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Brain Amino Acid Abnormalities in Pyruvate Carboxylase Deficiency

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Amino acids were measured in several regions of autopsied brain from an infant who died with congenital lactic acidosis due to pyruvate carboxylase deficiency (McKusick 26615), as well as in cerebrospinal fluid (CSF) and plasma of four living infants with this disorder. Glutamine content was greatly reduced in all brain regions, while glutamic acid and proline contents were elevated. The γ-aminobutyric acid (GABA) content was normal in brain. Glutamine concentrations in CSF and plasma were also decreased in the living patients. Glutamine may serve as a pool to provide glutamate and GABA for use as neurotransmitters, and to provide α-ketoglutarate for the tricarboxylic acid cycle when oxaloacetate can no longer be formed directly from pyruvate.

Congenital lactic acidosis in infants can be produced by genetically determined deficiencies of either of two enzymes involved in pyruvate metabolism, pyruvate dehydrogenase (EC 1.2.4.1), and pyruvate carboxylase (EC 6.4.1.1) (Blass, 1983). The most common inherited form of lactic acidosis is that due to pyruvate dehydrogenase deficiency, with a majority of affected infants having a deficiency of the first component of the pyruvate dehydrogenase complex, pyruvate decarboxylase. A minority of patients also have deficiencies of α-keto glutarate dehydrogenase and the branched chain keto acid dehydrogenase complexes (Robinson and Sherwood, 1984). Pyruvate carboxylase deficiency (McKusick 26615) is a second important form of congenital lactic acidosis, and it has further been subdivided into two types. In one (CRM +ve), a biotinylated, cross-reaching pyruvate carboxylase protein can be immunoprecipitated by antipyruvate carboxylase antiserum, while in the second (CRM −ve), no protein corresponding to pyruvate carboxylase can be demonstrated. In addition, (CRM −ve) patients not only have lactic acidosis, but also have elevated plasma concentrations of ammonia, lysine and citrulline (Robinson and Sherwood, 1984).

To our knowledge, no one has yet described the biochemical changes occurring in the brains of infants dying with any form of lactic acidosis, even though psychomotor retardation, seizures, and other neurological abnormalities are regularly present in these patients. We describe here the amino acid abnormalities that we found in autopsied brain of a child who died with pyruvate carboxylase deficiency (CRM +ve) (McKusick 26615) and we call attention to similar abnormalities observed in the cerebrospinal fluid (CSF) of four additional living infants with this form of pyruvate carboxylase deficiency.

MATERIALS AND METHODS

Patients

G.M. and D.M. were Amerindian siblings, described originally as having lactic acidosis due to deficiency of the pyruvate dehydrogenase enzyme complex (Haworth *et al.*, 1976). Later, however, enzyme assays of their cultured fibroblasts showed that their lactic acidosis was instead due to pyruvate carboxylase deficiency (Robinson *et al.*, 1984). G.B. and R.S. were unrelated Amerindian infants with pyruvate carboxylase deficiency who were reported earlier (Haworth *et al.*, 1981). These four patients are respectively patients 2, 3, 5 and 6 in the study of Robinson *et al.* (1984). During life, all four exhibited markedly increased concentrations of alanine and proline in plasma, but they did not have elevated levels of blood ammonia, or of citrulline and lysine in plasma (Robinson *et al.*, 1984). These four patients also excreted greatly increased amounts of pyruvate and α-ketoglutarate in their urine (Haworth *et al.*, 1976, 1981). CSF specimens were obtained from all four infants, and their CSF amino acid abnormalities are reported here.

J.K. was an additional unrelated Amerindian infant not previously described. She had repeated episodes of severe metabolic acidosis and showed delayed neurological development. Her lactic acidosis was also due to pyruvate carboxylase deficiency, as demonstrated by

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enzyme assays of cultured fibroblasts which had $< 5\%$ of the activity in control cell lines. CSF, plasma, and urine were not available to us, but when she died at the age of 3 years, frozen brain was obtained for biochemical study. The interval between the infant's death and the time brain was frozen was 31 h. Histological abnormalities found at autopsy were limited to severe fatty changes in the liver, and cerebral oedema.

