Cerebrospinal fluid levels of catechols in patients with neurogenic orthostatic hypotension

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ABSTRACT

In multiple system atrophy (MSA) and pure autonomic failure (PAF), orthostatic hypotension (OH) results from deficient noradrenaline release from sympathetic nerves during standing. Post-mortem findings have indicated loss of central noradrenergic cells in both diseases. The present study sought in vivo neurochemical evidence for central noradrenergic deficiency in patients with OH due to MSA or PAF. A total of 28 patients with OH (18 with MSA; 10 with PAF) had cerebrospinal fluid and blood sampled for levels of noradrenaline and its neuronal metabolite dihydroxyphenylglycol. A control group of 44 subjects included 10 elderly normal volunteers, 10 patients with Alzheimer’s disease, 18 patients with dysautonomia (postural tachycardia syndrome or neurocardiogenic syncope) and six patients with MSA in the absence of OH. Patients with OH had lower cerebrospinal fluid concentrations of noradrenaline (0.53 ± 0.07 nmol/l) and dihydroxyphenylglycol (6.52 ± 0.46 nmol/l) than did control subjects (0.90 ± 0.09 and 9.64 ± 0.46 nmol/l respectively; P = 0.0001). The MSA + OH group had higher plasma levels of both catechols (noradrenaline, 1.31 ± 0.16 nmol/l; dihydroxyphenylglycol, 5.08 ± 0.43 nmol/l) than did the PAF group (noradrenaline, 0.38 ± 0.08 nmol/l; dihydroxyphenylglycol, 2.53 ± 0.30 nmol/l; P < 0.001), despite similarly low cerebrospinal fluid levels. Among MSA patients, those with OH had lower cerebrospinal fluid levels of noradrenaline and dihydroxyphenylglycol than those without OH (noradrenaline, 1.71 ± 0.64 nmol/l; dihydroxyphenylglycol, 10.41 ± 1.77 nmol/l respectively; P = 0.006). The findings are consistent with central noradrenergic deficiency in both MSA + OH and PAF. In MSA, central noradrenergic deficiency seems to relate specifically to OH.

INTRODUCTION

In chronic primary autonomic failure, blood pressure often falls during standing, due to inadequate reflexive, sympathetically mediated release of noradrenaline in response to decreased cardiac filling [1]. Conversely, orthostatic hypotension (OH) constitutes a cardinal sign of failure of the sympathetic nervous system.

OH occurs typically in multiple system atrophy (MSA) and in pure autonomic failure (PAF) [2]. MSA is a progressive disease that involves autonomic failure and clinical evidence of Parkinsonism, cerebellar atrophy or degeneration of other central neural pathways. The combination of MSA and OH was formerly termed the Shy–Drager syndrome [3]. In unusual cases, MSA occurs without OH, but with urinary retention and

Key words: dihydroxyphenylglycol, multiple system atrophy, noradrenaline, pure autonomic failure, sympathetic nervous system.

Abbreviations: MSA, multiple system atrophy; OH, orthostatic hypotension; PAF, pure autonomic failure.

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incontinence, erectile failure in men, constipation and slurred speech. A hallmark of PAF, which also has been called Bradbury–Eggleston syndrome [4] or idiopathic OH [5], is severe OH associated with neurochemical [1,6], neuropharmacological [7] and neuroimaging [8] evidence of diffuse loss of sympathetic noradrenergic terminals.

Patients with OH in the setting of chronic primary autonomic failure usually do not have the normal increase in blood pressure at the end of phase II of the Valsalva manoeuvre, nor the normal overshoot of blood pressure during phase IV after release of the manoeuvre [9]. This pattern is identical in MSA with OH and in PAF.

The results of many studies have led to the concept that PAF entails a post-ganglionic lesion, with loss of sympathetic terminal innervation, whereas in MSA sympathetic post-ganglionic innervation remains intact [8]. PAF does not include clinical evidence of central noradrenergic dysfunction [10]; however, in both diseases, post-mortem studies have reported the loss of noradrenaline-producing cells in the brainstem [5,11].

Researchers in neurology and psychiatry have long viewed the cerebrospinal fluid as a ‘window on the brain’. Little is known about relationships among the levels of noradrenaline and its metabolites in the cerebrospinal fluid in chronic primary autonomic failure. One report noted decreased cerebrospinal fluid levels of noradrenaline in MSA [12], but did not stratify patients in terms of the occurrence of OH. MSA patients have decreased cerebrospinal fluid concentrations of methoxyhydroxyphenylglycol, a deaminated, O-methylated metabolite of noradrenaline [12–14]; however, cerebrospinal fluid concentrations of this metabolite depend crucially on circulating methoxyhydroxyphenylglycol traversing the blood–brain barrier [15]. Previous studies have not examined cerebrospinal fluid levels of dihydroxyphenylglycol, the main neuronal metabolite of noradrenaline [16], in patients with MSA, nor levels of noradrenaline or dihydroxyphenylglycol in those with PAF. PAF patients have approximately normal summed rates of entry of noradrenaline and its metabolites into internal jugular venous plasma [17]. Previous studies also have not stratified MSA patients in terms of the occurrence of OH.

