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Aromatic L-Amino Acid Decarboxylase Deficiency: Diagnostic Methodology

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Aromatic L-amino acid decarboxylase (EC. 4.1.1.28) deficiency is a newly described inborn error of metabolism that affects serotonin and dopamine biosynthesis. The major biochemical markers for this disease are increases of L-dopa, 3-methoxytyrosine, and 5-hydroxytryptophan in urine, plasma, and cerebrospinal fluid together with decreased cerebrospinal fluid concentrations of homovanillic acid and 5-hydroxyindoleacetic acid. In addition, concentrations of vanillactic acid are increased in the urine. Specific HPLC and gas chromatography–mass spectrometry methods are described that permit the identification and measurement of these metabolites in the above body fluids. Simplified assays for human plasma L-dopa decarboxylase and liver L-dopa and 5-hydroxytryptophan decarboxylase, used to demonstrate the enzyme deficiency, are also reported.

Additional Keyphrases: *chromatography, reversed-phase · gas chromatography/mass spectrometry · heritable disorders · neurotransmitters · dopamine · serotonin · L-dopa*

Recently (*1, 2*), we described the biochemical and clinical features of the first reported cases of deficiency in aromatic L-amino acid decarboxylase (AADC; dopa decarboxylase, hydroxytryptophan decarboxylase, EC 4.1.1.28).[2] This enzyme converts L-3,4-dihydroxyphenylalanine (L-dopa) to dopamine and 5-hydroxytryptophan (5HTP) to serotonin (*3*); lack of the enzyme leads to deficiency of these neurotransmitters and to severe neurological problems (*2*). During our investigations we developed several new methods specifically for diagnosing and monitoring this disease.

Diagnostic markers for AADC deficiency are increases of 5HTP, L-dopa, and 3-methoxytyrosine (3MT) in urine, cerebrospinal fluid (CSF), and plasma (*1, 2*). L-Dopa and 5HTP are the substrates for the enzyme, and the 3MT accumulates after the catechol *O*-methyltransferase-catalyzed methylation of L-dopa (*4*). 3MT has a very long half-life in plasma and CSF, and high concentrations of 3MT accumulate when L-dopa concentrations are increased (*5*). Here we describe methods that allow these metabolites to be measured in a single chromatographic run and provide positive identification of 3MT in plasma and urine from the patients by gas chromatography/mass spectrometry (GC-MS).

The patients, monozygote twins, presented with features of biogenic amine deficiency. Decreased CSF concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA) together with low whole-blood serotonin and plasma catecholamines confirmed a severe deficiency of dopamine and serotonin in both the central and peripheral nervous systems (*1, 2*). The measurement of HVA and 5HIAA in CSF by HPLC with electrochemical detection is standard for investigating neurological diseases of unknown origin in our laboratory (*6*). We have now adapted the assay to detect 3MT in the same chromatographic run that is used to measure the biogenic amine metabolites. The new protocol allows the differentiation of AADC deficiency from other conditions that may lead to decreased turnover of dopamine or serotonin. We also developed a simplified method for the assay of AADC activity in either plasma or liver. The new method allows the detection of dopamine or serotonin (the products of the reaction) in either plasma or liver samples with the only sample cleanup step being the acidic removal of protein. All methods required for the initial detection and positive diagnosis of AADC deficiency are described here.

Materials and Methods

Materials

The following compounds were obtained from Sigma, Poole, UK: 5-HIAA, HVA, 3MT, L- and D-dopa, 5HTP, pargyline, α-methyltyrosine, 4-hydroxy-3-methoxyphenyllactic acid (vanillactic acid, VLA), 4-hydroxy-3-methoxyphenylpyruvic acid (vanilpyruvic acid, VPA), and pyridoxyl 5′-phosphate. Octyl sodium sulfate was obtained from Kodak (Liverpool, UK) and HPLC-grade methanol, *dl-n*-butylamine, and acetonitrile were from BDH (Poole, UK). The acetonitrile used for derivitization reactions was dried over a 5-μm molecular sieve (Sigma). SCX Bond Elut cation-exchange columns were from Analytichem (Harbor City, CA). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) and TriSil/BSA [*N,O*-bis(trimethylsilyl)acetamide in pyridine] were from Pierce Europe B.V. (Oud Beijerland, The Netherlands). All other chemicals were of Analar grade or higher.