Amino acid analyses of plasma and CSF

Concentrations of amino acids in fasting plasma and CSF were determined for the first four lactic acidosis patients, and for suitable infant control subjects, as previously described (Perry *et al.*, 1975). The amino acid analyses were performed on a Technicon automatic amino acid analyser, using a single long cation exchange column and a lithium citrate elution buffer system (Perry *et al.*, 1968). This technique gives an optimal resolution of the wide variety of amino compounds present in physiological fluids, and in particular makes possible accurate quantitation of aspartic acid and glutamic acid, and of their corresponding amides.

Amino acid analyses of brain

Frozen autopsied brain from patient J.K., and from 17 other infants who died from various causes, was dissected at $-10°C$ into various regions. Before and after dissection, all brain specimens were stored at $-70°C$. Weighed specimens were later homogenized and deproteinized in 0.4 M perchloric acid, as described elsewhere (Perry *et al.*, 1971, 1981; Perry, 1982), and amino compounds were then quantitated on the amino acid analyzer using the same chromatographic system as for physiological fluids (Perry *et al.*, 1968).

RESULTS

Amino acid concentrations in CSF and fasting plasma

Table 1 shows the concentrations of four amino acids found during life in pyruvate carboxylase deficiency patients G.M., D.M., G.B. and R.S. Alanine and proline concentrations were always markedly elevated in fasting plasma, and concentrations of glutamic acid were sometimes elevated. Glutamine concentrations in plasma, by contrast, were reduced. Alanine concentrations were markedly elevated in the infants' CSF, while glutamine concentrations were always decreased. Levels of glutamic acid in CSF were usually normal, while those of proline were normal or elevated. Amino acids other than those listed in Table 1 were present in normal concentrations in the four patients' plasma and CSF, and in particular concentrations of citrulline and lysine were never elevated.

Amino acid contents of autopsied brain

Table 2 shows the contents of seven selected amino acids in six different regions of the autopsied brain of patient J.K. and of control infants of roughly similar ages. Levels of some amino acids in infant brain are substantially different from those in adult human brain (Perry, 1982). It was necessary, therefore, to use infants with various brain disorders as controls for patient J.K., since autopsied brain from infants dying without neurological disorders was not available to us, and use of adult non-neurological brain would have been inappropriate. Among the control infants, none had metabolic disorders known to involve abnormalities of the compounds listed in Table 2.

Brain contents of taurine, glutamic acid, and glutamine are not affected by delays between death and the time at which the brain is frozen (Perry *et al.*, 1981). Therefore, the contents of these three compounds found in J.K.'s autopsied brain are likely to have approximated their contents during life. γ-Aminobutyric acid (GABA) content increases rapidly after death in unfrozen human brain, reaching a maximum by 2 h which is stable for at least 96 h (Perry *et al.*, 1981). Since the death-to-freezing intervals for the patient's brain and the control infant brains were 2 h or more, GABA values are properly comparable in Table 2. Contents of proline, alanine, and tyrosine all rise steadily as delays between death and freezing of the brain are prolonged. The control brains used in compiling the data shown in Table 2 had shorter death-to-freezing intervals than did the autopsied brain of patient J.K., but the marked elevation of proline, alanine, and tyrosine contents in the lactic acidosis patient were far greater than could be accounted for by delay in freezing her brain after death.