The main purpose of the present study was to determine if patients with OH have decreased cerebrospinal fluid levels of noradrenaline and dihydroxyphenylglycol. Such a finding would be consistent with a central noradrenergic deficiency.

METHODS

The study protocol was approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke, and all patients gave informed written consent before participating.

A total of 28 patients referred for primary chronic autonomic failure and who had OH (18 with MSA and 10 with PAF) underwent cerebrospinal fluid sampling by lumbar puncture and arterial blood sampling by brachial arterial cannulation, for assays of cerebrospinal fluid and arterial plasma levels of catechols.

There were four control groups (a total of 44 control subjects). Of these, 18 patients were referred for dysautonomia without OH, 10 had Alzheimer’s disease [17a] and six had MSA without OH. Criteria for dysautonomia without OH were: (1) a referral diagnosis, confirmed clinically by us, of postural tachycardia syndrome, frequent episodes of neurocardiogenic syncope or presyncope, or reflex sympathetic dystrophy; (2) lack of OH; (3) a normal pattern of blood pressure responses in both phase II, phase III and phase IV of the Valsalva manoeuvre; and (4) a normal (at least 60%) orthostatic increment in the plasma noradrenaline level.

MSA without OH was characterized by urinary retention and incontinence, erectile failure in men, constipation and slurred speech. The fourth control group comprised 10 elderly normal volunteers.

The subjects were not on drugs known to elicit OH (Levodopa treatment did not produce OH [18–21]).

To obtain cerebrospinal fluid for neurochemical assays, patients underwent lumbar puncture. In most cases, the lumbar puncture was done under fluoroscopic guidance. Six 1 ml aliquots of fluid were collected into chilled 1.5 ml plastic sample tubes, which were frozen immediately in solid CO₂ and then stored at −70 °C until the samples were assayed. The sixth aliquot was used for neurochemical assays.

For arterial blood sampling, a brachial arterial cannula was inserted after local anaesthesia of the overlying skin. Arterial blood (approx. 6 ml) was obtained after the patient had been resting supine for at least 20 min. Antecubital venous rather than arterial blood was obtained from patients with Alzheimer’s disease and elderly normal volunteers. Blood samples were centrifuged at 4 °C, and the plasma was separated and frozen at −70 °C.

Plasma and cerebrospinal fluid were assayed for catechols, by batch alumina extraction and liquid chromatography with electrochemical detection, in our laboratory [22].

The four control groups without OH did not differ significantly with regard to cerebrospinal fluid or plasma concentrations of noradrenaline or dihydroxyphenylglycol (Table 1), although patients with MSA in the absence of OH had relatively high and variable levels. Thus, for most statistical comparisons, the data from the four control groups were combined. Statistical testing consisted of factorial ANOVA, with post hoc testing for particular group differences using the Fisher PLSD (protected least significant difference) test,

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Table 1 Cerebrospinal fluid concentrations of catechols in the control groups

Numbers in parentheses are numbers of subjects.∗. Data from [17a].

<table>
<thead>
<tr>
<th>Group</th>
<th>Noradrenaline (nmol/l)</th>
<th>Dihydroxyphenylglycol (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.81 ± 0.05 (44)</td>
<td>9.70 ± 0.40 (44)</td>
</tr>
<tr>
<td>Dysautonomia</td>
<td>0.81 ± 0.08 (18)</td>
<td>9.50 ± 0.75 (18)</td>
</tr>
<tr>
<td>Elderly normal volunteers</td>
<td>0.69 ± 0.07 (10)</td>
<td>9.96 ± 0.67 (10)</td>
</tr>
<tr>
<td>Alzheimer’s disease∗</td>
<td>0.89 ± 0.11 (10)</td>
<td>9.74 ± 0.65 (10)</td>
</tr>
<tr>
<td>MSA without OH</td>
<td>1.71 ± 0.64 (6)</td>
<td>10.41 ± 1.77 (6)</td>
</tr>
</tbody>
</table>

Figure 1 Levels of noradrenaline (NA) (upper panel) and dihydroxyphenylglycol (DHPG) (lower panel) in the cerebrospinal fluid (CSF) of the various groups

The groups comprise patients with PAF, patients with MSA + OH, elderly control subjects (Old Control), patients with Alzheimer’s disease (Alz) [17a], patients with chronic orthostatic intolerance (COI), and patients with MSA in the absence of OH (MSA w/o OH). Significant differences ∗P < 0.05 compared with all controls by Fisher’s PLSD post hoc test; ∗∗∗∗P < 0.0001 compared with all controls by independent-means t test. The broken lines show mean values in control subjects (all four control groups combined).

