Dopamine β-hydroxylase immunoreactivity in human cerebrospinal fluid: properties, relationship to central noradrenergic neuronal activity and variation in Parkinson’s disease and congenital dopamine β-hydroxylase deficiency

Daniel T. O’CONNOR¹,², Justine H. CERVENKA¹,², Richard A. STONE¹,²,³, Gail L. LEVINE¹,², Robert J. PARMER¹,², Rebecca E. FRANCO-BOURLAND⁴, Ignacio MADRAZ⁴, Philip J. LANGLAIS²,³, David ROBERTSON⁶ and Italo BIAGGIONI⁶

Departments of ¹Medicine and ⁴Neuroscience, University of California, San Diego, California, U.S.A., ²Department of Veterans Affairs Medical Center, San Diego, California, U.S.A., ³Departamento de Bioquimica, Instituto Nacional de la Nutricion Salvador Zubiran, Mexico D.F., Mexico, ⁴Departamento de Investigacion Clinica Neurologia y Neurocirugia, Centro Medico Siglo XXI, Instituto Mexicano del Seguro Social, Mexico D.F., Mexico, and ⁶Department of Medicine, Vanderbilt University, Nashville, Tennessee, U.S.A.

(Received 15 March/20 August 1993; accepted 26 August 1993)

1. Dopamine β-hydroxylase is stored and released with catecholamines by exocytosis from secretory vesicles in noradrenergic neurons and chromaffin cells. Although dopamine β-hydroxylase enzymic activity is measurable in cerebrospinal fluid, such activity is unstable, and its relationship to central noradrenergic neuronal activity in humans is not clearly established. To explore the significance of cerebrospinal fluid dopamine β-hydroxylase, we applied a homologous human dopamine β-hydroxylase radioimmunoassay to cerebrospinal fluid, in order to characterize the properties and stability of cerebrospinal fluid dopamine β-hydroxylase, as well as its relationship to central noradrenergic neuronal activity and its variation in disease states such as hypertension, renal failure, Parkinsonism and congenital dopamine β-hydroxylase deficiency.

2. Authentic, physically stable dopamine β-hydroxylase immunoreactivity was present in normal human cerebrospinal fluid at a concentration of 313 ± 1.4 ng/ml (range: 18.5-52.5 ng/ml), but at a 283-fold lower concentration than that found in plasma. Cerebrospinal fluid and plasma dopamine β-hydroxylase concentrations were correlated (r = 0.67, P = 0.001). Some degree of local central nervous system control of cerebrospinal fluid dopamine β-hydroxylase was suggested by incomplete correlation with plasma dopamine β-hydroxylase (with an especially marked dissociation in renal disease) as well as the lack of a ventricular/lumbar cerebrospinal dopamine β-hydroxylase concentration gradient.

3. Cerebrospinal fluid dopamine β-hydroxylase was not changed by the central α₂-agonist clonidine at a dose that diminished cerebrospinal fluid noradrenaline, nor did cerebrospinal fluid dopamine β-hydroxylase correspond between subjects to cerebrospinal fluid concentrations of noradrenaline or methoxyhydroxyphenylglycol; thus, cerebrospinal fluid dopamine β-hydroxylase concentration was not closely linked either pharmacologically or biochemically to central noradrenergic neuronal activity.

4. Cerebrospinal fluid dopamine β-hydroxylase was not changed in essential hypertension. In Parkinson’s disease, cerebrospinal fluid dopamine β-hydroxylase was markedly diminished (16.3 ± 2.9 versus 31.3 ± 1.4 ng/ml, P < 0.001) and rose by 58 ± 21% (P = 0.02) after adrenal-to-caudate chromaffin cell autografts. In congenital dopamine β-hydroxylase deficiency, lack of detectable dopamine β-hydroxylase immunoreactivity in cerebrospinal fluid or plasma suggests absent enzyme (rather than a catalytically defective enzyme) as the origin of the disorder.

5. We conclude that cerebrospinal fluid dopamine β-hydroxylase immunoreactivity, while not closely linked to central noradrenergic neuronal activity, is at least in part derived from the central nervous system, and that its measurement may be useful in both the diagnosis and treatment of neurological disease.
INTRODUCTION

Dopamine β-hydroxylase (DBH; EC 1.14.2.1), the enzyme catalysing the conversion of dopamine to noradrenaline, is stored and released with catecholamines from vesicles in the adrenal medulla and sympathetic axons [1, 2]. DBH is also present in noradrenergic neurons of the brain [3–5] and its activity in cerebrospinal fluid (CSF) has been proposed as a reflection of central noradrenergic neuronal activity [6–9].