Biological Samples

The first 0.5 mL of lumbar CSF collected was frozen on solid CO_2 at bedside. Samples were stored at −70 °C until analyzed. Control CSF samples were from patients with diverse neurological diseases who had no clinical

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[2] Nonstandard abbreviations: AADC, aromatic L-amino acid decarboxylase; L-dopa, L-3,4-dihydroxyphenylalanine; 5HTP, 5-hydroxytryptophan; 3MT, 3-methoxytyrosine; CSF, cerebrospinal fluid; GC-MS, gas chromatography/mass spectrometry; HVA, homovanillic acid; 5HIAA, 5-hydroxyindoleacetic acid; VLA, vanillactic acid; VPA, vanilpyruvic acid; ODS, octadecylsilyl; MTBSTFA, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide; and TBDMS, *tert*-butyldimethylsilyl.

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or biochemical indications of dopamine or serotonin abnormalities. The 24-h urine samples were collected into 6 mol/L HCl and stored at −20 °C. Blood was collected into heparin-containing tubes, and plasma was separated and stored at −70 °C within 15 min. Liver biopsies from the infants were obtained percutaneously and frozen without delay at −70 °C.

Procedures

Measurement of neurotransmitter metabolites in CSF. For HPLC we used a mobile phase of 0.05 mol/L sodium phosphate buffer, pH 2.2, containing, per liter, 220 mL of methanol, 5 mmol of octyl sodium sulfate, and 48 μmol of EDTA; an Isochrom isocratic pump (Spectra-Physics, San Jose, CA), a Rheodyne (Cotati, CA) 7125 injector; and a 25 cm × 4.5 mm (i.d.) Apex 5-μm octadecylsilyl (ODS) reversed-phase column (Jones Chromatography, Llanbradach, UK) protected by a 1-cm-long Spherisorb 5-μm ODS guard column (HPLC Technology, Macclesfield, UK). HVA, 5HIAA, and 3MT were detected by using the second electrode of an ESA 5010 dual-cell electrochemical detector (Bedford, MA) with the analytical electrodes set at +0.05 V and +0.35 V. The gain was set at 1000 and the output (to a Spectra-Physics Chromjet integrator) at 10 mV. The flow rate was 1.3 mL/min and the column temperature was maintained at 35 °C by using a column oven. The system was calibrated by injecting 10 μL of a solution containing 500 nmol of 5HIAA, HVA, and 3MT in 0.1 mol/L HCL.

Measurement of AADC. Incubation procedures for the assay of L-dopa decarboxylase and 5HTP decarboxylase in plasma and liver were adapted from previously described methods (*7, 8*).

Plasma L-dopa decarboxylase. Pyridoxal 5′-phosphate (50 μL of a 0.7 mmol/L solution) and 100 μL of plasma were added to 300 μL of sodium phosphate buffer (167 mmol/L, pH 7.0), containing, per liter, 0.167 mmol of EDTA and 39 mmol of dithioerythritol. The contents were mixed and incubated at 37 °C for 2 h. The assay was started by adding 50 μL of 20 mmol/L L-dopa in 0.01 mmol/L HCl. After 90 min at 37 °C, the reaction was stopped by adding 500 μL of 0.8 mol/L perchloric acid. Blanks were included in which either the L-dopa or the plasma was omitted or in which the L-dopa was replaced by D-dopa. After 10 min the samples were centrifuged in an Eppendorf microfuge, and 25 μL of the supernate was injected onto the HPLC.

Liver L-dopa decarboxylase and 5HTP decarboxylase. Liver samples were thawed and homogenized (1 mg/100 μL wet weight) in 167 mmol/L sodium phosphate buffer containing EDTA (0.167 mmol/L). The assay conditions for L-dopa decarboxylase were the same as for plasma except that the buffer volume was increased to 350 μL and 50 μL of homogenate was used. The preincubation time was 10 min and the incubation time after the addition of L-dopa was 20 min. The reaction was stopped and the supernate was prepared before HPLC as was done for plasma. Liver 5HTP decarboxylase was assayed similarly except that the incubation buffer was 167 mol/L sodium phosphate buffer, pH 8.0, and the reaction was started by adding 50 μL of 5 mmol/L L-5HTP.

