Fits, pyridoxine, and hyperprolinaemia type II

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Abstract

The rare inherited disorder hyperprolinaemia type II presents with fits in childhood, usually precipitated by infection. A diagnosis of hyperprolinaemia type II and vitamin B_6 deficiency was made in a well nourished child with fits. It is thought that pyridoxine deficiency was implicated in her fits and was the result of inactivation of the vitamin by the proline metabolite, pyrroline-5-carboxylate.

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Hyperprolinaemia type II (McKusick 23591) is a rare autosomal recessive disorder caused by deficiency of Δ^1-pyrroline-5-carboxylate dehydrogenase. The biochemical abnormalities are a 10–15-fold increase in plasma proline, accumulation of pyrroline-5-carboxylate, and increased urinary proline, hydroxyproline, and glycine.[1] Clinical presentation is with convulsions in childhood, usually precipitated by infection. In one reported case these were associated with an erythematous rash. Most affected adults and some children detected by family screening do not have fits and there is probably no association with learning difficulties or developmental delay.[1 2]

The child reported was found to have both hyperprolinaemia type II and vitamin B_6 deficiency from amino acid and organic acid abnormalities when she presented with fits and encephalopathy. We speculate that pyridoxine deficiency was implicated in her fits and that it was caused by inactivation of the vitamin by the proline metabolite pyrroline-5-carboxylate. This would be a unique cause of increased vitamin dependency. It would account for the fits in hyperprolinaemia type II, which are currently unexplained.

Table 1 Diagnostic biochemical investigations

	Value	*Reference range*
Hyperprolinaemia type II		
Plasma (μmol/l) (n = 5)		
Proline	2290–2900	89–281
Hydroxyproline	6–90	< 10
Ornithine	131–145	27–103
Urine (μmol/mmol creatinine) (n = 5)		
Proline	3700–47500	< 13
Hydroxyproline	85–420	< 13
Glycine	1432–4055	< 356
Ornithine	18–33	< 8
N-(pyrrole-2-carboxyl)-glycine	Present	Not detected
Vitamin B_6 deficiency		
Plasma (nmol/l) (n = 2)		
Pyridoxal phosphate	23.5; 22.7	24.3–81.0
Pyridoxic acid	1.0; 2.7	10.9–27.3
Urine (μmol/mmol creatinine) (n = 1)		
Pyridoxic acid	0.4	0.5–2.3
	Urine (μmol/mmol creatinine)	
	Basal (reference)	*Peak*
Tryptophan load test		
Xanthurenic acid	12.4 (0.3–1.5)	3165.9*
Kynurenic acid	2.5 (0.8–3.0)	524.9*
Kynurenine	3.1 (1.1–3.7)	545.5*
2-Aminoacetophenone	Not detected	Present†

Plasma vitamin B_6 was measured by high performance liquid chromatography (Dunn Nutrition Centre, Cambridge; department of clinical biochemistry, Glasgow Royal Infirmary). Tryptophan metabolites and pyridoxic acid were measured in urease treated extracted urine by gas chromatography mass spectrometry with single ion monitoring. Basal values are compared with in house paediatric reference ranges.
*Reference is less than 10-fold increase above basal; †Not reported

Case report

A previously well girl had a febrile convulsion at 18 months. She recovered completely. At 20 months she became sleepy, lethargic, and fed poorly in association with pneumonia. She had three convulsions within 15 hours, two lasting more than five minutes and one 20 minutes. After these, she was encephalopathic with back arching and purposeless movements, areflexic, but breathing normally. An electroencephalogram (EEG) six hours after her fits did not show any discharges; slow activity was quite prominent with spindles appropriate for sleep. On brief arousal, low activities were seen with no excess of slow activity to indicate an overt encephalopathy. However, 24 hours later, when she was awake and not fitting, the EEG was very abnormal (fig 1). Large irregular slow activities (2–3 Hz, 200 μV) mixed with rather repetitive sharp/polysharp waves and occasional spikes were seen over both hemispheres with variable localisation. Computerised brain tomography was normal. She recovered slowly over five days. The EEG became normal. Three months later she had a severe nappy rash for three to four weeks, which was resistant to local treatment. There have been no more fits (see addendum). After demonstrating pyridoxine deficiency, she was treated with 50 mg pyridoxine orally daily from 32 months of age for five weeks, reducing to 10 mg daily long term. At 3 years of age, she is growing along the 50th centile for height, 25th centile for weight, and is neurologically normal except for slight speech delay. She has a good, mixed diet and there have never been feeding or intestinal problems.

