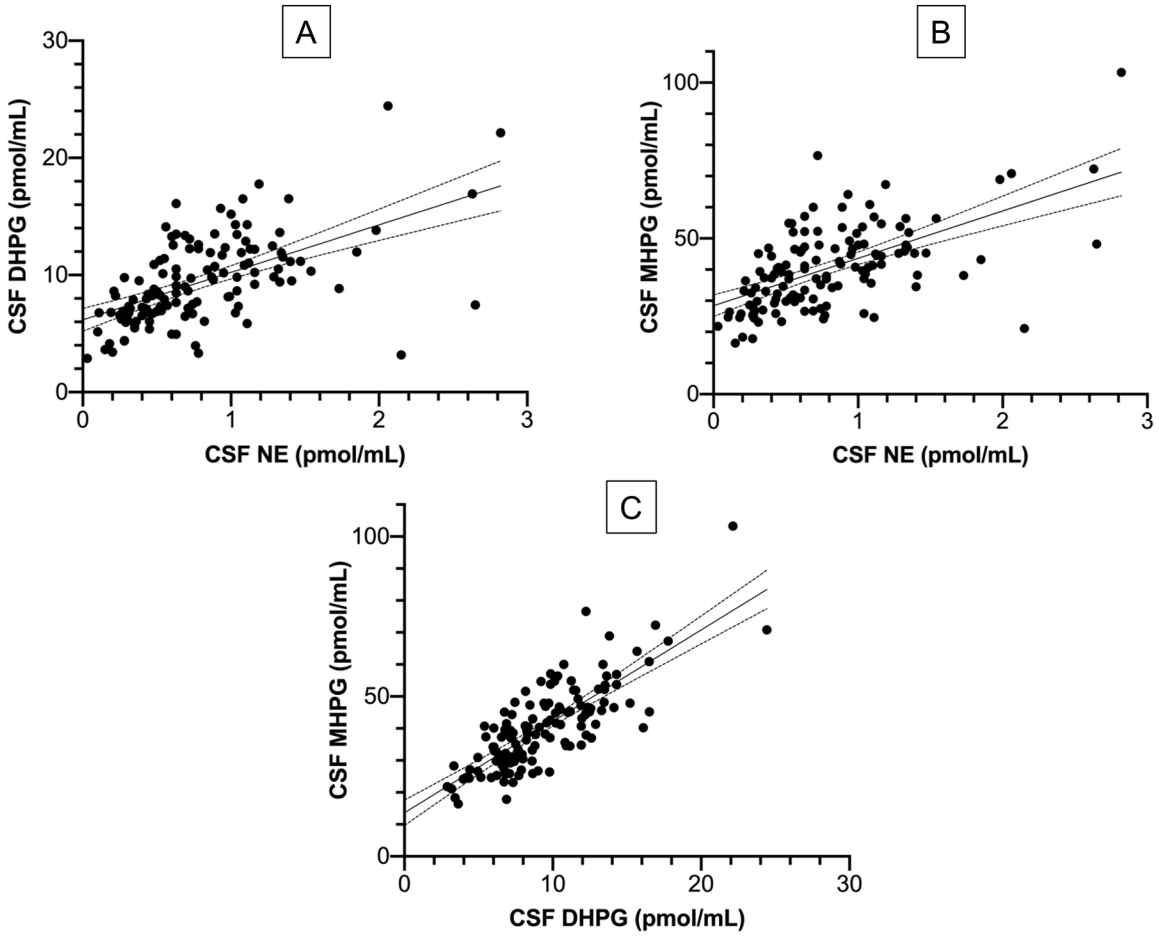

Figure 3: Scatter plots comparing individual values for (A) CSF DHPG vs. NE, (B) CSF MHPG vs. NE and (C) CSF MHPG vs. DHPG across all subjects (N=130).

Lines of best fit with 95% confidence intervals are shown. Abbreviations: DHPG=3,4-dihydroxyphenylglycol; MHPG=3-methoxy-4-hydroxyphenylglycol; NE=norepinephrine. There are strong positive inter-correlations among the 3 central noradrenergic indices. The lines of best fit have their y-intercept values above the origin.