The data shown in Table 2 indicate that in this form of lactic acidosis, alanine levels are greatly increased in brain, as in plasma and CSF, and that brain proline levels are increased as markedly as in plasma. Glutamic acid, an important excitatory neurotransmitter in brain, was present in clearly increased amounts in all brain regions. The contents of GABA, the key inhibitory neurotransmitter in brain, and of taurine, a possible inhibitory neurotransmitter or neuromodulator, were normal or higher. Tyrosine contents were clearly elevated in the patient's brain. Normally phenylalanine contents increase in autopsied brain at the same rate as tyrosine contents with increasing death-to-freezing intervals (Perry *et al.*, 1981), and tyrosine/phenylalanine molar ratios in autopsied brain are usually less than one. In patient J.K.'s brain, tyrosine/phenylalanine molar ratios were at least 2–3 in all regions examined. Finally, Table 2 shows that the contents of glutamine were markedly reduced in all brain regions, to an extent even greater than we found in the CSF and plasma of living pyruvate carboxylase deficiency patients (Table 1).

No abnormalities were observed in the contents of 30 other amino acids and related compounds measured in patient J.K.'s brain, but not listed in Table 2. In particular, it was impossible to estimate whether or not aspartic acid contents had been reduced in brain during life, since increases in brain aspartate content are very large after long death-to-freezing intervals (Perry *et al.*, 1981), due both to autolysis of proteins and to hydrolysis of *N*-acetylaspartate.

DISCUSSION

It has been suggested (Blass, 1983) that pyruvate carboxylase deficiency can impair the supply of 4-carbon compounds in the brain to prime the

Table 1 Concentrations of amino acids in plasma and CSF

	Fasting plasma				*CSF*			
Patients	*Glutamic acid*	*Glutamine*	*Proline*	*Alanine*	*Glutamic acid*	*Glutamine*	*Proline*	*Alanine*
Control infants	75 ± 37 (20)	548 ± 75 (21)	161 ± 72 (21)	235 ± 93 (21)	1.2 ± 0.8 (21)	504 ± 135 (21)	0.6 ± 1.7 (22)	30.7 ± 9.0 (22)
Patient G.M. (5 weeks–4 months)	111–262	395–499	271–544	741–946	0.7	365	5.7	124
Patient D.M. (2 weeks–14 months)	15–141	306–494	366–528	727–775	0.1–6.2	342–394	0–6.4	92–107
Patient G.B. (6 months)	65	274	500	537	0.3	229	0	93
Patient R.S. (5 months)	75	336	505	1105	0.5	317	3.0	111

Amino acid concentrations are expressed in μmol l^{-1}. Means ± S.D. are shown for control infants, with number in parentheses. Controls ranged from 2 weeks to 12 months in age, and had various illnesses, none of them known to involve the amino acids listed above, Where plasma or CSF were repeatedly sampled in the lactic acidosis patients, the range of values is shown. (Amino acids were quantitated in plasma on four occasions in patients G.M. and D.M., and in CSF on two occasions in patient D.M.)

Table 2 Regional contents of selected amino acids in autopsied brain of patient J.K.

	Frontal cortex		Occipital cortex		Cerebellar cortex		Caudate nucleus		Putamen		Globulus pallidus	
Amino acid	*J.K.*	*Control* (17)	*J.K.*	*Control* (15)	*J.K.*	*Control* (13)	*J.K.*	*Control* (11)	*J.K.*	*Control* (6)	*J.K.*	*Control* (6)
Taurine	3.74	2.07 ± 0.78	4.44	2.23 ± 1.33	8.88	4.97 ± 1.39	5.42	2.18 ± 0.64	6.02	2.69 ± 0.87	5.71	2.35 ± 0.85
Glutamic acid	10.78	6.68 ± 2.55	10.90	6.60 ± 2.79	11.23	6.85 ± 3.06	12.54	8.17 ± 2.89	15.43	7.35 ± 2.06	7.98	3.28 ± 1.08
Glutamine	0.03	4.43 ± 2.54	0.99	5.04 ± 3.24	2.39	5.89 ± 1.71	0.85	4.47 ± 2.05	0.60	4.23 ± 2.11	0.95	4.22 ± 3.02
Proline	1.24	0.27 ± 0.20	1.32	0.34 ± 0.32	2.24	0.43 ± 0.25	1.08	0.31 ± 0.18	1.25	0.28 ± 0.15	1.73	0.25 ± 0.11
Alanine	7.28	1.18 ± 0.72	6.71	1.23 ± 0.65	7.87	1.52 ± 0.72	4.67	1.66 ± 1.23	8.85	1.67 ± 1.06	5.17	1.59 ± 1.46
Tyrosine	0.88	0.17 ± 0.09	0.85	0.19 ± 0.12	0.69	0.25 ± 0.17	0.67	0.19 ± 0.12	0.50	0.15 ± 0.07	0.51	0.15 ± 0.06
GABA	2.32	1.88 ± 0.58	2.42	1.98 ± 0.73	1.55	1.47 ± 0.65	2.81	2.23 ± 0.94	4.13	3.15 ± 1.18	11.19	6.72 ± 1.45