RESULTS

The main catechols detected in cerebrospinal fluid were (in descending order of concentration) dihydroxyphenylglycol, l-dopa, dihydroxyphenylacetic acid and noradrenaline. Adrenaline and dopamine levels in cerebrospinal fluid were very low, and often below the detection limits of the assay (approx. 5 pg/ml, or 0.03 nmol/l). Among the control subjects, the mean cerebrospinal fluid adrenaline concentration of 0.0038 ± 0.0018 nmol/l was less than 3% of the mean plasma level (0.1455 ± 0.0256 nmol/l).

Mean values for noradrenaline and dihydroxyphenylglycol concentrations in cerebrospinal fluid differed highly significantly between patients with OH and controls (Figures 1 and 2). Post hoc testing showed that both groups with OH (MSA and PAF) had significantly lower cerebrospinal fluid levels than did the control group. The group with MSA + OH had significantly lower cerebrospinal fluid levels of both noradrenaline and dihydroxyphenylglycol than did the group with MSA lacking OH. Cerebrospinal fluid levels
Scatterplots relating individual values of cerebrospinal fluid (CSF; upper panel) and plasma (lower panel) levels of dihydroxyphenylglycol (DHPG) and noradrenaline (NA) in control subjects (○), patients with MSA + OH (■) and patients with PAF (●). Regression equations and lines of best fit are for control subjects, excluding outlying data from two control subjects with high plasma levels.

Regression equation: $y = 5.38 + 3.26x$; $r = 0.68$

Regression equation: $y = 3.05 + 1.08x$; $r = 0.60$

**Figure 3** Scatterplots relating individual values of cerebrospinal fluid (CSF) and plasma levels of dihydroxyphenylglycol (DHPG) and noradrenaline (NA) in control subjects (○), patients with MSA + OH (■) and patients with PAF (●).

Regression equations and lines of best fit are for control subjects, excluding outlying data from two control subjects with high plasma levels.

of both catechols correlated strongly and positively with plasma levels.

Cerebrospinal fluid concentrations of dihydroxyphenylglycol correlated strongly and positively with those of noradrenaline (Figure 3). Plasma noradrenaline levels varied highly significantly with diagnostic group ($F = 21.3$, $P < 0.001$), with those in the MSA + OH group being significantly lower than those in the control group, and also lower than those in the groups with MSA with or without OH. Levels of dihydroxyphenylglycol also varied highly significantly with diagnostic group ($F = 12.6$, $P < 0.001$), again with those in the PAF group lower than in the control and MSA groups. Plasma dihydroxyphenylglycol levels correlated strongly and positively with those of noradrenaline.

**DISCUSSION**

In the present study, patient groups with primary chronic autonomic failure and OH, i.e. with MSA or PAF, had low cerebrospinal fluid concentrations of both noradrenaline and its main neuronal metabolite, dihydroxyphenylglycol, compared with levels in four control groups: patients with chronic orthostatic intolerance without OH, Alzheimer’s disease, MSA without OH and elderly normal volunteers. These findings are consistent with central noradrenaline deficiency in both MSA + OH and PAF, although, as noted below, theoretically an alternative mechanism can explain low cerebrospinal fluid concentrations of noradrenaline and dihydroxyphenylglycol in PAF.

In marked contrast with the neurochemical findings in the cerebrospinal fluid, the MSA + OH and PAF groups differed clearly in terms of arterial plasma concentrations of noradrenaline and dihydroxyphenylglycol. Patients with MSA + OH had normal levels of these catechols, whereas patients with PAF had low plasma levels of both catechols. These results replicate those published previously in different patient cohorts [6]. Since the groups differed markedly in circulating noradrenaline and dihydroxyphenylglycol levels, yet had virtually identically low levels of both compounds in cerebrospinal fluid, the results were consistent with decreased production of noradrenaline in the central nervous system in these diseases.

The production of methoxyhydroxyphenylglycol from noradrenaline depends on the actions of three enzymes – monoamine oxidase, aldehyde reductase and catechol O-methyltransferase. Low levels of methoxyhydroxyphenylglycol in cerebrospinal fluid therefore are consistent with, but do not necessarily indicate, decreased noradrenaline production. The present finding of low levels of noradrenaline itself in cerebrospinal fluid excludes decreased enzyme activity as an explanation for low methoxyhydroxyphenylglycol levels in patients with MSA [13], and supports the notion of central noradrenaline deficiency.