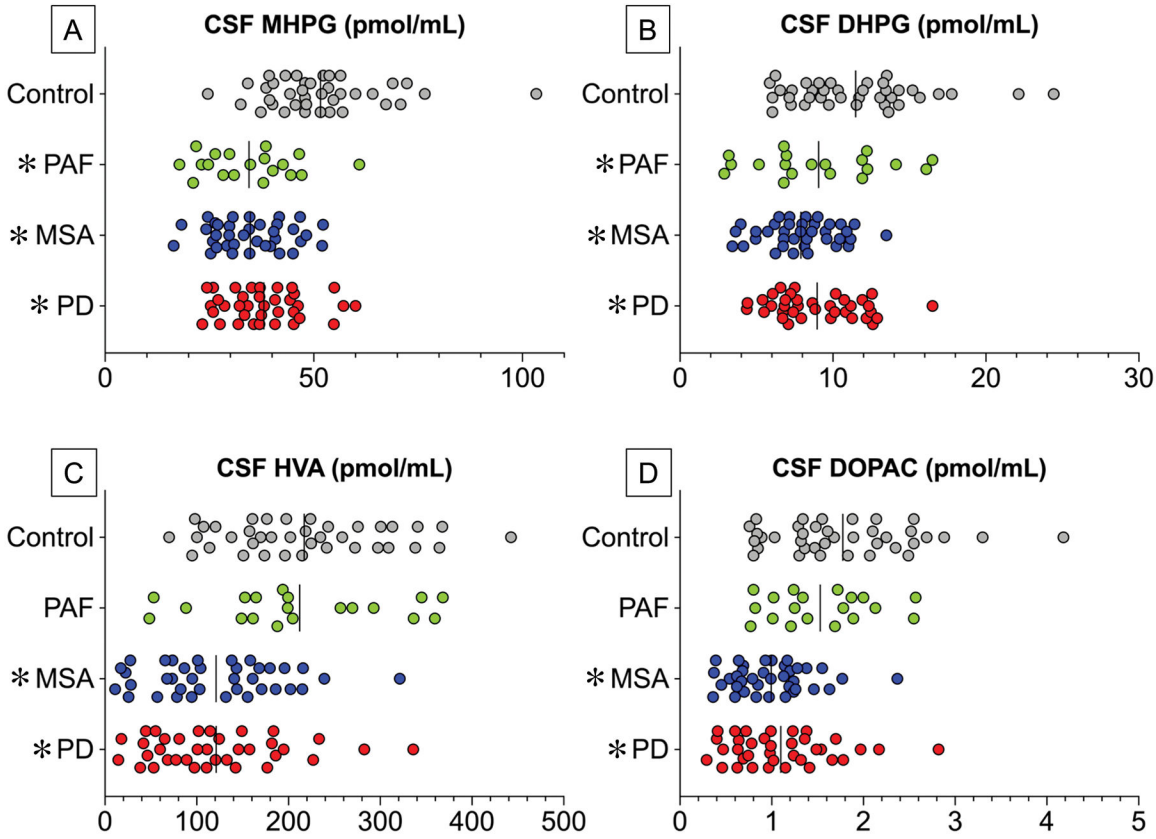

Figure 4: Individual values for cerebrospinal fluid (CSF) levels of (A) MHPG, (B) DHPG, (C) HVA, and (D) DOPAC in synucleinopathies and control subjects.

Abbreviations: DHPG=3,4-dihydroxyphenylglycol; DOPAC=3,4-dihydroxyphenylacetic acid; HVA=homovanillic acid; MHPG=3-methoxy-4-hydroxyphenylglycol; MSA=multiple system atrophy (N=32, no CSF MHPG in 1 MSA patient) ; PAF=pure autonomic failure (N=19). PD=Parkinson disease (N=36). PD data are in red, MSA in blue, PAF in green, and control subjects in gray (N=32). Vertical lines indicate group mean values. CSF MHPG and DHPG are decreased in the 3 synucleinopathy groups, whereas CSF HVA and DOPAC are decreased in PD and MSA but not PAF.