Chromatography. For HPLC we used a mobile phase of 0.1 mol/L sodium phosphate buffer, pH 3.45, containing, per liter, 250 mL of methanol, 5 mmol of octyl sodium sulfate, and 48 μmol of EDTA and an SP8770 isocratic pump (Spectra-Physics); the injector, reversed-phase column, and guard column were as described above. Dopamine and serotonin were detected by using an ESA 5011 high-sensitivity dual-cell electrochemical detector operated in the redox mode, with the analytical electrodes set at +0.15 V and −0.3 V. The gain was set between 10 × 100 and 95 × 100, depending on the sample, and the output (to a Spectra-Physics SP 4270 integrator) was set to 10 mV. The flow rate was 1.3 mL/min and the column temperature was maintained at 30 °C for detection of dopamine (45 °C for serotonin) by using a column oven. The system was calibrated by injecting 25 μL of a 1 μmol/L solution of either dopamine or serotonin in 0.4 mol/L perchloric acid.

Measurement of L-dopa, 5HTP, and 3MT in plasma and CSF. Chromatography was performed with a mobile phase of 0.05 mol/L acetate buffer, pH 4.85, containing, per liter, 0.048 μmol of EDTA and 2 μmol of *dl-n*-butylamine; a Spectra-Physics isocratic SP8770 isocratic pump; a Rheodyne 7125 injector; and a 25 cm × 4.5 mm (i.d.) Techsphere 5-μm ODS reversed-phase column protected by a 1-cm-long Spherisorb 5-μm ODS guard column. Detection was by an LS3 spectrofluorometer (Perkin-Elmer Ltd., Beaconsfield, UK) with excitation and emission wavelengths set at 278 and 325 nm, respectively. Peaks were quantified by using a Spectra-Physics SP4270 computing integrator. The flow rate was 1.3 mL/min, and the column temperature was maintained at 35 °C by using a column oven. The system was calibrated by injecting 100 μL of a solution containing, per liter, 0.45 μmol (100 μg) of 5HTP, 1.09 μmol (250 μg) of 3MT, and 1.27 μmol (250 μg) of L-dopa. CSF was injected directly onto the column. Plasma was prepared for chromatography by adding an equal volume of 0.8 mol/L perchloric acid. After 10 min the protein was removed by centrifugation, and 100 μL of the supernate was injected onto the column without delay.

Measurement of L-dopa, 5HTP, and 3MT in urine. We added 20 μL of a 1 g/L solution of α-methyltyrosine to 1 mL of urine as an internal standard. The pH was adjusted to 1.5 with 2 mol/L HCl, and the urine was applied to a 3-mL SCX Bond Elut column that had been preconditioned with 3 mL of methanol followed by 3 mL of 10 mL/L acetic acid. The column was washed with 1 mL of methanol followed by 3 mL of 10 mL/L acetic acid. The retained compounds were then eluted with 10 mL of saturated barium chloride solution into tubes containing 10 mg of ascorbic acid. The eluates were acidified with 50 μL of concentrated HCl and could be stored for at least 1 week at −20 °C until analysis without loss of L-dopa, 5HTP, 3MT, or α-methyltyrosine. Chromatographic conditions were the same as for the analysis of

CSF and plasma samples; 100 μL of barium chloride eluate was injected.

Identification of 5HTP and 3MT in urine and plasma by GC-MS. Amino acids were extracted from the acidified urine by using an SCX column as described above. The column was then washed with 5 mL of water and the amino acids were eluted with 2 mL of 3 mol/L aqueous ammonia and dried under nitrogen. The residue was then redissolved in 100 μL of dichloromethane, dried under nitrogen, and derivatized by dissolving them in 50 μL of acetonitrile, adding 50 μL of MTBSTFA, and heating to 60 °C for 20 min. Amino acids were extracted from plasma by using a modification of the cation-exchange cleanup procedure described by Adams (*9*) and analyzed by GC-MS of their *tert*-butyldimethylsilyl (TBDMS) derivatives (*10*). Plasma samples (300 μL) were acidified with 150 μL of an equivolume mixture of water and acetic acid, supplemented with 38.5 nmol of α-methyltyrosine as internal standard, and passed through an SCX column that had been primed by washing with 5 mL of methanol and 5 mL of 10 mL/L acetic acid. After the column was washed with 30 mL of water, the amino acids were eluted with ammonia and dried and derivatized as described for the urine samples.

