Clinical Utility of Monoamine Neurotransmitter Metabolite Analysis in Cerebrospinal Fluid

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BACKGROUND: Measurements of monoamine neurotransmitters and their metabolites in plasma and urine are commonly used to aid in the detection and monitoring of neuroblastoma and pheochromocytoma and the evaluation of hypotension or hypertension. Measurements of these neurotransmitters and metabolites can also be helpful in the investigation of disorders that primarily affect the central nervous system, but only when the measurements are made in cerebrospinal fluid (CSF).

CONTENT: I describe CSF profiles of monoamine metabolites in the primary and secondary defects affecting serotonin and catecholamine metabolism. I outline the methods required to analyze these metabolites together with details of specific sample handling requirements, sample stability, and interfering compounds, and I emphasize a need for age-related reference intervals.

SUMMARY: Measured values of monoamine metabolites in CSF provide only a single-time snapshot of the overall turnover of the monoamine neurotransmitters within the brain. Because these measurements reflect the average concentrations accumulated from all brain regions plus the regional changes that occur within the spinal cord, they may miss subtle abnormalities in particular brain regions or changes that occur on a minute-to-minute or diurnal basis. Clearly defined diagnosed disorders are currently limited to those affecting synthetic and catabolic pathways. In many cases, abnormal monoamine metabolite concentrations are found in CSF and an underlying etiology cannot be found. Molecular screening of candidate genes related to steps in the neurotransmission process, including storage in presynaptic nerve vesicles, release, interaction with receptors, and reuptake, might be a fruitful endeavor in these cases.

Peripheral measurements of monoamine neurotransmitters (serotonin and the catecholamines, dopamine and norepinephrine) and their metabolites in either plasma or urine have been used most often to look at mechanisms in hypotension or hypertension, and as screening procedures to detect and monitor neuroblastoma and pheochromocytoma (1–3). Measurement of these neurotransmitters and metabolites in peripheral fluids, although useful for these disorders, rarely has benefit for the investigation of disorders that primarily affect the central nervous system (CNS).1 This review is therefore not designed to cover the use of monoamine and metabolite analysis in the periphery, but rather is limited to the clinical utility of their measurement in cerebrospinal fluid (CSF) as this relates to human disease within the CNS.

Pathways for the synthesis and catabolism of serotonin and the catecholamines are shown in Fig. 1. The major metabolites of these neurotransmitters that appear in human CSF are homovanillic acid (HVA) for dopamine, 5-hydroxyindoleacetic acid (5HIAA) for serotonin, and 3-methoxy,4-hydroxyphenylglycol (MHPG) for norepinephrine; the concentrations of these are thought to reflect the overall turnover of the neurotransmitters within the CNS (4). Measurement of monoamine metabolites in CSF can therefore be used to establish a “snapshot” of serotonin and catecholamine metabolism at one particular time that may provide an indication of disease mechanisms that affect the neurotransmitter pathways.

The monoamine neurotransmitters are involved in the control of a wide variety of neuronal functions. They regulate, among other things, psychomotor function (through involvement in the regulation of motor coordination), reward-driven learning, arousal, processing of sensory input, memory, appetite, emotional stability, sleep, mood, vomiting, sexual behavior, and secretion of anterior pituitary and other hormones.

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Nonstandard abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; HVA, homovanillic acid; 5HIAA, 5-hydroxyindoleacetic acid; MHPG, 3-methoxy,4-hydroxyphenylglycol; BH4, tetrahydrobiopterin; PLP, pyridoxal 5'-phosphate; AADC, aromatic L-amino acid decarboxylase; PNP0, pyridox(am)ine phosphatase oxidase; EC, electrochemical.
In the field of neuropsychiatry, changes in CSF monoamine metabolite concentration have been linked to numerous disease phenotypes including depression (7), Alzheimer’s disease and Parkinson’s disease (8), obsessive compulsive disorder (9), and suicide (10). In these disorders, metabolite changes can be subtle, and often only statistical analysis of large populations allows differentiation from controls. For this reason, the measurement of monoamine metabolites in CSF from these patient populations is not of great clinical utility. Their measurement is of importance, however, for the diagnosis and monitoring of inherited disorders that affect the synthesis, action, and degradation of serotonin and the catecholamines. In these conditions, changes in monoamine metabolites are normally profound, particular patterns may be suggestive for individual conditions, and concentrations of metabolites can be used to monitor treatment outcomes (11).