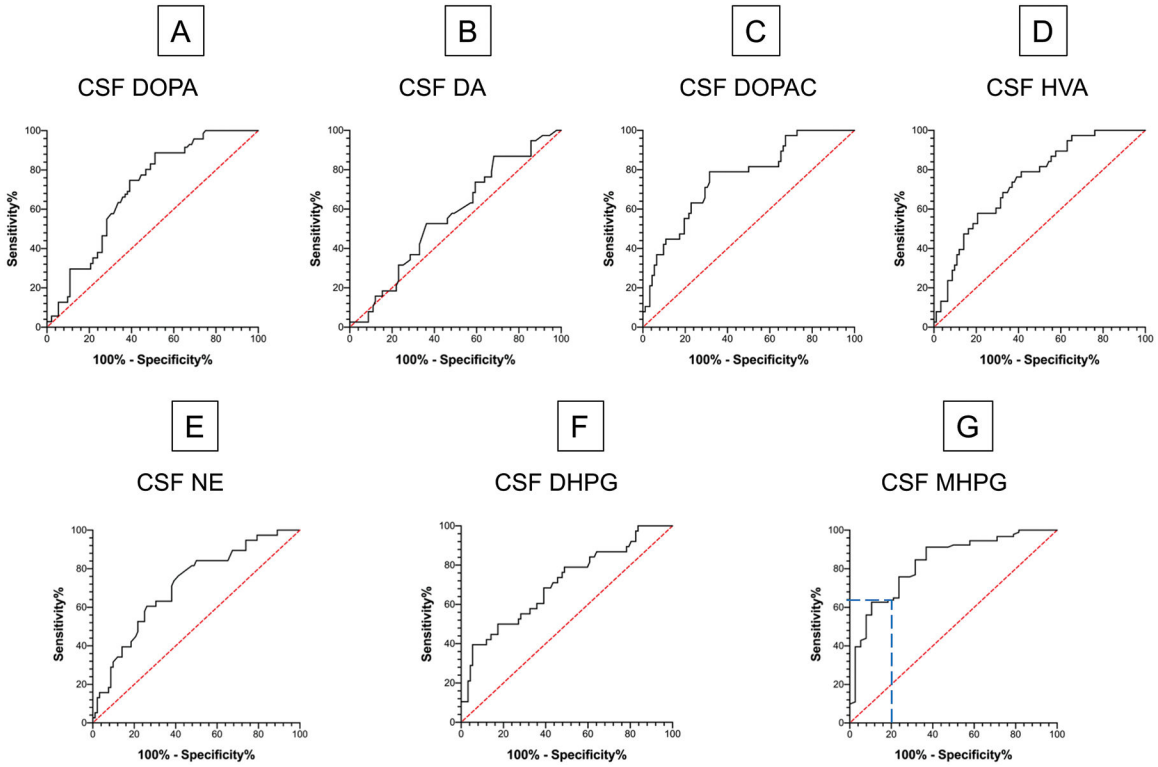

Figure 5: Receiver operating characteristic (ROC) curves for distinguishing synucleinopathy patients (N=92) from controls (N=24) by CSF levels of (A) DOPA, (B) DA, (C) DOPAC, (D) HVA, (E) NE, (F) DHPG, and (G) MHPG.

Abbreviations: DA=dopamine; DHPG=3,4-dihydroxyphenylglycol; DOPA=3,4-dihydroxyphenylalanine; DOPAC=3,4-dihydroxyphenylacetic acid; HVA=homovanillic acid; MHPG=3-methoxy-4-hydroxyphenylglycol; NE=norepinephrine. Red line indicates predictions from the null hypothesis of no distinction between the groups. Blue dashed line shows that for CSF MHPG sensitivity is 65% and specificity 80%.