GC-MS was performed with an HP 5890 gas chromatograph (with an HP 7673 autosampler) coupled to an HP 5970 mass selective detector and ChemStation data system (all from Hewlett-Packard Co., Palo Alto, CA). The column was a 30 m × 0.25 mm (i.d.) fused-silica capillary column coated with a 0.25-μm-thick chemically bonded DB1 stationary phase (J&W Scientific, Folsom, CA); the carrier gas was helium (2 mL/min). Samples of 1 μL (still in the derivatizing reagent) were injected by using the fast-injection mode of the autosampler into a split/splitless injector heated to 270 °C. After 2 min with the column at 120 °C, the split valve was opened and the column oven was heated by 20 °C/min to 190 °C, then by 15 °C/min to 190 °C, and finally by 20 °C/min to 270 °C. The mass selective detector was used in the scan mode to produce complete mass spectra of chromatographic peaks. For quantification, we used reconstructed ion chromatograms to compare the area of the *m/z* 302 peak from 3-methoxytyrosine with that of *m/z* 316 from α-methyltyrosine.

Measurement of VLA in urine. We diluted 2 mL of urine with 8 mL of water, saturated this with sodium chloride, and adjusted the sample to pH 2.0 with HCl. The phenolic acids (and other organic acids) were extracted by shaking twice with 25 mL of ethyl acetate. After drying, the organic acids were derivatized by using TriSil BSA in pyridine (50 μL); 1 μL was injected into the gas chromatograph (with the injector conditions as above). The column temperature was 80 °C for 2 min, then was increased by 4 °C/min to 300 °C. VLA was also quantified by reversed-phase HPLC with electrochemical detection (*11*).

Results

Measurement of CSF HVA, 5HIAA, and 3MT. We adapted our standard method for measuring biogenic amine metabolites so that we could separate 5HIAA, HVA, and 3MT from other electroactive species in CSF. To establish a reference range, we determined 3MT concentrations in CSF samples taken from 21 children with various neurological diseases (Figure 1). The concentration of 3MT decreases rapidly in the first year of life and then remains relatively constant (<50 nmol/L) up to age 10 years. The CSF 3MT concentrations in the twins were 1650 and 1585 nmol/L at diagnosis at 10 months of age, decreasing to 430 and 330 nmol/L at 18 months of age (Figure 1). Both sets of values greatly exceeded the age-related reference range. Figure 2 shows the chromatograms obtained from standards, normal infant CSF, and CSF from a patient with AADC deficiency. The chromatogram obtained from the AADC sample clearly demonstrates the decrease in HVA and 5HIAA and the large increase in 3MT.

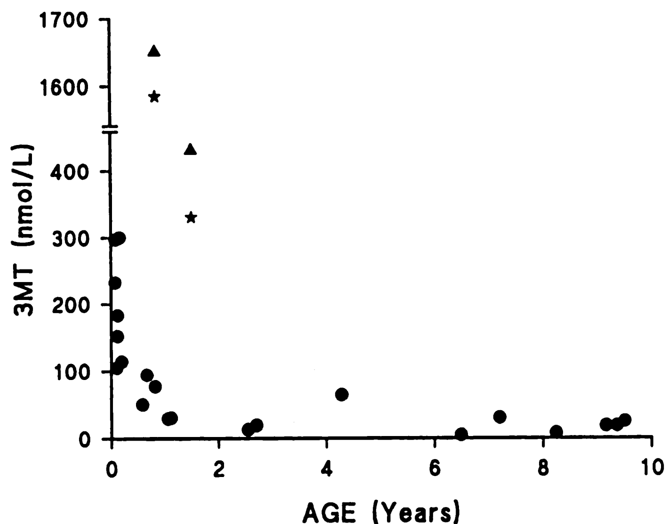

Fig. 1. Effect of age on CSF 3MT concentration
●, control subjects; ▲, ★, infants with AADC deficiency