In experimental animals, the response of CSF DBH activity and noradrenaline to pharmacological manipulation [6–15] suggests that CSF DBH may change in parallel with central noradrenergic neuronal activity. In man, the significance of CSF DBH is less clear, in part because of the extreme lability (instability) of DBH enzymic activity in human CSF [8, 9, 16–23].

We therefore developed and applied a homologous human DBH r.i.a. to CSF, in order to evaluate the properties and stability of CSF DBH, as well as its relationship to central noradrenergic neuronal activity and disease states such as hypertension and Parkinsonism. Our results suggest that CSF DBH is not closely linked to biochemical or pharmacological indices of central noradrenergic neuronal activity. While CSF DBH correlates with plasma DBH, their concentrations dissociate markedly under circumstances such as renal failure. A diminution of CSF DBH in Parkinson's disease may be of value both in diagnosis and in monitoring therapy.

MATERIALS AND METHODS

Human DBH r.i.a.

Human DBH was isolated from catecholamine storage vesicles of pheochromocytoma as described previously [24, 25], and rabbit polyclonal antisera to human DBH were generated by repeated intradermal immunization [26]. The assay antibody recognizes DBH immunohistochemically in primate (human and monkey) brain noradrenergic axons [3–5].

The human DBH r.i.a. was performed as described previously [26, 27]. DBH immunoreactivity is unaffected even by enzymic inactivation of DBH after chelation of its copper co-factor [26]. For measurements of DBH in CSF, volumes of 100–300 µl were freeze-dried and reconstituted with water at 10–30 µl (that is, 10-fold concentration) before assay. Plasma samples (0.1–10 µl) were measured directly. The lower limit of detection of DBH in this assay (determined at B/B₀ = 80%) is approximately 2 ng/assay tube. The assay has intra- and inter-assay coefficients of variation of 10% and 12% [27].

Human adrenergic tissues

Catecholamine storage vesicles were prepared from fresh pheochromocytoma and normal (autopsy) adrenal medulla as described previously [28]. Sympathetic nerve, dissected from autopsy spleen, was homogenized as described previously [29].

Immunoblotting

To confirm the target of the anti-DBH antibody used in the r.i.a., human chromaffin vesicle soluble core proteins were separated by SDS/PAGE and immunoblotted with the anti-human DBH intact molecule antibody (1:5000 dilution), as described previously [30]. Control antisera were directed against other catecholamine storage vesicle peptides. Anti-chromogranin A was raised as described previously [30, 31] against a chromogranin A N-terminal synthetic peptide (human/bovine [17-tyrosine]chromogranin A 1–17 peptide; LPVNSPMNKGDTEVMKY), while anti-chromogranin B was raised as described in [32] against a chromogranin B N-terminal synthetic peptide (human [16-tyrosine]chromogranin B 1–16 peptide; MPVDRNRNHNEGMVTRY).

DBH activity assay

Heparinized plasma samples (50 µl in duplicate) were assayed for 30 min at 37°C by the spectrophotometric method [33] as previously used by us [24–27]. The results are reported as i.u./l of plasma, where 1 i.u. represents conversion of 1 µmol of tyramine substrate to octopamine product/min. N-Ethylmaleimide (NEM; 30 mmol/l) was included to overcome endogenous inhibitors of DBH activity [33]. This assay was not sufficiently sensitive to detect DBH activity in human CSF [34]. DBH 'homospecific activity' [26, 27, 35] in a given sample is defined as i.u. of DBH enzymic activity/mg of immunoreactive DBH protein (determined by r.i.a.).

DBH inhibition assay

To determine whether some plasma samples (renal failure) contained DBH activity inhibitors not overcome by 30 mmol/l NEM, samples (control and renal failure) were assayed at serial twofold dilutions (from 50 µl down to 6.25 µl) or after 1:1 (25 µl:25 µl) mixing of control and renal failure plasmas.

CSF protocol

CSF was obtained in the morning (07.00–10.00 hours) after an overnight fast in awake subjects by lumbar (L4–L5) puncture, under lidocaine anaesthesia, while subjects were in the lateral decubitus position. Upon emergence from the subarachnoid space, CSF was collected in four separate tubes as ml1, ml2–4, ml5–7 and ml8–10. Only atraumatic (crystal clear, not pink or xanthochromic) CSF
samples were evaluated. CSF was immediately placed on ice after lumbar puncture, then frozen at 
−70°C before assay. A heparinized plasma sample was obtained just before lumbar puncture. Blood pressure [taking the phase V Korotkoff sound (dis-
appearance) as diastolic blood pressure (DBP), on a mercury sphygmomanometer] and heart rate were measured in triplicate in the supine position just before blood and CSF sampling.