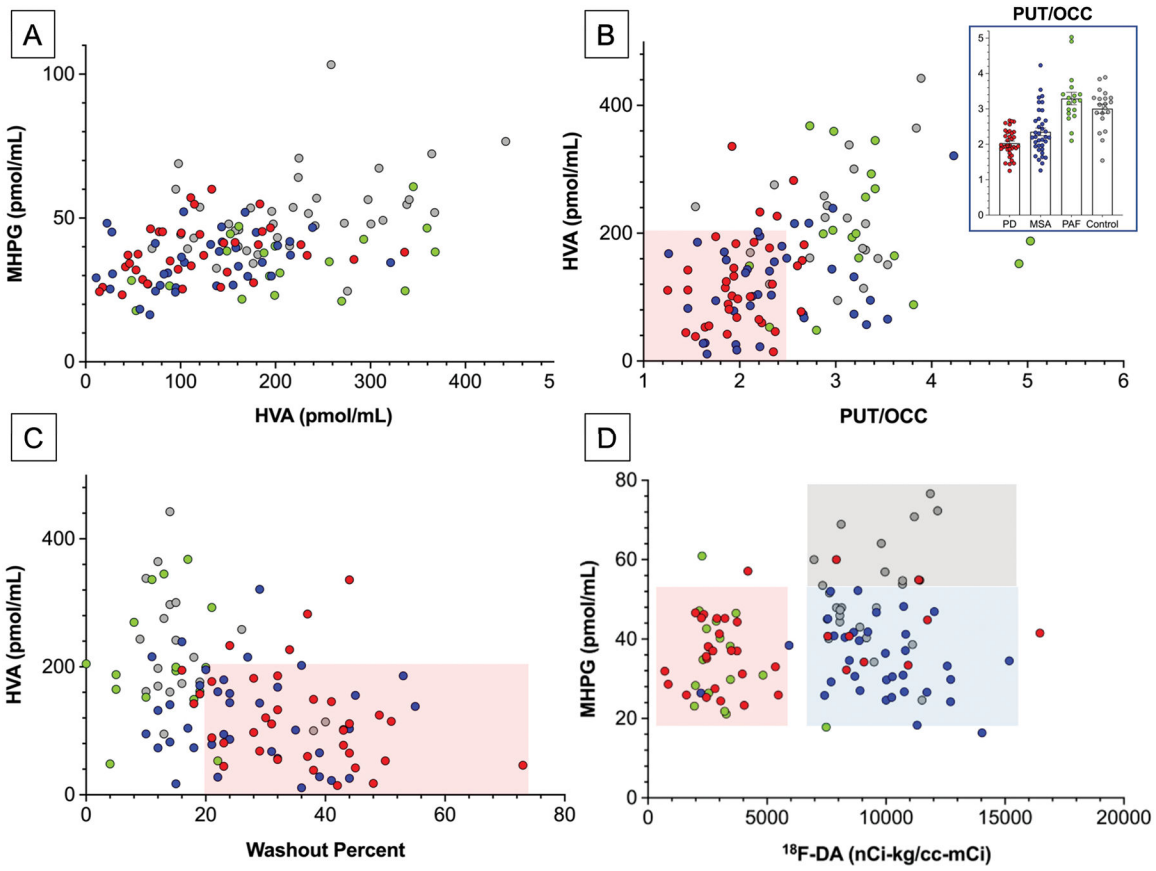

Figure 6: Individual values for cerebrospinal fluid (CSF) levels of (A) MHPG as a function of HVA, (B) HVA as a function of the putamen/occipital (PUT/OCC) ratio of 18F-DOPA-derived radioactivity, (C) HVA as a function of the washout percent of 18F-DOPA-derived radioactivity, and (D) MHPG as a function of septal myocardial 18F-dopamine- (18F-DA-) derived radioactivity in synucleinopathies and control subjects.

Abbreviations: HVA=homovanillic acid; MSA=multiple system atrophy (total N=37, blue); PAF=pure autonomic failure (total N=19). PD=Parkinson disease (Total N=36). PD data are in red, multiple system atrophy, MSA blue, PAF green, and control subjects gray (Total N=24). Inset in (B) shows individual PUT/OCC ratios, with means ± SEM. Pink rectangles in (B) and (C) placed visually to emphasize low HVA, PUT/OCC ratios, and increased 18F-DOPA washout precents in PD and MSA. In (D), gray rectangle placed visually to depict normal values. The pink and blue rectangles in (D) are placed visually to indicate low 18F-DA-derived radioactivity in PD and PAF and normal radioactivity in MSA.