**PRIMARY INHERITED DISORDERS AFFECTING SEROTONIN AND CATECHOLAMINE METABOLISM**

The synthesis of serotonin and the catecholamines requires participation of 2 cofactors, tetrahydrobiopterin (BH4) for the activity of tyrosine hydroxylase and tryptophan hydroxylase and pyridoxal 5’-phosphate (PLP) for the activity of aromatic L-amino acid decarboxylase (AADC) (Fig. 1). Tetrahydrobiopterin is formed in a 3-step pathway from GTP and involves the activities of GTP cyclohydrolase, 6-pyruvoyltetrahydropterin synthase, and sepiapterin reductase. After its oxidation in the 2 hydroxylase reactions, it is reduced back to the active form by the action of pterin-4α-carbinolamine dehydratase and dihydropteridine reductase (Fig. 1).
Pathogenic mutations have been described in all the known enzymes involved in BH4 metabolism. As these mutations affect synthesis of both serotonin and the catecholamines, concentrations of 5HIAA, HVA, and MHPG are, in general, significantly decreased in CSF. An exception is found in dominantly inherited GTP cyclohydrolase deficiency (dopa-responsive dystonia); in this disorder, it appears that the major impact is on the dopaminergic system, as an isolated decrease in HVA is observed.

Primary defects affecting the AADC gene also lead to decreases in all 3 CSF metabolites, as this enzyme is likewise involved in both pathways. In addition, there are elevations of L-dopa and 5-hydroxytryptophan, which are the substrates for the enzyme. L-Dopa can also be methylated using S-adenosylmethionine as the methyl group donor, and the product, 3-O-methyldopa, accumulates and provides a major indicator for decreased activity of AADC. Pyridox(am)ine phosphate oxidase (PNPO) is required for the maintenance of PLP levels within the CNS, and mutations in this enzyme lead to a deficiency of PLP and consequently to decreased activity of AADC and a similar metabolite pattern. Elevations of CSF threonine and glycine can also be seen, as PLP is required for their catabolism. In both primary AADC deficiency and PNPO deficiency, the accumulating 3-O-methyldopa can be further metabolized via transamination to form vanillactic acid, which can be detected on a urine organic acid screen.

Tyrosine hydroxylase deficiency leads to specific decreases in HVA and MHPG but normal levels of 5HIAA, as serotonin synthesis is unaffected. Mutations in tryptophan hydroxylase have yet to be described, but an isolated decrease in 5HIAA can be predicted. Formation of norepinephrine relies on the activity of dopamine β-hydroxylase. This is a copper-containing enzyme, and deficiency of dopamine β-hydroxylase leads to increased HVA levels due to the accumulation of dopamine and its subsequent conversion to this metabolite. Catabolism of serotonin requires the activity of monoamine oxidase, and low levels of 5HIAA are predicted in this disorder.

Catabolism of the catecholamines requires monoamine oxidase and catechol-O-methyltransferase (Fig. 1), and again deficiencies of these enzymes likely lead to low CSF concentrations of HVA and MHPG. Monoamine metabolite profiles seen, or expected, in these disorders are summarized in Table 1 and indicated in Fig. 1.

**SECONDARY ABNORMALITIES OF SEROTONIN AND CATECHOLAMINES**

It is important to emphasize that changes in CSF serotonin and catecholamine metabolites can occur as a consequence of problems in other areas of metabolism. Secondary changes have been described in posthypoxia, epilepsy, febrile convulsions, liver disease and viral infections, abnormalities of folate metabolism, Lesch Nyhan syndrome, Down syndrome, urea cycle disorders, Rett syndrome, Menkes disease, and deficiencies of arginase and phenylalanine hydroxylase. Isolated low concentrations of HVA have also been found in many neurological conditions where etiology is unclear. This list is not comprehensive but does serve to demonstrate that other possibilities should be considered before making a diagnosis of a primary disorder of neurotransmitter monoamine metabolism.

**Table 1.** Metabolite patterns observed in CSF in the inherited disorders affecting catecholamine and serotonin metabolism.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>HVA</th>
<th>5-Hydroxyindoleacetic acid</th>
<th>MHPG</th>
<th>3-O-methyldopa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorders of BH4 synthesis (recessive)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>GTP cyclohydrolase (dominant)</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Tyrosine hydroxylase</td>
<td>↓</td>
<td>N</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>Tryptophan hydroxylase*</td>
<td>N</td>
<td>↓</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>AADC</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>PNPOb</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Dopamine β-hydroxylase</td>
<td>↑</td>
<td>N</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>Monoamine oxidase</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
</tr>
</tbody>
</table>

N, normal; ↓, decreased; ↑, elevated.

* Predicted.

* This pattern is not always found. Elevations of threonine and glycine can also be seen in PNPO deficiency.
metabolism when abnormal concentrations of CSF metabolites have been found.