Stability in vitro

The stability of CSF DBH immunoreactivity was evaluated in response to the following manipula-
tions: freeze-drying followed by water reconstitution, repeated freezing and thawing (from −70°C to room temperature; up to five cycles), prolonged (up to 6 days) incubation at 37°C, and boiling (100°C for 5 min).

Other assays

Total protein (plasma and CSF) was measured spectrophotometrically [36], using BSA as standard. Noradrenaline was measured radioenzymically [29] in CSF samples that had been collected on to reduced glutathione (final concentration 1 mg/ml). Methoxyhydroxyphenylglycol (MHPG) was mea-
sured by reverse-phase h.p.l.c. with electrochemical detection [37]. Serum creatinine was measured by autoanalyser.

Subjects

Some of these subjects were also studied as part of an evaluation of CSF chromogranin A [37a]. Samples (CSF, or CSF plus plasma) were obtained from the following subject groups.

Normal control subjects (n = 15). These were otherwise healthy normotensive (DBP consistently <90 mmHg) subjects who were about to undergo spinal anaesthesia for elective lower body surgery (herniorrhaphy, transurethral resection of the pro-
state, resection of local transitional cell bladder carcinoma or circumcision). None had a history of cardiovascular or neuropsychiatric disease, renal insufficiency (serum creatinine ≥1.6 mg/dl) or acute urinary retention, and none was on medications known to influence blood pressure or autonomic function. Their blood pressure just before lumbar puncture was 122 ± 3/71 ± 2 mmHg.

Essential hypertension (n = 17). These subjects had a diagnosis of mild to moderate uncomplicated essential hypertension [DBP (Korotkoff sound V) = 95–115 mmHg in the outpatient clinic] after an evaluation that included a normal haemogram, urina-
lysis, serum chemistries [creatinine (≤1.6 mg/dl), urea nitrogen, electrolytes, calcium, phosphorus], and 24 h urine collection (catecholamines, meta-
nephrines, vanillylmandelic acid). They were either previously untreated or had been off anti-
hypertensive medications for at least 2 weeks. Their blood pressure just before lumbar puncture was 149 ± 5/95 ± 2 mmHg.

Renal disease (n = 11). These subjects had either renal insufficiency (serum creatinine ≥2.5 mg/dl; n = 6) preceding the onset of hypertension (DBP > 90 mmHg) or significant (> 50%) unilateral renal artery stenosis on renal arteriogram (n = 5). They had been off all antihypertensive medications for at least 3 days before the study. Their blood pressure just before lumbar puncture was 170 ± 6/91 ± 4 mmHg.

Parkinson's and other neurological disease. Nine Mexican subjects (seven male, two female; age range 34–58 years) with advanced Parkinson's disease refractory to customary medical management were studied with and without L-dopa/carbidopa, before adrenal-to-caudate autotransplantation, as described previously [38]. After surgery, lumbar and ventricu-
lar (via an implanted Ommaya reservoir) CSF, obtained on the same day, were studied serially at 1 month intervals for up to four visits.

Lumbar CSF was also obtained from four Mexican subjects with central nervous system cysticercosis.

Congenital DBH deficiency (n = 2). These pre-
viously described [39] subjects had selective norad-
renergic failure with disabling orthostatic hypoten-
sion as well as no measurable DBH activity, noradrenaline or adrenaline in plasma or CSF [39].

Pharmacological study: central α₂-adrenergic agonist (clonidine)

Clonidine has been used as a tool to diminish central noradrenergic neuronal activity in man [40]. Essential hypertensive subjects (n = 5) were studied at baseline (placebo antihypertensive treatment for 2 weeks) and again after 6 weeks of antihypertensive monotherapy with the central α₂-agonist clonidine, given twice daily at a dosage (0.27 ± 0.04 mg/day) individually titrated to achieve a target DBP of < 90 mmHg. In these subjects, noradrenaline and MHPG were measured in lumbar CSF, in addition to DBH.

Statistics

Results are reported as means ± SEM. Inter-group and intra-group comparisons were made by t-test or analysis of variance, as appropriate. Variables that were not normally distributed were analysed by the non-parametric Wilcoxon signed rank test. Statistical and graphical manipulations were performed on a Macintosh microcomputer with the software packages Statworks and Cricketgraph (Cricket Software, Malvern, PA, U.S.A.).
Fig. 1. Immunoblots of catecholamine storage vesicle soluble core proteins (50 μg) from normal bovine or human pheochromocytoma chromaffin vesicle soluble proteins. The antibody dilutions (v/v) were: anti-DBH, 1:5000; anti-chromogranin A (anti-CgA), 1:500; anti-chromogranin B (anti-CgB), 1:100. The antigens against which rabbit antisera were raised are described in the Materials and methods section. Lane I, size standards; lane 2, bovine adrenal medullary chromaffin vesicle soluble proteins; lane 3, human pheochromocytoma vesicle soluble proteins.