Biochemical investigations

Hyperprolinaemia type II was diagnosed from persisting amino acid and organic acid abnormalities (table 1). DNA analysis has excluded the mutation present in a large Irish kindred.[3] Further DNA studies are planned. A skin biopsy for fibroblast enzyme confirmation is not justifiable at present. Novel findings in the acute urine sample were two metabolites of tryptophan: xanthurenic acid and a volatile degradation product of kynurenine, 2-aminoacetophenone. These abnormalities suggested vitamin B_6 deficiency.[4] Plasma

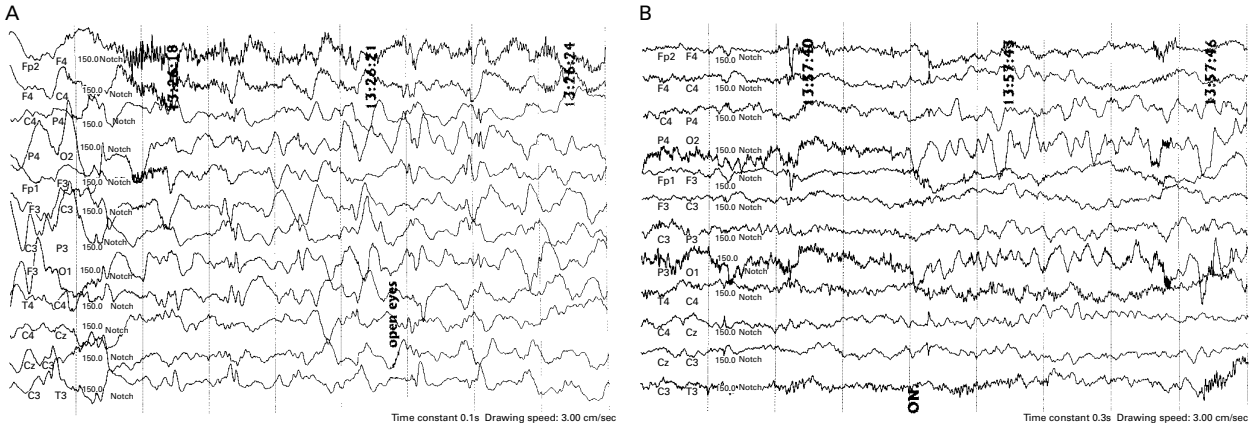

Figure 1 (A) The second electroencephalogram (EEG) 30 hours after admission, which was taken while the patient was awake and not fitting, shows runs of rather repetitive sharp/polysharp discharges mixed with excess slow activity (Δ). (B) Normal EEG on recovery. "On" marks passive eye closure with appearance of posterior rhythmic activity appropriate for age.

analysed by two laboratories when the child was well at 22 months and 28 months showed marginally low pyridoxal phosphate and greatly reduced pyridoxic acid, to concentrations that were barely detectable. Pyridoxic acid is the end product of vitamin B_6 catabolism and low values indicate a deficiency. An oral L-tryptophan loading test (100 mg/kg body weight) was carried out at 28 months of age to stress vitamin B_6 metabolism. Random urine samples were collected for 7.5 hours after loading. Basal excretion of xanthurenic acid was increased and the response to loading was excessive,[5] confirming vitamin B_6 deficiency. In a follow up test after five weeks of 50 mg pyridoxine orally daily, basal urine xanthurenic acid was normal (1.1 μmol/mmol creatinine), as was the post load excretion (9.8 μmol/mmol creatinine). Co-incubation of pyridoxal, pyridoxal phosphate, and pyridoxamine with pyrroline-5-carboxylate at physiological pH in vitro produced new compounds demonstrable with gas chromatography mass spectrometry and nuclear magnetic resonance spectroscopy. Studies to characterise these are in progress (see addendum).

Discussion

Because vitamin B_6 occurs widely in food, symptomatic deficiency is rare. Nutritional deficiency in infants aged 6 weeks to 6 months fed autoclaved cows' milk caused irritability and generalised convulsions with EEG abnormalities. Adults treated with vitamin B_6 antagonists and low pyridoxine diets also had epileptiform convulsions and sometimes a red scaly rash involving the eyes, nose, and perineum.[4] Vitamin B_6 deficiency would explain the fits in our child and might have contributed to the severe nappy rash. The unusual EEG abnormalities in her acute illness resemble those in pyridoxine dependent epilepsy.[6]

Nutritional deficiency was not the explanation here. Alternatives are antagonism or inactivation of one of the physiological forms of vitamin B_6 (pyridoxal, pyridoxine, or pyridoxamine, or their phosphates). Antagonists are generally structural analogues of the B_6 vitamins or agents that complex with them.[7] Pyrroline-5-carboxylate is a reactive compound that accumulates in hyperprolinaemia type II. We propose that it links covalently with one of the B_6 vitamins and inactivates it. Pyridoxal or pyridoxal phosphate are likely candidates because they have a very reactive aldehyde group. Our preliminary studies in vitro indicate that such an interaction is possible. We know of no other instance of an endogenous metabolite causing similar vitamin dependency.

We intend to treat our patient with pyridoxine (10 mg/day) until she is 10 years old when the risk of fits should have decreased.[2] Other children with the defect might also benefit from supplements and their plasma pyridoxal phosphate and pyridoxic acid should be measured.

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Addendum

The child was admitted in December 1999 (aged 4 years 7 months) with a lower respiratory tract infection. She had fits, was unrousable, and encephalopathic as before. She had not been receiving vitamin B_6 at home. She was given 110 mg pyridoxine intravenously in divided doses. She was responsive within 16 hours and was back to normal within 36 hours.

We have now characterised the conjugate of pyridoxal phosphate and pyrroline-5-carboxylate, and are preparing a further [analytical] publication.

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