Because monoamine metabolite profiles can be nonspecific, an absolute diagnosis of a primary defect in monoamine metabolism requires further investigation. Pituitary prolactin secretion is regulated by neurosecretory dopamine neurons in the hypothalamus that normally inhibit prolactin secretion. A finding of an increased serum prolactin can therefore provide further evidence of a central dopamine deficiency when low CSF HVA concentrations have been found (37). For tryptophan hydroxylase and tyrosine hydroxylase deficiency, genomic sequencing is required for absolute diagnosis, as there is no easily accessible tissue that can be used to measure enzyme activity. The CSF pattern seen in AADC deficiency is almost always diagnostic but may not differentiate between a primary AADC defect or secondary inhibition of the enzyme activity due to lack of PLP; PLP measurement in CSF may allow distinction between the two (18). AADC activity can be measured in plasma, and a finding of low activity is diagnostic for AADC deficiency. In the case of PNPO deficiency, plasma AADC activity can be increased, probably owing to upregulation of apoenzyme synthesis in the absence of the PLP cofactor. Genomic sequencing for AADC and PNPO may be used for final definitive confirmation.

Confirmation of monoamine oxidase-A deficiency is made by measurement of the enzyme activity in dexamethasone-stimulated fibroblasts or by sequencing (21). In patients with dopamine β-hydroxylase deficiency, activity of this enzyme is absent in plasma; however, there is genetically determined interindividual variation in plasma dopamine β-hydroxylase, with 3% to 4% of the normal adult population having near-zero concentrations (38). The disorder can be confirmed by the evaluation of the norepinephrine-to-dopamine ratio in plasma. Normally this ratio is around 10, but in patients with dopamine β-hydroxylase deficiency it is reduced to <0.1 (20). Genomic sequencing is also available (39, 40).

**ANALYTICAL METHODS**

Many different methods have been used to analyze serotonin, the catecholamines, and their metabolites in biological fluids. These include capillary electrophoresis (41), HPLC/MS (42), GC/MS (43), and HPLC with either fluorescence or electrochemical (EC) detection (44, 45). The current methods used to analyze CSF monoamines and metabolites in clinical and research laboratories rely mostly on reversed-phase HPLC coupled with EC detection (19, 46–52). These systems are highly sensitive and selective. Electrochemical detectors are now extremely stable and metabolites can be detected in the femtomole range. Selectivity is clearly demonstrated, as in many procedures CSF can be directly injected onto an HPLC column without prior derivatization of the samples (47, 49, 50).

Methods in general have used isocratic conditions and reversed-phase columns with or without the incorporation of ion pairing in the mobile phase (19, 46–51). Changes in pH and organic modifier (usually methanol or acetonitrile) concentration markedly affect retention times of all metabolites and can be used to obtain optimum separation of any desired components. Accurate measurement of HVA and 5HIAA is easily obtained in all the systems described, but, MHPG is present in relatively small concentrations in CSF, and separation from other compounds can be challenging. The difficulties of measuring this compound accurately were evident in the large interlaboratory variations noted in a pilot quality control program (53). Partial purification of samples before analysis may alleviate this problem (19). Alternately, multielectrode coulometric electrochemical cells in series, with or without gradient elution, can be used to help resolve coeluting compounds by their current/voltage characteristics (54).

As well as the measurement of HVA, 5HIAA, and MHPG, there are occasions when other metabolite analysis may become important. As previously described, blockage of AADC leads to accumulation 3-O-methyldopa, which is the primary accumulating metabolite and, as such, it acts as a marker for this disease. In addition there is accumulation of 5-hydroxytryptophan and l-dopa (17). All of these compounds can be resolved and measured using a simple isocratic system (55) (Fig. 2).

When investigating CSF for a possible defect in monoamine neurotransmitter metabolism, it is critical that one also examine CSF concentrations of the cofactor BH4 and its precursor neopterin. This is also achieved using HPLC with either fluorescence (14) or, in series, EC and fluorescence detection (56). Normally inherited defects in BH4 metabolism are detected at the time of newborn screening, as BH4 is also required for the activity of phenylalanine hydroxylase in the liver and a deficiency of the cofactor leads to hyperphenylalaninemia (14). In dominantly inherited GTP cyclohydrolase deficiency and in defects affecting sepiapterin reductase (the third enzyme in the BH4 synthesis pathway), hyperphenylalaninemia is absent. These conditions can be detected only by abnormal monoamine metabolite and pterin profiles in CSF (57).