RESULTS

CSF DBH immunoreactivity

The r.i.a. antibody recognized authentic human (although not bovine) DBH (dimer at 150 kDa) on immunoblots of catecholamine storage vesicles (Fig. 1), and showed no cross-reactivity with the other major catecholamine storage vesicle soluble proteins, chromogranins A or B.

The assay detected DBH immunoreactivity in CSF, plasma and adrenergic tissues, as evidenced by parallel displacement of labelled DBH standard from anti-DBH antibody by pure DBH, catecholamine storage vesicles (from pheochromocytoma, adrenal medulla and sympathetic nerve), plasma and CSF (Fig. 2).

CSF DBH immunoreactivity was unchanged by freeze-drying (from 46.1 ± 4.8 to 42.3 ± 4.6 ng/ml, n = 9, P = 0.3), repeated freezing and thawing (from 44.4 ± 4.5 to 49.1 ± 4.2 ng/ml over five freeze/thaw cycles, n = 5, P = 0.617) or prolonged incubation at 37°C (from 52.0 ± 6.3 to 52.3 ± 5.3 ng/ml over 6 days, n = 6, P = 0.45). Boiling (5 min at 100°C) diminished apparent immunoreactivity by 45% (from 36.8 ± 4.7 to 20.2 ± 4.3 ng/ml, n = 4, P = 0.03).

DBH immunoreactivity showed no local concentration gradient during emergence of CSF from the subarachnoid space (Fig. 3).

In 32 subjects without neurological or renal disease, immunoreactive DBH was present in CSF at a concentration of 31.3 ± 1.4 ng/ml (range 18.5–52.5 ng/ml). By contrast, plasma DBH concentration was 9.24 ± 1.11 μg/ml, a value 283 ± 27-fold higher than that in matched CSF samples. DBH immunoreactivity/mg of total protein was also consistently greater (P < 0.001) in plasma (110 ± 14 ng/mg) than in CSF (54.8 ± 2.6 ng/mg). CSF DBH immunoreactivity correlated with plasma DBH immunoreactivity (r = 0.67, n = 22, P = 0.001; Fig. 4a) and
plasma DBH enzymic activity \((r=0.81, n=18, P<0.001; \text{Fig. 4b})\). In turn, plasma DBH enzymic activity correlated with plasma DBH immunoreactivity \((r=0.86, n=15, P<0.001; \text{Fig. 4c})\). Plasma DBH homospecific activity was 3.58 ± 0.42 i.u. of DBH enzymic activity/mg of DBH immunoreactive protein \((n=15)\).

CSF DBH did not correlate with age \((r=0.23, n=32, P=0.135; \text{range, 30–87 years})\).

### CSF DBH and central noradrenergic neuronal activity (Table I)

In five essential hypertensive subjects treated with clonidine monotherapy (Table I), DBP \((P=0.017)\) and heart rate \((P=0.009)\) fell, together with a 53% decline in CSF noradrenaline (from \(310±111\) to \(147±26\) pg/ml, \(P<0.05\)). CSF MHPG also declined marginally (from \(6.74±2.09\) to \(4.89±1.33\) ng/ml, \(P=0.363\)). By contrast, CSF DBH was unchanged (from \(25.7±3.8\) to \(28.8±3.8\) ng/ml, \(P=0.547\)). Plasma DBH and noradrenaline were also unchanged \((P>0.1)\). Lack of decline in plasma noradrenaline after clonidine was an unexpected finding \([40]\), and may perhaps reflect the small number of subjects studied \((n=5)\).

CSF DBH did not correlate with other biochemical measures putatively influenced by central noradrenergic neuronal activity, such as CSF noradrenaline \((r=0.05, n=11, P=0.885)\), CSF MHPG \((r=-0.175, n=18, P=0.124)\), plasma noradrenaline