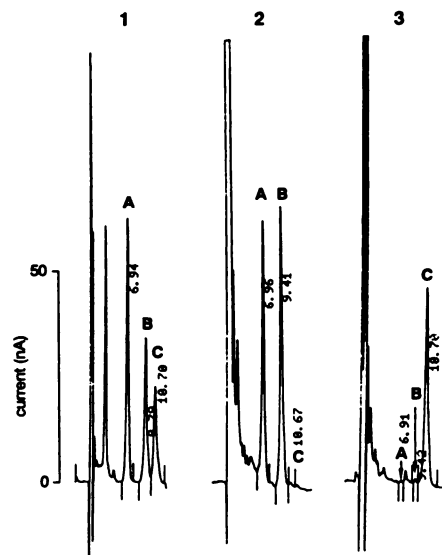

Fig. 2. Electrochemical detection of 5HIAA, HVA, and 3MT in CSF
1, standard, 500 nmol/L each for 5HIAA (*A*), HVA (*B*), and 3MT (*C*); *2*, normal CSF; *3*, CSF from patient with AADC deficiency. Injection volume 10 μL

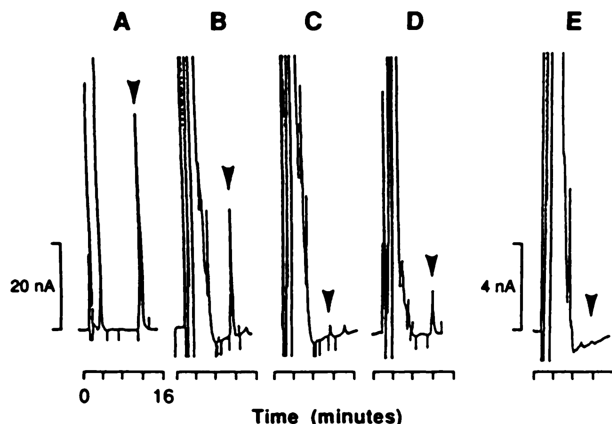

Fig. 3. Chromatograms obtained by using HPLC with electrochemical detection for L-dopa decarboxylase activity in plasma and liver
A, standard, 1 μmol/L dopamine; *B*, control subject's plasma; *C*, patient's plasma; *D*, control subject's liver; *E*, patient's liver. Injection volume 25 μL. ▼ indicates dopamine formed

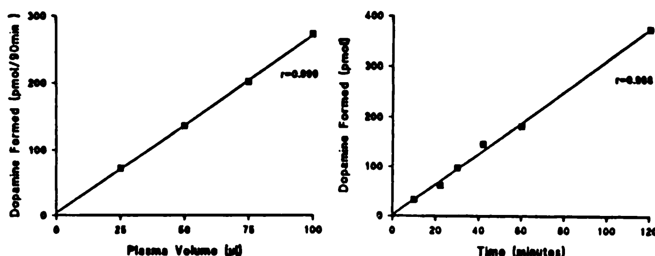

Fig. 4. Effect of plasma volume and incubation time on dopamine formation

Measurement of L-dopa decarboxylase. Conditions were established that allowed baseline resolution of dopamine from the L-dopa substrate and from all other endogenous peaks found in either plasma or liver (Figure 3). Occasionally a contaminant in the L-dopa coeluted with dopamine; however, the contaminant could be separated by a slight adjustment in pH. The activity of L-dopa decarboxylase in human plasma increased linearly with time of incubation for at least 2 h and with plasma volume to at least 100 μL (Figure 4). In liver the activity increased linearly for at least 20 min with at least 200 μg of tissue per incubation (not shown). The minimum detection limit for dopamine was 100 fmol (signal to noise ratio = 3).