Fig. 2 provides example chromatograms obtained from the analysis of CSF from patients with deficiencies of BH4, tyrosine hydroxylase, and aromatic l-amino acid decarboxylase using a simple isocratic reversed-phase HPLC system with coulometric EC detection. This system has been in use for >15 years in our clinical
Fig. 2. Example chromatograms.

(a), Patients with aromatic \( \alpha \)-amino acid decarboxylase deficiency; note the elevations of 3-O-methyldopa, 5-hydroxytryptophan, and \( \alpha \)-dopa and the virtual absence of 5-hydroxyindoleacetic acid and homovanillic acid. (b), Patients with tyrosine hydroxylase deficiency; note the normal concentration of 5-hydroxyindoleacetic acid and the virtual absence of homovanillic acid. (c), Patients with tetrahydrobiopterin deficiency; note the absence of homovanillic acid and 5-hydroxyindoleacetic acid. (d), A patient with normal concentrations of 5-hydroxyindoleacetic acid and homovanillic acid who is receiving acetaminophen (*). (e), A mixture of standard compounds. 1, 3-methoxy-4-hydroxyphenylglycol; 2, \( \alpha \)-dopa; 3, 5-hydroxyindoleacetic acid; 4, homovanillic acid; 5, 3-O-methyldopa; 6, 5-hydroxytryptophan. Chromatographic conditions: 20 \( \mu \)L CSF was injected onto a 250 by 4.6 mm Phenomenex, SpheraClone 5 \( \mu \)m ODS (2) column using an ESA 542 autosampler cooled to 4 °C. Compounds were eluted under isocratic conditions at a flow rate of 1.3 mL/min using an ESA 582 pump and mobile phase consisting of 0.05M potassium phosphate buffer (pH 2.7) containing 1 mmol/L octyl sodium sulfate, 54 \( \mu \)mol/L EDTA, and 14% methanol. Compounds were detected using an ESA Coulouchem III dual electrode electrochemical detector. Electrode 1 was set at \(-0.05\) mV, and compounds were oxidized at electrode 2, which was set at \(+400\) mV.
laboratory. More than 1000 samples can be analyzed without the need to change the analytical column. This system does not allow detection of MHPG, but in most instances where MHPG concentrations might be low, specific measurement of this compound is not required, as changes in HVA concentration are always also observed.

**SAMPLE COLLECTION**

Measurement of neurotransmitter metabolites in CSF is of little value unless the method of collection and sample handling are carefully controlled. Age, height, diet, motility, concentration gradients, site of puncture, diurnal variations, and even season at time of birth have been suggested to affect measured concentrations (58, 59).

Obviously, in the clinical setting, it is not possible to control for all of the above factors, but these confounding issues should be considered if large research studies are being conducted. One of the most important factors in the clinical arena is the rostrocaudal gradient for HVA and 5HIAA within the spinal cord (11, 19, 60, 61). Values double with approximately every 5–10 mL of CSF drawn. For this reason, it is essential that patient data is compared to reference intervals obtained using the same fraction of CSF. Values for MHPG do not vary substantially with increased CSF volume drawn, so they presumably reflect spinal cord
concentrations rather than those within the brain (19). It is necessary that every laboratory define its own specific collection protocol and ensure that samples are then collected appropriately.

It is also necessary to have age-related reference intervals to which results can be compared, as metabolite concentrations are high in the newborn period, rapidly drop during the first few months of life, and then slowly decrease with age (Fig. 3, a and b; Table 2) (47, 62). A clear linear relationship exists between concentrations of 5HIAA and HVA in CSF (19, 63), and this relationship does not change with the fraction of CSF collected. There is, however, a change in slope of the regression line for this relationship between data obtained from individuals 0 to 6 months of age and older than 6 months (Fig. 3, c and d) (19). This change reflects a more rapid age-related drop in 5HIAA than HVA in the first 6 months of life. Provided HVA/5HIAA ratios are compared to the appropriate reference ratios, they can prove useful in detecting abnormalities in monoamine metabolism in situations where CSF has not been collected in an optimal fashion (19).

**SAMPLE STABILITY**

Neurotransmitter metabolites are relatively stable in CSF, but blood contamination during collection of CSF can lead to oxidation of metabolites if the red blood cells are allowed to hemolyze. Blood contaminated samples should, therefore, be centrifuged as soon as possible after sample collection, and the clear CSF should be transferred to a new container before freezing. Metabolite concentrations during analysis are stable for at least 24 h if kept at 4 °C (19), and samples can be frozen and thawed several times without changes in 5HIAA and HVA (64). When stored at <−70 °C, all the metabolites are stable for at least 5 years without a need for antioxidant addition (K.H., unpublished observation).