### Table I. Effects of the central \(\alpha_2\)-agonist clonidine on CSF DBH in essential hypertension \((n=5)\). Results were obtained at baseline (placebo antihypertensive treatment for 2 weeks) and again after antihypertensive monotherapy for 6 weeks with oral clonidine, twice daily, at a dose \((0.27±0.04\) mg/day\) individually titrated to normalize DBP (target DBP < 90 mmHg). Abbreviation: SBP, systolic blood pressure. Values are means ± SEM.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Placebo</th>
<th>Clonidine</th>
<th>(P)</th>
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<tr>
<td>SBP (mmHg)</td>
<td>139 ± 3</td>
<td>131 ± 11</td>
<td>0.48</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>89 ± 3</td>
<td>76 ± 3</td>
<td>0.017</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>76 ± 3</td>
<td>61 ± 3</td>
<td>0.009</td>
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<tr>
<td>CSF DBH (ng/ml)</td>
<td>25.7 ± 3.8</td>
<td>28.8 ± 3.8</td>
<td>0.547</td>
</tr>
<tr>
<td>Noradrenaline (pg/ml)</td>
<td>310 ± 111</td>
<td>147 ± 26</td>
<td>&lt;0.05</td>
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<tr>
<td>MHPG (ng/ml)</td>
<td>6.74 ± 2.09</td>
<td>4.89 ± 1.33</td>
<td>0.363</td>
</tr>
<tr>
<td>Plasma DBH (µg/ml)</td>
<td>13.7 ± 3.8</td>
<td>16.8 ± 7.2</td>
<td>0.48</td>
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<tr>
<td>DBH activity (i.u./l)</td>
<td>31.1 ± 8.1</td>
<td>39.3 ± 10.2</td>
<td>0.257</td>
</tr>
<tr>
<td>Noradrenaline (pg/ml)</td>
<td>199 ± 36</td>
<td>164 ± 24</td>
<td>0.47</td>
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<tr>
<td>Adrenaline (pg/ml)</td>
<td>25 ± 5</td>
<td>21 ± 4</td>
<td>0.04</td>
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Table 2. Effects of renal disease on CSF DBH. Subjects with renal disease (either serum creatinine >2.5 mg/dl, or >50% unilateral renal artery stenosis on arteriogram) are compared with control subjects without neurological or renal disease (serum creatinine ≤1.6 mg/dl). Although all of the subjects with renal disease had hypertension (either systolic blood pressure >140 mmHg, or DBP >90 mmHg, or both), and some of the control subjects had essential hypertension (DBP >90 mmHg), none of the subjects was receiving antihypertensive drugs at the time of lumbar puncture (see Materials and methods section). All of these subjects were male. Values are means ± SEM. Statistical significance: *P < 0.001, **P = 0.017 comparing control and renal disease groups.

<table>
<thead>
<tr>
<th></th>
<th>Normal renal function</th>
<th>Renal disease</th>
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<tr>
<td></td>
<td>(n=32)</td>
<td>(n=11)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 ± 2.4</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Race (White/Black)</td>
<td>28/4</td>
<td>9/2</td>
</tr>
<tr>
<td>Plasma DBH (ng/ml)</td>
<td>31.3 ± 1.4</td>
<td>34.7 ± 5.1</td>
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<tr>
<td>MHPG (ng/ml)</td>
<td>6.85 ± 1.55</td>
<td>9.1 ± 2.8</td>
</tr>
<tr>
<td>Plasma DBH (µg/ml)</td>
<td>9.24 ± 1.11</td>
<td>22.2 ± 3.9*</td>
</tr>
<tr>
<td>DBH activity (i.u./l)</td>
<td>35.2 ± 6.4</td>
<td>44.5 ± 8.3</td>
</tr>
<tr>
<td>DBH homospecific activity</td>
<td>3.58 ± 0.42</td>
<td>2.09 ± 0.25**</td>
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<tr>
<td>CSF DBH (units of DBH enzymic activity/mg of DBH immunoreactive protein)</td>
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<tr>
<td>Noradrenaline (µg/ml)</td>
<td>282 ± 80</td>
<td>617 ± 102***</td>
</tr>
<tr>
<td>Adrenaline (µg/ml)</td>
<td>72 ± 25</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.14 ± 0.05</td>
<td>4.0 ± 1.3*</td>
</tr>
<tr>
<td>Plasma/CSF DBH ratio</td>
<td>283 ± 27</td>
<td>702 ± 137*</td>
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(r = 0.366, n = 12, P = 0.184) or plasma adrenaline (r = -0.154, n = 12, P = 0.633).

CSF DBH in hypertension

CSF DBH concentration was no different (31.7 ± 1.7 versus 30.9 ± 2.15 ng/ml, P = 0.776) in normotensive control subjects (n=15) than in unmedicated essential hypertensive subjects (n=17), despite marked differences in blood pressure (122 ± 3/71 ± 2 versus 149 ± 5/95 ± 2 mmHg; P < 0.001 for both systolic blood pressure and DBP). CSF DBH did not correlate with either systolic blood pressure (r = -0.169, n = 32, P = 0.354) or DBP (r = -0.021, n = 32, P = 0.910). Plasma DBH immunoactivity was also not different between normotensive control subjects and essential hypertensive subjects (8.78 ± 1.42 versus 9.50 ± 1.57 µg/ml, P = 0.767).