The method was used to assay L-dopa decarboxylase activity in plasma and liver from the affected twins. Comparisons of the chromatograms with those obtained from normal plasma and liver are shown in Figure 3. The activity of L-dopa decarboxylase in the patients' plasma at age 17 months was <2% of the reference mean, with the absolute values being 1.23 and 1.15 $nmol \cdot min^{-1} \cdot L^{-1}$ for the twins compared with the control pediatric reference range of 36–128.8 $nmol \cdot min^{-1} \cdot L^{-1}$ (71.1 ± 34.9, mean ± SD; n = 7). Dopa decarboxylase activity in a liver biopsy from one of the twins at age 10 months was 4 pmol/min per milligram of protein, which is <2% of the mean value obtained from control liver biopsies from six infants, ages 4–36 months (mean 344 pmol/min per milligram of protein; range 124–695).

Measurement of 5HTP decarboxylase. The same mobile phase used to separate dopamine from L-dopa was used to separate serotonin from 5HTP. The retention time of serotonin was shortened by increasing the column temperature to 45 °C. Under these conditions, serotonin was resolved from 5HTP and eluted after 14 min (data not shown). The minimum detection limit was 150 fmol injected. The activity of 5HTP decarboxylase in control plasma was very low (~1 $nmol \cdot min^{-1} \cdot L^{-1}$); hence, we did not attempt accurate measurement of this in either the control or the patients' samples. 5HTP decarboxylase activity in control liver ranged from 5.6 to 23.2 pmol/min per milligram of protein (mean 13.1; n = 6). The activity within the patient's sample was below the detection limits of the system.

Adding pargyline (a monoamine oxidase inhibitor), 0.1 mmol/L, to the reaction mixtures had no effect on the final concentration of dopamine or serotonin. For all samples, the analytical recovery of dopamine and serotonin from the perchloric acid extract was essentially 100%.

Measurement of L-dopa, 5HTP, and 3MT. A deficiency of AADC would likely lead to a buildup of the substrates for this enzyme (5HTP and L-dopa) and to an increase of 3MT. The latter has a very long half-life in CSF and plasma and accumulates when L-dopa concentrations increase (*5, 12*). Chromatographic conditions were therefore established for measuring these three metabolites in plasma, CSF, and urine. CSF was injected directly onto the HPLC column. Preparation of plasma required the removal of protein by the addition of an equal volume of 0.8 mol/L perchloric acid. Analysis of urine required a more extensive sample cleanup with a commercially available SCX ion-exchange column. α-Methyltyrosine was used as the internal standard. Absolute recoveries from urine supplemented with 0.4–20 mg/L concentrations of L-dopa, 3MT, 5HTP, and α-methyltyrosine were 85–95%, 87–100%, 91–99%, and 88–100%, respectively. Relative to the internal standard, the recoveries were 88–104% for L-dopa, 96–106% for 3MT, and 97–107% for 5HTP. Within-day variation (CV) of the system was <4.3% (n = 7) and between-day variation was <7.1% for all three metabolites in urine samples containing added L-dopa (20 mg/L), 3MT (20 mg/L), and 5HTP (8 mg/L). Lower detection limits of the system were 1.5 pmol for L-dopa, 1.9 pmol for 3 MT, and 0.2 pmol for 5HTP, and detection response varied linearly with concentration to at least 2.5 nmol of each metabolite (not shown). The concentrations of all three metabolites were much greater in CSF, plasma, and urine from the twins than in similar samples from the control subjects (Figure 5).

Positive identification of metabolites. The identity of 3MT and 5HTP in urine was confirmed by GC-MS of TBDMS derivatives (*10*). Urine from both twins produced a peak similar in size to that of the tyrosine peak and with a retention time and mass spectrum identical with that for reference 3MT (Figure 6). No such peak could be detected in urine from age-matched control subjects. For quantification of 3MT, a linear calibration curve could be obtained by using α-methyltyrosine as the internal

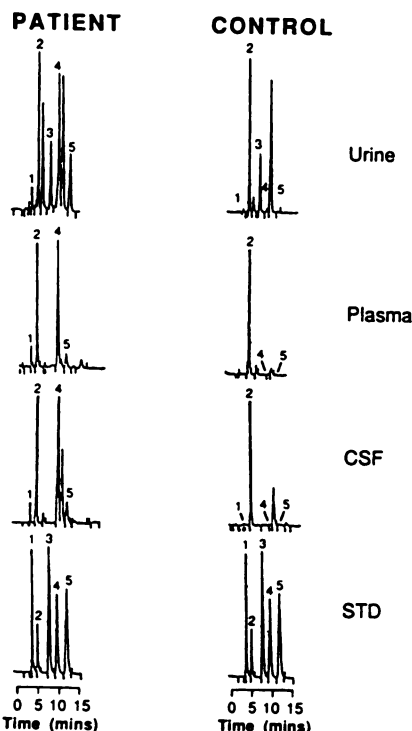

Fig. 5. Chromatograms obtained by HPLC with fluorescence detection of L-dopa, 3MT, and 5HT in standards and in CSF, plasma, and urine from the twins and the control subjects