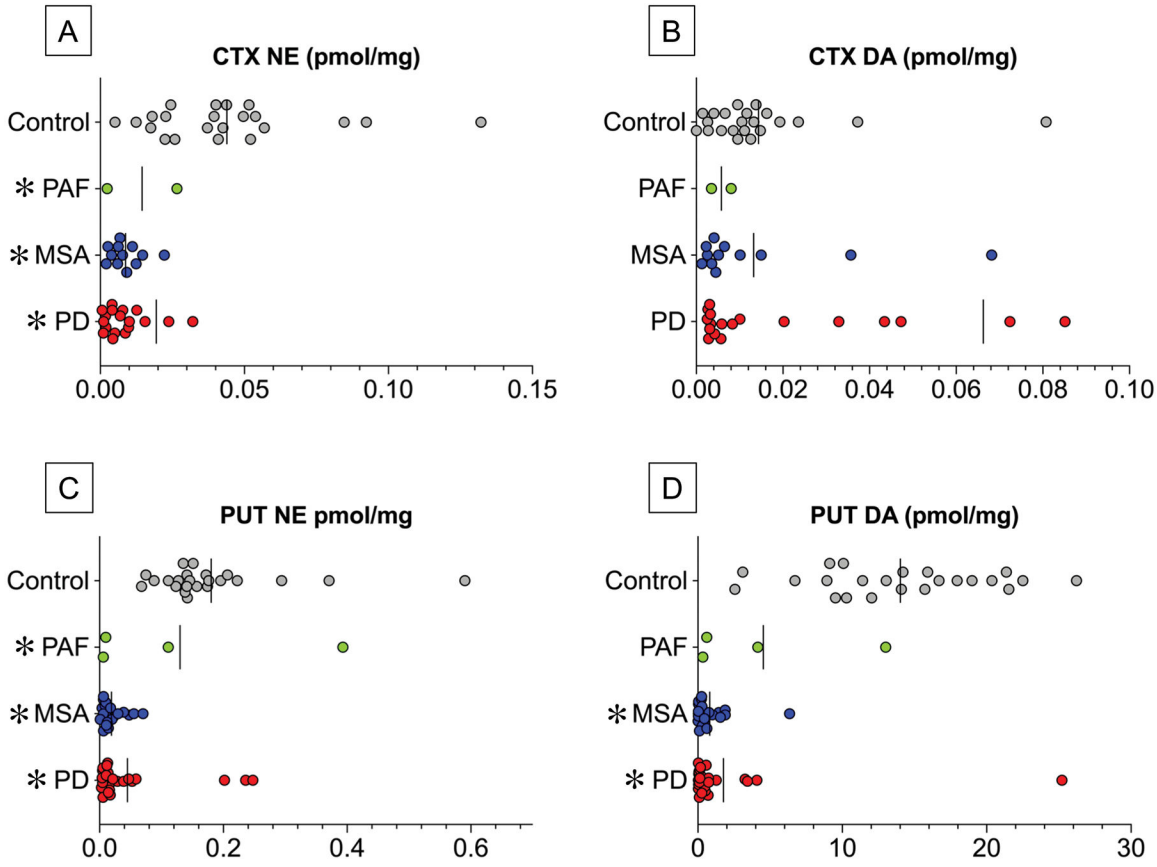

Figure 7: Individual data for frontal cortex (CTX) and putamen (PUT) concentrations of dopamine (DA) and norepinephrine (NE) in synucleinopathy patients and control subjects.

Patients with Parkinson disease (PD) are in red (Total N=19), multiple system atrophy (MSA) blue (Total N=12), pure autonomic failure (PAF) green (Total N=2 for CTX, 4 for PUT), and controls gray (Total N=22). Vertical lines indicate group mean values. Abbreviations: DA=dopamine; NE=norepinephrine. In (A) 1 data point for CTX NE in a control subject is outside the axis limit, and in (B) 1 data point for CTX DA in a PD patient is outside the axis limit. Note decreased CTX NE and normal CTX DA in the 3 synucleinopathy groups, whereas PUT DA is decreased in the PD and MSA groups and variable in the PAF group.

Table 1:

Published reports about cerebrospinal fluid (CSF) levels of catecholamines and catecholamine metabolites in Parkinson disease (PD), multiple system atrophy (MSA), or pure autonomic failure (PAF) and control groups.