CSF DBH in renal disease (Table 2)

CSF DBH was no different in subjects with renal disease compared with control subjects with normal renal function (34.7 ± 5.1 versus 31.3 ± 1.4 ng/ml, P = 0.374), nor did CSF DBH correlate with either serum creatinine (r = -0.027, n = 27, P = 0.894) or the reciprocal of serum creatinine (r = 0.016, n = 27, P = 0.936).

By contrast, plasma DBH was markedly higher in renal disease (22.2 ± 3.9 versus 9.24 ± 1.11 µg/ml, P < 0.001), and plasma DBH concentration correlated inversely with glomerular filtration rate, as estimated by the reciprocal of serum creatinine (r = -0.56, n = 33, P = 0.001), although this correlation depended critically on three subjects with the most advanced renal failure.

Despite higher plasma DBH immunoactivity in renal disease, plasma DBH enzymic activity was not significantly elevated (44.5 ± 8.3 versus 35.3 ± 6.4 i.u., P = 0.388), and the homospecific activity of plasma DBH was markedly diminished in renal disease (2.09 ± 0.25 versus 3.58 ± 0.42 i.u. of DBH enzymic activity/mg of immunoreactive DBH protein, P = 0.015). Indeed, plasma DBH homospecific activity declined as a function of decreasing glomerular filtration rate (r = 0.46, n = 26, P = 0.018).

In renal disease, the correlation of CSF with plasma DBH immunoactivity was weaker (r = 0.52, n = 10, P = 0.128) than that for subjects without renal disease (r = 0.67, n = 22, P = 0.001), and the ratio of plasma to CSF DBH was substantially higher (702 ± 137 versus 283 ± 27, P < 0.001).

To explore whether decreased DBH homospecific activity in renal failure resulted from a relatively inactive enzyme, rather than a uraemic inhibitor of DBH enzymic activity, dilution and mixing studies were performed on plasma DBH enzymic activity. Measured DBH activity was linear from zero to 50 µl (a realistic range of plasma concentrations for enzymic activity measurement in this DBH assay) in both normal and uraemic plasmas. One-to-one mixing studies generated comparable recoveries of DBH activity when comparing mixtures of uraemic/normal plasma (88 ± 2%) and normal/normal plasma (89 ± 8%).

CSF DBH in Parkinson's and other neurological disease

In Parkinson's disease, lumbar CSF DBH was 16.3 ± 2.9 ng/ml (n = 7), significantly (P < 0.001) less than the value in healthy subjects without neurological or renal disease (31.3 ± 1.4 ng/ml, n = 32).

When lumbar CSF DBH was evaluated in paired samples before and after withdrawal of anti-Parkinson medications, CSF DBH did not change (from 17.6 ± 3.5 to 15.1 ± 3.0 ng/ml, n = 5, P = 0.616).

When paired lumbar and ventricular CSF were sampled from the subjects, the values did not vary (13.8 ± 2.8 versus 16.6 ± 2.2 ng/ml, n = 6, P = 0.383).

In nine subjects subjected to adrenalin-to-caudate autograft for treatment of refractory Parkinson's disease, serial sampling of CSF revealed a mean DBH increment of 58 ± 21% after transplantation; the increment was sustained over a 3 month follow-up period (F = 3.93, P = 0.02).

In four subjects with central nervous system cysticercosis, CSF DBH (34.5 ± 9.8 ng/ml) did not differ (P = 0.53) from values in healthy control subjects.
CSF DBH in congenital DBH deficiency

In two subjects with congenital DBH deficiency, DBH activity [33] was not detectable in either CSF or plasma, nor was DBH immunoreactivity detectable in these specimens (up to 300μl of CSF and 50μl of plasma). By contrast, DBH immunoreactivity was easily detectable in comparable volumes of normal CSF or plasma (Fig. 5).

DISCUSSION

Several investigations have measured DBH activity in CSF of experimental animals and humans [6-18, 20-23, 41]. CSF DBH enzymic activity, while readily measurable by radioenzymic activity in humans, is quite unstable and rapidly degraded in vitro [8, 9, 16-23]. By contrast, we found that DBH immunoreactivity was imperturbable to a variety of physical insults, such as freeze-drying, repeated freezing and thawing and prolonged incubation in vitro; only denaturation of the protein by boiling diminished the immunoreactivity.