1, L-dopa; *2*, tyrosine; *3*, α-methyltyrosine (internal standard); *4*, 3MT; and *5*, 5HTP. Injection volume 100 μL

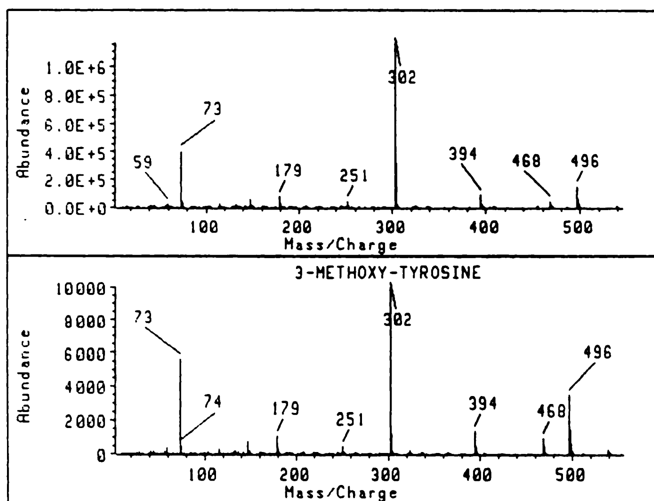

Fig. 6. Electron-impact mass spectrum of the TBDMS derivative of 3MT from the urine of one twin (*upper trace*) compared with the reference compound (*lower trace*)

standard. GC-MS analysis also revealed two small peaks with retention times and mass spectra identical to those produced by the reference 5HTP (data not shown). Mass spectra indicated that in the first peak the indole NH-group was not derivitized; in the second peak, this group was converted to the TBDMS derivative. Confirmation of the identity of L-dopa could not be achieved by the method described because it is labile at the alkaline pH used in the extraction. The presence of 3MT in the twins' plasma was also confirmed by GC-MS, and quantification produced a result similar to that by the HPLC method (data not shown).

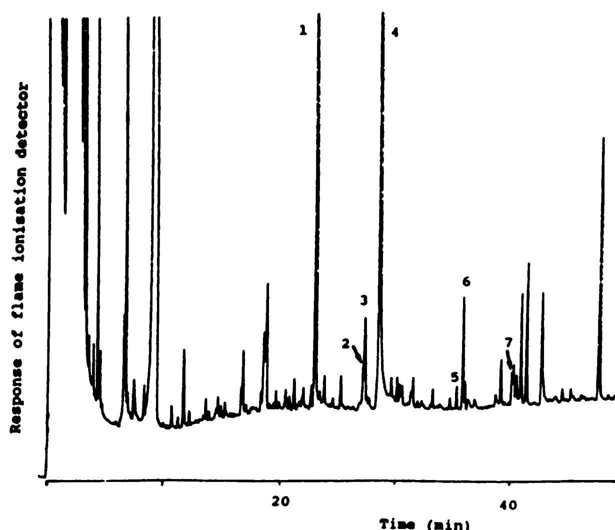

Fig. 7. Urinary organic acids of one twin analyzed by capillary GC after ethyl acetate extraction

Identities indicated by mass spectrometry were: *1*, 4-hydroxyphenylacetic acid; *2*, vanillic acid; *3*, homovanillic acid; *4*, hippuric acid; *5*, vanillactic acid; *6*, palmitic acid; and *7*, 5-hydroxyindoleacetic acid

Transamination of accumulating 3MT could be expected to yield VPA and its reduction product, VLA (*13*). These substances were sought in the urine by using GC-MS. Figure 7 shows the organic acid chromatogram. Mass spectrometry confirmed the identity of the VLA peak; both the compound in the urine and the reference compound showed the following ions: 428 $(M)^+$, 413 $(M - 15)^+$, 338 $(M - 90)^+$, 209, 147, and 73. VPA was not detected. The VLA excretion in control samples as determined by GC-MS was <5% of that of the twins. HPLC measurements of VLA excretion also showed that the twins were excreting considerably more of this compound than were the control subjects (*2*).