**QUALITY**

Commercial quality control materials are not available for use in the measurement of CSF monoamine metabolites, but they can easily be prepared by dilution or spiking of CSF. When preparing diluted specimens, it is important to realize that the dilution process decreases the endogenous antioxidants in CSF, which can lead to instability of metabolites. Preparation of quality control materials with low metabolite values should therefore be performed using artificial CSF containing 0.01 g/L ascorbic acid. In the clinical setting, suitable internal standards have not been found to ensure injection integrity. Therefore the use of spiked samples is recommended, where each sample is first run neat and then diluted 50:50 with the external standard and checked for appropriate recovery.

**INTERFERING COMPOUNDS AND DRUG EFFECTS**

Using the HPLC system defined in Fig. 2, I have analyzed >10 000 CSF samples from patients of all ages who have had a wide range of neurological and psychiatric conditions. It is extremely rare to observe other compounds that interfere with the measurement of 5HIAA and HVA. None of the currently used anticonvulsants generate electrochemically active compounds that appear on the neurotransmitter metabolite chromatogram. Acetaminophen elutes at around 5 min but does not affect measurement of the desired compounds (Fig. 2). Occasionally, small peaks that elute close to 3-O-methyldopa may be observed, but small changes in pH can be used to obtain clear separation if measurement of this compound is critical.

It is important to obtain a list of medications at the time of CSF collection. Sinemet (L-dopa with carbidopa) therapy leads to a large accumulation of 3-O-methyldopa. Also, serotonin reuptake inhibitors and certain antidepressants are known to influence CSF metabolite concentrations (65, 66), and it is likely that

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**Table 2. Age-related reference intervals for monoamine metabolites in lumbar cerebrospinal fluid.**

<table>
<thead>
<tr>
<th>Age, years</th>
<th>5-Hydroxyindoleacetic acid</th>
<th>HVA</th>
<th>3-O-methyldopa</th>
<th>l-Dopa</th>
<th>5-Hydroxytryptophan</th>
<th>MHPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.2</td>
<td>208–1159</td>
<td>337–1299</td>
<td>&lt;300</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>95–274</td>
</tr>
<tr>
<td>0.2–0.5</td>
<td>179–711</td>
<td>450–1132</td>
<td>&lt;300</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>52–136</td>
</tr>
<tr>
<td>0.5–2</td>
<td>129–520</td>
<td>294–1115</td>
<td>&lt;300</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>41–71</td>
</tr>
<tr>
<td>2–5</td>
<td>74–345</td>
<td>233–928</td>
<td>&lt;150</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>39–75</td>
</tr>
<tr>
<td>5–10</td>
<td>66–338</td>
<td>218–852</td>
<td>&lt;100</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>37–69</td>
</tr>
<tr>
<td>10–15</td>
<td>67–189</td>
<td>167–563</td>
<td>&lt;100</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>40–72</td>
</tr>
<tr>
<td>Adult</td>
<td>67–140</td>
<td>145–324</td>
<td>&lt;100</td>
<td>&lt;25</td>
<td>&lt;10</td>
<td>35–65</td>
</tr>
</tbody>
</table>

All values are nmol/L. CSF was collected from the first drop, and the first 0.5-mL fraction was used for the analysis of monoamine metabolites.
serotonin and catecholamine agonists and antagonists will affect metabolite concentrations.

Conclusions

The measurement of monoamine metabolites in CSF is not ideal, as it provides a only single snapshot of one time point, and the measured values probably reflect average concentrations accumulated from all brain regions together with the regional changes that occur within the spinal cord. Thus, subtle abnormalities—in particular brain regions or changes that occur on a minute-to-minute or diurnal basis—may be missed. This limits the clinical utility of metabolite measurement in CSF to situations where the monoamine pathways are affected in a global manner leading to large changes in metabolite concentrations. Despite these drawbacks, CSF monoamine neurotransmitter metabolite analysis has become fairly routine in many research laboratories and in specialized clinical laboratories that search for changes that might indicate problems in this area of neurochemistry. Clearly defined diagnosed disorders have been limited to those affecting synthetic and catabolic pathways. The process of neurotransmission, however, requires other steps including storage in presynaptic nerve vesicles, release, interaction with receptors, and reuptake. In most animal models where each of these steps has been knocked out, the intracellular (and presumably extracellular) concentrations of neurotransmitters are profoundly changed (67–71). It is certain that these disorders occur in humans. In many cases, abnormal monoamine metabolite concentrations are found in CSF and an underlying etiology cannot be found. These are likely candidates for which molecular screening of candidate genes might be a fruitful endeavor.

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Review