Two lines of evidence suggest that human CSF DBH does not record changes in central noradrenergic neuronal activity with high fidelity. First, the α2-agonist clonidine (Table 1) did not diminish CSF noradrenaline, as well as blood pressure and heart rate. Others have established clonidine as a valid perturbation of central noradrenergic neuronal activity in humans [40]. Secondly, CSF DBH failed to correlate with other putative biochemical indices of central noradrenergic neuronal activity, such as CSF noradrenaline [8, 9] or CSF MHPG [37, 40].

By contrast, in experimental animals such as the rabbit and cat, CSF DBH does change after pharmacological manipulation of central noradrenergic neuronal activity with α-adrenergic agents [6-10, 13] and intraventricular 6-hydroxydopamine [8, 9]; such responses may be specific for central noradrenergic neurons, as judged by the absence of changes in plasma DBH [8, 9]. In our study, it is conceivable that CSF DBH did not correlate with CSF noradrenaline or MHPG because of differences in diffusion of these substances of very different sizes from synaptic clefts to the lumbar CSF sampling site.

Is CSF DBH derived, at least in part, from plasma DBH, perhaps as a function of diffusion across the blood–brain barrier? Several lines of evidence support this position. First, both we and others [8, 9, 16-18, 20-23, 41] have found that there is far less DBH in human CSF than in plasma. In our healthy control subjects, the mean plasma/CSF DBH immunoreactivity ratio was 283±27 (n=22), and even the DBH/total protein ratio was lower in CSF than in plasma (54.8±2.6 versus 110±14 ng/ml, P<0.001). Even though DBH is a rather large protein with a Stokes radius of 7.4-7.6 nm [24], such proteins do diffuse into the CSF from plasma [42-45]. Based on the size of DBH one can calculate an expected plasma/CSF ratio of 10-2000:1 [42-45] resulting from diffusion alone; this is consistent with the observed 283±27:1 ratio. Secondly CSF DBH correlates (r=0.67, n=22, P=0.001; Fig. 4a) with plasma DBH. Thus, 45% (r²=0.45) of the inter-individual variance in CSF DBH can be accounted for by plasma DBH. Thirdly, clear-cut suppression of central noradrenergic neuronal activity (Table 1) did not affect CSF DBH. Fourthly, some investigators have found that stimulation of a peripheral nerve, such as the sciatic nerve, elevates CSF DBH [46].

In contrast, there are several lines of evidence against a sole or exclusive determination of CSF DBH by plasma DBH concentration. First, at the relatively large size of 7.4-7.6 nm Stokes radius and 300kDa [24, 25], plasma DBH would diffuse at best slowly into CSF. Indeed, the time course of equilibration of even the relatively small protein albumin (Stokes radius 3.6 nm [42-44]) from plasma into CSF is of the order of 24-72h [47]. Secondly, the correlation between plasma and CSF DBH (Fig. 4a; r=0.67) accounts for, at best, 45% (r²=0.45) of the inter-individual variance in CSF DBH, leaving roughly half of the DBH in CSF unexplained. The intersection of the regression line in Fig. 4a with the Y-axis at 22.4 ng of DBH/ml of CSF also suggests that a portion of CSF DBH immunoreactivity is independent of plasma DBH. Furthermore, the correlation of plasma and CSF DBH (r=0.67; Fig. 4a) does not establish that any of the CSF DBH arose from plasma; just as plasma DBH is largely genetically determined [48], so also the same gene(s) might influence central nervous system production and secretion of DBH into CSF. Thirdly, in renal disease, a substantial (2.5-fold) increment in plasma DBH is not accompanied by an increase in CSF DBH (Table 2). Fourthly, the equivalent amounts of DBH we observed in lumbar and ventricular CSF from subjects with Parkinson’s disease (19.0±2.3 versus 18.9±2.1 ng/ml, P=0.954) are unusual, in that the concentrations of most CSF proteins fall 1.5- to 3-fold from lumbar to ventricular fluid [44, 49]. The relatively high ventricular
CSF concentration of DBH may suggest local supratentorial release of DBH. Fifth, Lerner et al. [21] have noted a selective response of human CSF DBH (in the absence of consistent plasma DBH changes) to monoamine oxidase inhibition. Sixth, local introduction of chromaffin cells into the central nervous system in Parkinson’s disease resulted in sustained elevations of CSF DBH (by $58 \pm 21\%$, $P = 0.02$). Seventh, the DBH gene is transcribed in brain, indicating local biosynthesis of the protein [50].