Discussion

AADC deficiency is a new inborn error affecting both dopamine and serotonin biosynthesis. Early diagnosis is important, because our clinical studies have demonstrated that the disease may be treatable with agents capable of potentiating monoaminergic function (*1, 2*). The major diagnostic metabolite is 3MT, the methylated product of L-dopa (*4*). This compound is very stable and has a long half-life in plasma and CSF (*5*); in our cases of AADC deficiency, its concentrations in plasma were 5–12 μmol/L (reference range <80 nmol/L) and were 1–1.6 μmol/L in CSF (reference range <50 nmol/L) (*1, 2*). Despite the above-normal concentrations, routine measurements of plasma amino acids may fail to detect the 3MT because the patients' concentrations are near the limits of sensitivity of most standard clinical plasma amino acid analyzers.

The major metabolite of 3MT is VLA (*13*); we were able to show that a standard method of analysis for organic acids in urine can demonstrate the increased concentrations of VLA, thus permitting a tentative

diagnosis of AADC deficiency to be made. Confirmation can then be sought by using one of the special methods described in this paper.

When the twins were admitted, our laboratory was routinely measuring CSF concentrations of HVA and 5HIAA in children with undiagnosed neurological disease, and the initial indication of AADC deficiency was the very low concentrations of these biogenic amine metabolites in CSF in the absence of any abnormality of pterin or aromatic amino acid metabolism (*1, 2*). The chromatographic conditions used for the assay of 5HIAA and HVA did not demonstrate the presence of a large increase in 3MT. The current mobile phase separates all three metabolites and hence provides a rapid technique for discriminating between AADC deficiency and other diseases causing decreased HVA and 5HIAA concentrations. It is important to compare the 3MT concentrations with age-related reference values, because 3MT concentrations drop rapidly during the first 6 months after birth. This pattern is similar to that found for 5HIAA and HVA and probably reflects decreasing aminergic activity during this period (*14*).

CSF is not always available, even in cases of obvious neurological dysfunction; hence methods were developed whereby the diagnosis can be made by using plasma or urine. 3MT, 5HTP, and L-dopa were separated isocratically and detected by their natural fluorescence. The measurement of these compounds in plasma is simple, requiring only the acidic removal of protein before injecting the sample onto the HPLC column. Measurement in urine requires sample cleanup with SCX cation-exchange columns and the addition of an internal standard. Nonetheless, sample preparation still takes only a few minutes.

A clinical history should always be obtained to exclude the possibility that the patient is receiving L-dopa therapy, which also leads to a large increase in 3MT (*5*). Similarly, 3MT concentrations are increased in dopamine β-hydroxylase (EC 1.14.17.1) deficiency; however, these cases do not present with neurological problems in childhood, and there are no deficiencies of serotonin or dopamine (*15*).

Absolute diagnosis of AADC deficiency requires confirmation by enzyme assay. The assay described for measuring plasma and liver L-dopa decarboxylase activity has been greatly simplified compared with previously described procedures (*7, 8*). The activity of L-dopa decarboxylase in plasma is very low and all prior assays have required extensive sample purification to remove the L-dopa substrate that would otherwise obscure the small quantity of dopamine formed. The present method overcomes this problem by the use of redox coulometric electrochemical detection of the dopamine product. This technique effectively screens out the signal from L-dopa and allows the detection of dopamine in plasma, with the only sample cleanup being the acidic removal of protein. This method is also suitable for measuring L-dopa decarboxylase and 5HTP decarboxylase in liver biopsy tissue, and we used it to demonstrate an intracellular deficiency of the enzyme in liver from one of the twins and prenatal diagnosis of an at-risk fetus by analysis of a fetal liver biopsy (*1, 2*).

In conclusion, the analytical procedures described provide a specific series of tests that can be used if AADC deficiency is suspected.

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