Disease	N	CSF DOPA	CSF DA	CSF DOPAC	CSF HVA	CSF NE	CSF DHPG	CSF MHPG	First Author	Year	PMID
PD	1				Decreased				Bernheimer	1966	4229554
	2				Decreased				Guldberg	1967	5588903
	3				Decreased				Johansson	1967	6035772
	4				Normal				Olsson	1968	5668438
	5				Decreased				Gottfries	1969	5808100
	6							Normal	Wilk	1971	5571114
	7							Decreased	Granerus	1974	4854088
	8							Normal	Davidson	1977	591981
	9				Decreased				Cunha	1983	6839227
	10							Normal	Mann	1983	6644314
	11							Normal	Mena	1984	6204498
	12					Normal		Normal	Turkka	1987	3816883
	13				Decreased			Normal	Hartikainen	1992	1347220
	14					Decreased		Normal	Martignoni	1992	1320891
	15				Decreased				Strittmatter	1992	1378766
	16								Lewitt	1993	8420188
	17				Decreased	Normal		Normal	Chia	1993	8336158
	18			Normal	Normal			Normal	González-Quevedo	1993	7521168
	19			Decreased	Decreased	Normal		Normal	Mashige	1994	7914240
	20	Normal	Normal	Decreased		Decreased			Eldrup	1995	7484057
	21				Decreased				Cheng	1996	9617787
	22				Decreased				Kanemaru	1998	9605500
	23		Normal	Decreased	Normal				Engelborghs	2003	12834252
	24		Normal	Decreased					Goldstein	2008	18325818
	25	Decreased			Decreased				Ishibashi	2010	20002007
	26				Normal				Lewitt	2011	21784416
	27	Decreased	Normal	Decreased		Normal	Decreased		Goldstein	2012	22451506
	28	Decreased	Normal	Decreased		Normal	Decreased		Engelborghs	2012	22451506
	29				Decreased			Normal	Herbert	2013	24122060
	30	Decreased	Decreased	Decreased	Decreased			Normal	Andersen	2017	28244186
	31	Decreased	Normal	Decreased	Decreased	Decreased	Decreased	Decreased	Goldstein	This study	
MSA	1						Decreased	Decreased	Polinsky	1984	6539879
	2					Decreased		Decreased	Martignoni	1992	1320891
	3								Goldstein	2003	12540289

Disease	N	CSF DOPA	CSF DA	CSF DOPAC	CSF HVA	CSF NE	CSF DHPG	CSF MHPG	First Author	Year	PMID
	4				Decreased			Decreased	Abdo	2007	17448720
	5		Normal	Decreased					Goldstein	2008	18325818
	6	Decreased	Decreased	Decreased		Normal	Decreased		Goldstein	2012	22451506
	7	Decreased	Normal	Decreased	Decreased	Decreased	Decreased	Decreased	Goldstein	This study	
PAF	1							Decreased	Polinsky	1984	6539879
	2						Decreased		Goldstein	2003	12540289
	3	Decreased	Decreased	Decreased		Decreased	Decreased		Goldstein	2012	22451506
	4	Normal	Normal	Normal	Normal	Decreased	Decreased	Decreased	Goldstein	This study	

Abbreviations: DA=dopamine; DHPG=3,4-dihydroxyphenylglycol; DOPA=3,4-dihydroxyphenylalanine; DOPAC=3,4-dihydroxyphenylacetic acid; HVA=homovanillic acid; MHPG=3-methoxy-4-hydroxyphenylglycol; NE=norepinephrine; PMID=PubMed ID number.

Table 2:

Autopsy findings in idiopathic orthostatic hypotension or pure autonomic failure.

Reference	No.	SNS LBs	LC Neuronal Loss	SN Neuronal Loss
(Johnson *et al.* 1966)	Case 1	Eosinophilic bodies	0	0
(Vanderhaeghen *et al.* 1970)	Case 1	1	1	1
	Case 2	1	0	0
(Roessmann *et al.* 1971)	Case 1	1	1	1
(Schober *et al.* 1975)	Case 2	1	1	1
(Black & Petito 1976)	Case C	1	Blank TH	Normal TH
(Petito & Black 1978)	Patient 3	Hyaline bodies	1	1
(van Ingelghem *et al.* 1994)	1	1	0	0
(Hague *et al.* 1997)	1	1	Mild, WNL	Mild, WNL
(Arai *et al.* 2000)	1	1	No comment	0
(Miura *et al.* 2001)	1	No ganglia found	1	1
(Terao *et al.* 1993)	1	1	1	1
(Kaufmann *et al.* 2004)	Case 1	1	1 (Pallor)	1 (Pallor)
	Case 2	1	1 (Pallor)	1 (Pallor)

Abbreviations: Brstem=brainstem; LB=Lewy body; TH=tyrosine hydroxylase; WNL=within normal limits.