By contrast with man, the relative amounts of CSF and plasma DBH activity are similar in magnitude in experimental animals, such as the rabbit and the cat [6–15], in which CSF DBH clearly changes in response to manipulation of central noradrenergic neuronal activity [6–15]. Perhaps the lack of effect of central noradrenergic neuronal activity manipulation on human CSF DBH (Table 1) is the result of an inordinate (across species lines) influence of plasma DBH on CSF DBH in man. Human plasma DBH is largely determined by heredity [48], and is only slightly influenced by manipulation of peripheral sympathoadrenal activity [48].

In essential hypertension, CSF DBH was not different from control values ($30.9 \pm 2.15$ versus $31.7 \pm 1.7$ ng/ml, $P = 0.776$). Abundant other evidence indicates altered central noradrenergic neuronal activity in essential hypertension [34, 51–53]. However, lack of response of human CSF DBH to changes in central noradrenergic neuronal activity (Table 1) suggests that human CSF DBH will not be informative about central nervous mechanisms in human hypertension. In contrast to our results, Cubeddu et al. [16] found a reduction in CSF DBH activity in essential hypertensives. However, that study [16] differed from ours in several ways: (a) some of the normal control subjects had neurological disease (epilepsy or headaches); (b) the essential hypertensive subjects were older than the normotensive control subjects, and some studies [17, 20] have suggested a change in CSF DBH enzymic activity with increasing age; (c) rather unstable CSF DBH enzymic activity was measured, compared with the physically stable DBH immunoreactivity measured in the present study; (d) the ratio of plasma to CSF DBH was greater than 2000:1, as compared with $283 \pm 27:1$ in our subjects, further suggesting a substantial loss of unstable CSF DBH activity in the former study.

In renal disease, the decline in plasma DBH homospecific activity (Table 2) could represent either a catalytically inactive enzyme, or inhibition of a catalytically normal DBH enzyme. The results of mixing and dilution studies weighed against inhibition, and suggested that a portion of plasma DBH immunoreactivity in renal disease consists of a catalytically inactive enzyme (perhaps the result of degradation, or retention of immunoreactive fragments of DBH).

We found a marked reduction in CSF DBH in subjects with Parkinson’s disease ($16.3 \pm 2.9$ versus $31.3 \pm 1.4$ ng/ml in control subjects, $P < 0.001$), confirming previous findings of reduced DBH activity [22, 54], although not all investigators have found such a diminution [55]. The augmentation of CSF DBH after adrenal-to-caudate chromaffin cell autografts (by $58 \pm 21\%$, $P = 0.02$) may represent either exocytotic secretion by the transplanted cells or a non-specific disturbance in the blood–brain barrier to protein entry after surgical trauma. It is also conceivable that decreased CSF DBH in Parkinson’s disease results from relative immobility with decreased sympathetic activity, and conversely the increase after autograft from increased activity. Nonetheless, the results raise the possibility that CSF DBH can be monitored to follow therapeutic interventions in central nervous system disease. Diminished CSF DBH in Parkinson’s disease may be a diagnostic finding of some specificity, in that CSF DBH is not reported to be consistently diminished in other neuropsychiatric disorders, such as Alzheimer’s disease [55], amyotrophic lateral sclerosis [55], schizophrenia [56], or central nervous system cysticercosis (the present study). However, our patients had especially severe Parkinson’s disease, which prompted the radical therapy of adrenal-to-caudate autograft [38]; it is not clear whether CSF DBH by our assay would be so decreased in less severe cases.

In congenital DBH deficiency, lack of immunologically detectable DBH in either plasma or CSF (Fig. 5) suggests that this syndrome results from absent (or at least greatly diminished) DBH protein, rather than from normal amounts of a catalytically inactive enzyme. Since this DBH r.i.a. relies on a polyclonal antiserum, it is unlikely that a subtle alteration (or mutation) in DBH would negate DBH detection.

In conclusion, authentic, physically stable DBH immunoreactivity is found in human CSF. Its concentration is in part determined by the concentration of circulating plasma DBH, but in part CSF DBH is released from a central nervous system source. Its concentration is not closely linked to the activity of central noradrenergic neurons. While CSF DBH is not changed in hypertension or renal disease, a decline in CSF DBH may be of diagnostic value and a useful tool to follow neural transplant therapy in Parkinson’s disease. Lack of DBH immunoreactivity in congenital DBH deficiency indicates that this syndrome results from absent enzyme, rather than a catalytically inactive enzyme.

**ACKNOWLEDGMENTS**

Dr Ronald P. Frigon developed and Ms Annie Chen performed the DBH r.i.a. We are grateful for the assistance of the nursing staff of the Special Diagnostic and Treatment Unit of the Department of Veterans Affairs Medical Center, San Diego. Dr Juan A. Barbosa assisted with the immunoblot. Drs
REFERENCES


