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Short Communication

Aromatic Amino Acid Decarboxylase Deficiency in Twins

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Much information about the effects of serotonin and catecholamine deficiency has been obtained from the study of inborn errors of metabolism which affect the biosynthesis of these amines. To date, all of the defects described have been those affecting the synthesis and salvage of tetrahydrobiopterin (BH4), the cofactor required in the rate-limiting biosynthetic steps (Kaufman, 1987). To establish the frequency of abnormal serotonin and catecholamine metabolism in patients presenting with symptoms similar to those of BH4 deficiency, and to ascertain the aetiology, a protocol was developed which allows the metabolism of these neurotransmitter amines to be investigated both centrally and peripherally. The protocol permitted the detection of defective amine synthesis in twins and the localization of the defect to an inborn error of aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28).

CASE REPORT

Male monozygotic twins born to first cousin parents presented at the age of two months with severe hypotonia, developmental delay and 'fits'. The hypotonia was associated with an almost complete absence of spontaneous movements but with normal tendon reflexes. Both twins exhibited oculogyric crises and occasional choreoathetoid movements of the extremities. EEGs were unremarkable and CT and MRI scans showed mild cerebral atrophy only.

Treatment with tranylcypromine (monamine oxidase inhibitor), bromocriptine (dopamine agonist) and pyridoxine (cofactor for AADC) in combination led to striking improvement in tone and spontaneous movements. At 17 months the twins have almost complete head control on pulling to sit. They can feed themselves from a bottle, clap hands in imitation and are saying their first words. They are no longer irritable and have no oculogyric crises. An untreated sibling died at 9 months.

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SAMPLES AND METHODS

Lumbar CSF was collected, stored and analysed for aromatic amino acids, homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and pterins as previously described (Hyland *et al.*, 1985; Howells and Hyland, 1987; Hyland *et al.*, 1988). Whole blood was frozen at the bedside and serotonin measured by HPLC with fluorescence detection (Joseph and Lofthouse, 1987). Plasma was separated within 15 min of venepuncture and stored at − 70°C. 24 h urine was collected into 6 mmol/L HCl and stored at −20°C. Amniotic fluid supernatant was stored at −20°C. Fetal liver was immediately frozen on dry ice and stored at −70°C. Plasma catecholamines were assayed by HPLC with electrochemical detection (Dr D. Perret, London). Plasma and liver AADC activities, and CSF, plasma, amniotic fluid and urine concentrations of L-DOPA, 5-hydroxytryptophan (5-HTP) and 3-methoxytyrosine (3-MT) were analysed by HPLC with electrochemical and fluorescence detection respectively using new methodology to be described elsewhere. Confirmation of the identity of 5-HTP and 3-MT in urine was achieved by extraction of amino acids from acidified urine on a cation exchange resin, elution with 3 mol/L ammonia and analysis by GC–MS of *t*-butyldimethylsilyl (TBDMS) derivatives.

RESULTS AND DISCUSSION

Results from a single twin are presented, as there was essentially no difference between them. A monoamine neurotransmitter deficiency was confirmed by the finding of greatly decreased concentrations of CSF HVA (11 ng/ml, controls 96 ± 13.2), and 5-HIAA (2 ng/ml, controls 54 ± 30.3), plasma noradrenaline (< 0.1 nmol/L, controls 0.46–3.08, adult range) and whole blood serotonin (11.8 ng/ml, controls 279 ± 30). Biopterins, neopterins and aromatic amino acids were normal in CSF and no anatomical lesions were found to explain the amine deficiency. Plasma profiles of vitamin B6 metabolism were normal.

These results suggested the possibility of a deficiency of AADC, the enzyme catalysing the conversion of 5-HTP to serotonin and L-DOPA to dopamine. Further evidence for AADC deficiency was sought by analysis of 3-MT concentrations. 3-MT is the major metabolite of L-DOPA in patients receiving an inhibitor of AADC (Sharpless and McCann, 1971). The concentration of 3-MT was found to be very high in CSF, plasma (Table 1) and urine (results not shown). The presence of raised

Table 1 Plasma and CSF concentrations of L-dopa, 5-HTP and 3-methoxytyrosine (all values expressed as ng/ml)

	L-DOPA	*5HTP*	*3MT*
CSF	60	30.6	378
Control values	< 5	< 2	5.3 ± 1.6
Plasma	257	55	2232
Control values	< 10	< 4	< 20

concentrations of 3-MT in urine was confirmed by GC–MS. High concentrations of L-DOPA and 5-HTP were also found in CSF and plasma (Table 1).

To prove that the defect was in AADC, an HPLC/electrochemical assay for the enzyme was developed. At diagnosis, plasma L-DOPA decarboxylase activity was 7.5% of controls; seven months later this had fallen to 1.7%. To prove intracellular deficiency of AADC, a liver biopsy was performed. Activity in this tissue was 1% of the control mean (Table 2). Liver 5-HTP decarboxylase activity was also deficient (not shown). Mixing experiments provided no evidence for endogenous inhibitors.

Table 2 L-DOPA decarboxylase activity in plasma and liver

Sample	*Age*	*L-DOPA decarboxylase activity* (pmol min^{-1} ml^{-1})
Plasma		
Patient	10 months	5.32
	13 months	3.12
	17 months	1.23
Controls	0.5–36 months	71.1 ± 34.9 (mean ± SD, $n = 7$)
Mother	21 years	5.3
Father	31 years	6.1
Controls	23–45 years	32.0 ± 6.43 (mean ± SD, $n = 8$)
Liver		(nmol min^{-1} (mg protein)$^{-1}$)
Patient	10 months	0.004
Controls	4–36 months	0.344 ± 0.208 ($n = 6$)
Fetal controls	11–24 weeks	1.78 ± 0.75 ($n = 7$)

Treatment with agents designed to stimulate AADC activity (pyridoxine) and potentiate aminergic neurotransmission (tranylcypromine and bromocriptine) led to marked improvement in the twins' condition, confirming that AADC deficiency in the CNS was responsible for the presenting symptoms. Our results support the view that a single enzyme catalyses the decarboxylation of L-DOPA and 5-HTP in the CNS and the periphery in man.

The twins' parents were asymptomatic but had biochemical profiles consistent with their being heterozygous for AADC deficiency. Their plasma AADC activity was 15–20% of controls (Table 2) but they showed only slight, variable elevation of plasma 3-MT (18.9 to 60 ng/ml), L-DOPA (10 to 22 ng/ml) and 5-HTP (2.6 to 14 ng/ml). This is explained by the fact that the decarboxylase step is not rate-limiting. The results also indicate that for prenatal diagnosis, accumulation of 3-MT, 5-HTP and L-DOPA in amniotic fluid or fetal plasma should be used as a back-up for measurement of AADC activity in a fetal liver biopsy. (AADC is high in fetal liver (Table 2), no activity is present in fibroblasts or chorionic villi; 3-MT, 5-HTP and L-DOPA are virtually undetectable in normal amniotic fluid.)

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