In patients with PAF the situation is more complex. First, there is the potential complication of an imperfect blood–brain barrier for noradrenaline and the likely absence of a barrier for dihydroxyphenylglycol. Low cerebrospinal fluid levels of noradrenaline and dihydroxyphenylglycol in patients with PAF might then reflect decreased delivery of these catechols from the plasma, and not a central noradrenergic deficiency. Patients with phaeochromocytoma can have increased cerebrospinal fluid noradrenaline levels [23,24], although hypertension can interfere with the blood–brain barrier. On the other hand, hardly any of injected radiolabelled noradrenaline enters most of the central nervous system (at least acutely) [25]. The present findings supported the notion of a highly effective blood–brain barrier for catecholamines, because among control subjects the cerebrospinal fluid adrenaline concentration averaged only approx. 3% of the plasma concentration. Even after adjustment of cerebrospinal fluid levels for a
10% contribution of plasma noradrenaline and a 95% contribution of plasma dihydroxyphenylglycol, the PAF group still had decreased cerebrospinal fluid levels of these catechols (results not shown).

A more difficult issue is whether some of the noradrenaline released from sympathetic terminals might enter the interstitial fluid and then the cerebrospinal fluid space, without entering the bloodstream. For instance, cells of the choroid plexus, the source of cerebrospinal fluid production, possess sympathetic post-ganglionic innervation [26] and actively take up noradrenaline, although this uptake appears to be from the epithelial side [27]. Consistent with a post-ganglionic contribution to cerebrospinal fluid noradrenaline, ganglion blockade decreases cerebrospinal fluid noradrenaline levels in anaesthetized dogs [28], and bilateral superior cervical ganglionectomy decreases cerebrospinal fluid levels of both noradrenaline and dihydroxyphenylglycol in dogs and rats (E. Mamalaki, M. Herkenham and D. S. Goldstein, unpublished work). If an analogous situation occurred in humans, then a generalized loss of sympathetic post-ganglionic innervation in patients with PAF could result in correlated decreases in cerebrospinal fluid and plasma levels of noradrenaline, as in the present study, without necessarily indicating a central noradrenergic deficiency state. No data exist in humans about the actual contributions of sources outside the central nervous system to cerebrospinal fluid levels of either noradrenaline or dihydroxyphenylglycol, and the literature with regard to laboratory animals is inconsistent [28,29]. Accordingly, the present results cannot be taken as providing unequivocal support for central noradrenergic deficiency in PAF.

Patients with MSA in the absence of OH had, if anything, increased cerebrospinal fluid levels of both noradrenaline and dihydroxyphenylglycol, and an increased contribution of the brain to cerebrospinal fluid levels. The database for this group was small, because most patients with MSA also had OH. The findings in patients with MSA in the absence of OH suggest that, in MSA, OH may be related to a central deficiency specifically of noradrenaline. Previous studies have not considered separately MSA patients with and without OH.

Dihydroxyphenylglycol concentrations were highly positively correlated with concentrations of noradrenaline in both cerebrospinal fluid and plasma. In the periphery, under resting conditions, most dihydroxyphenylglycol production results from net leakage of noradrenaline from storage vesicles into the neuronal cytoplasm, by oxidative deamination of noradrenaline that escapes re-uptake into the vesicles. The y-intercept value for the relationship between plasma dihydroxyphenylglycol and noradrenaline concentrations therefore is normally above the origin, as with the control subjects in the present study. The y-intercept value for plasma levels in PAF patients was closer to the origin, as one would expect in the presence of noradrenergic denervation. For cerebrospinal fluid, the y-intercept value for the MSA + OH group seemed also to be above the origin, which may indicate decreased exocytotic release of noradrenaline from intact terminals as the basis for low cerebrospinal fluid noradrenaline levels in this group. The results were too variable in the PAF group to draw any inference.

In all subject groups, the dihydroxyphenylglycol concentration in cerebrospinal fluid substantially exceeded that in arterial plasma. In the periphery, much of the dihydroxyphenylglycol entering the interstitial fluid undergoes uptake by non-neuronal cells, followed by enzymic O-methylation to form methoxyhydroxyphenylglycol, before the dihydroxyphenylglycol can exit the tissue [30]. It is possible that the amount of enzymic O-methylation of dihydroxyphenylglycol is less in the central nervous system than in the periphery, because most central neurons do not appear to contain catechol O-methyltransferase [31].

In conclusion, the conditions of MSA + OH and PAF both feature low cerebrospinal fluid concentrations of noradrenaline and dihydroxyphenylglycol, despite clear differences in plasma concentrations. These findings indicate central noradrenergic deficiency in MSA + OH; however, because of the possibility of a post-ganglionic contribution to cerebrospinal fluid concentrations of noradrenaline and dihydroxyphenylglycol, the low concentrations in PAF do not necessarily reflect central noradrenergic deficiency. In MSA, the presence of OH seems to be related specifically to central noradrenergic deficiency, as a result of decreased exocytotic noradrenaline release from generally intact terminals.

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