Values are expressed in μmol g wet weight $^{-1}$. Means ± SD are shown for controls, with number of controls in parentheses. Control patients ranged in age from 2 months to 3 years, and died with a variety of illnesses, none of them known to involve the amino acids listed

tricarboxylic acid cycle, and that failure to synthesize oxaloacetate from pyruvate would be expected to lead to a brain deficiency of three putative neurotransmitter amino acids, aspartate, glutamate, and GABA. As explained above, we cannot exclude the possibility that our autopsied patient had a brain aspartate deficiency during life. Neurochemical examination of brain from future pyruvate carboxylase deficiency patients, whose brains have been frozen sooner than 4 h after death (Perry *et al.*, 1981), should answer this question.

However, the present study shows no evidence of any brain deficiency of either glutamic acid or GABA in this form of congenital lactic acidosis. Indeed, glutamate contents in brain were elevated. This is what might have been expected from the greatly increased urinary excretion of α-ketoglutarate we observed in four patients during life (Haworth *et al.*, 1976, 1981), and the elevated concentrations of glutamate sometimes present in plasma (Table 1). The elevated proline concentrations present routinely in plasma and sometimes in CSF, as well as the markedly increased proline contents in all brain regions, were probably secondary to increased levels of glutamate, since glutamic acid and proline are interconvertible in mammalian tissues. The increased tyrosine content observed in the autopsied patient's brain may have resulted from terminal hepatic failure (evident histologically). Tyrosine concentrations were not abnormally high in plasma and CSF of the four infants examined during life.

Puzzling abnormalities in lactic acidosis due to pyruvate carboxylase deficiency which we feel other investigators have not so far adequately explained are the elevations of glutamate and proline in physiological fluids, and the increased urinary excretion of α-ketoglutarate. The deficiency of glutamine which we describe here in plasma and CSF, and most notably in brain, may account for these abnormalities.

Glutamate accumulated in glial cells in the brain is largely converted to glutamine, and glutamine synthetase activity in brain is confined to astrocytes. These glial cells are also rich in activities of glutaminase, which converts glutamine to glutamate, and in the glutamate-metabolizing enzymes, glutamate dehydrogenase and glutamate oxaloacetate aminotransferase, which convert glutamate to α-ketoglutarate (Hertz, 1982). The activity of pyruvate carboxylase is normally high in brain and apparently is confined to glial cells, particularly astrocytes (Shank and Campbell, 1983). Absence of pyruvate carboxylase activity in brain could be compensated for by glutamine, with this amino acid acting as a pool to provide α-ketoglutarate for neurons. α-Ketoglutarate in turn would act as a metabolic source of the glutamate and GABA utilized by neurons for neurotransmission, as well as providing oxaloacetate through the tricarboxylic acid cycle for aspartate synthesis. Thus glutamine stores may be reduced both in brain and in peripheral tissues to replace oxaloacetate which is in short supply as a result of pyruvate carboxylase deficiency, and to keep the tricarboxylic acid cycle running.

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