Porphyrin Precursors in Blood, Urine and Cerebrospinal Fluid in Acute Porphyria

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SUMMARY

Four patients suffering from variegate porphyria were investigated during acute attacks. Porphyrin and porphyrin precursor concentrations were determined in the urine, serum, cerebrospinal fluid (CSF) and stools. Levels of delta-aminolaevulinic acid (ALA) and porphobilinogen were found to be very much lower in CSF than in serum sampled concurrently, and were well below levels at which these substances have been shown to exert effects on neural tissue *in vitro*. These findings cast doubt on the possibility that ALA or porphobilinogen is responsible for the production of the neural manifestations of acute porphyria.

The hereditary hepatic porphyrias have both clinical and biochemical features in common. Patients with these disorders are subject to acute attacks which are generally recognized as being largely neurological or neuropsychiatric in nature. During these attacks excessive amounts of the porphyrin precursors, delta-aminolaevulinic acid (ALA) and porphobilinogen are excreted in the urine. These features have given rise to speculation about possible aetiological relationships between overproduction of porphyrin precursors and the clinical manifestations.[1-3]

Previous experimental studies indicated that both ALA and porphobilinogen were innocuous substances,[1,2] but the question has recently been receiving further attention. On the one hand, ALA and porphobilinogen have now been shown to be capable of affecting neurotransmission *in vitro*,[4-6] while on the other, experiments in intact animals suggest that the blood-brain barrier is relatively impermeable to these compounds.[7,8]

What is the situation in acute porphyria? There is abundant information on the urinary excretion rates of ALA and porphobilinogen during the acute attack, but data on blood, cerebrospinal fluid (CSF) or tissue concentrations of porphyrin precursors are scant.[9-11] We have recently had the opportunity of investigating 4 patients with variegate porphyria during acute attacks, and report our findings in this article.

PATIENTS AND METHODS

Patient 1

A 30-year-old White married woman was admitted to

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Tygerberg Hospital on 29 July 1975. According to information supplied by her general practitioner and family, she was known to suffer from variegate porphyria. The illness had begun about 4 days previously, when she had complained of low backache. For this she received treatment with analgesics and diazepam. On the day before admission she developed acute abdominal pain and her urine became dark in colour. She was brought to hospital the next day after a convulsion and subsequent deterioration in her mental condition.

On admission she was stuporous. No localizing neurological signs were present. The Watson-Schwartz test for urinary porphobilinogen was strongly positive. Her plasma sodium concentration was 114 mmol/l. Symptomatic treatment with Pethilorfan (Roche), chlorpromazine, diazepam and intravenous fluids was instituted.

The patient first came to our notice on the seventh day after admission. At this stage she was still complaining of abdominal pain and was mentally confused. The plasma sodium concentration had returned to normal on the third day. An electro-encephalogram indicated diffuse cerebral changes compatible with a metabolic disturbance. It was repeated a week later but, although it showed improvement, it was still abnormal. Electromyography revealed signs of a minor degree of peripheral neuropathy. By the fifteenth day the patient had improved sufficiently to be discharged. She was subsequently seen as an outpatient, and had apparently made a good clinical recovery.

Patient 2

A 39-year-old White married woman presented with nausea, vomiting, severe abdominal pain and constipation. She was treated symptomatically by her general practitioner. She improved, but about 10 days after the onset of her illness she began to feel weak and noticed that her urine was dark. She was admitted to Tygerberg Hospital on 29 July 1976 with a suspected diagnosis of acute porphyria. No history of previous attacks was forthcoming. However, the patient was aware that one of her brothers had been shown to be porphyric.

On examination she was found to have marked weakness of the trunk and upper and lower limbs. There was no sensory loss. She was fully conscious and well orientated. At this stage her respiration was not embarrassed, but she had difficulty in swallowing, speaking and coughing. The Watson-Schwartz test for urinary porphobilinogen was strongly positive.

During the following few days her condition deteriorated. She became quadriplegic, and from 2 August needed a tracheostomy and assisted ventilation. Supportive therapy and tube feeding of a high-carbohydrate diet were in-

stituted. Other complications requiring treatment at different times were hyponatraemia, diarrhoea, urinary infection and bronchopneumonia. Latterly, a tracheo-oesophageal fistula developed, and was judged unsuitable for surgical repair. Aspiration of gastric contents ensued on several occasions, leading to death on 28 November. At autopsy, bilateral aspiration bronchopneumonia was demonstrated.

Patient 3

The patient, a 30-year-old Coloured woman known to be porphyric, had given birth to a normal infant 1 month before admission to Tygerberg Hospital on 15 January 1977. She had experienced a previous attack of acute porphyria after the birth of her first child 4 years previously. She complained of abdominal pain, constipation, weakness of the limbs and discoloration of the urine.

On examination she was found to have marked weakness of the arms and moderate weakness of the legs and trunk. Her voice was hoarse, but she had no difficulty in swallowing or with respiration. Sensation was normal. She was fully conscious and well orientated. Bullous lesions, scars and hyperpigmentation were evident on the face, the dorsa of the hands and the lower legs. The Watson-Schwartz test for urinary porphobilinogen was strongly positive. She was treated symptomatically with chlorpromazine and placed on a high-carbohydrate diet. Her condition improved steadily, and at the time of discharge on 23 February 1977 residual motor weakness was slight.

Patient 4

A 60-year-old Coloured widow was admitted to Tygerberg Hospital on 29 March 1977. She had been treated by her general practitioner, initially with penicillin and cloxacillin, for cellulitis of the lower legs. Later, co-trimoxazole had been substituted, and 10 days later she had developed weakness of the legs and arms. No family history of porphyria or of previous acute attacks was forthcoming.

On admission she was found to have marked weakness of both lower limbs and a lesser degree of weakness of the arms and trunk muscles. At this stage there was no impairment of breathing or swallowing. The patient was fully conscious and well orientated. Sensation was normal, but she complained of paraesthesiae. A few scars were evident on the dorsa of the hands and lower legs. The Watson-Schwartz test for urinary porphobilinogen was strongly positive.

Two days after admission respiratory embarrassment developed, necessitating a tracheostomy and intermittent positive pressure ventilation. Supportive therapy and feeding of a high-carbohydrate diet were instituted. Other complications included hyponatraemia, uraemia, hypo-albuminaemia and urinary infection. On the fourteenth day after admission the patient became dyspnoeic. X-ray films revealed pulmonary oedema and the electrocardiogram indicated an anterior myocardial infarction. Treatment with diuretics and digitalis produced satisfactory improvement. However, a second episode of acute pul-

monary oedema, accompanied by marked hypotension, occurred on 9 May. Despite resuscitative measures, the patient died shortly afterwards. No improvement in her neurological condition had been noted at any stage.

Laboratory Investigations

Porphyrin precursors in urine were determined according to Mauzerall and Granick,[12] and in serum and CSF by the method of Marver et al.,[13] except that in patients 2, 3 and 4 porphobilinogen in serum and CSF was measured using the procedure of Miyagi et al.[14] Urinary porphyrins were analysed according to Rimington[15] and stool porphyrins according to Holti et al.[16]

RESULTS

Representative figures for urinary and stool porphyrin excretion are given in Table I. The results of serial analyses for porphyrin precursors in urine, serum and CSF are shown in Table II. The findings are consistent with the acute attack of variegate porphyria in all 4 patients.

TABLE I. URINE AND STOOL PORPHYRINS IN PATIENTS WITH ACUTE PORPHYRIA

	Urine		Stool	
	Uro (mg/l)	Copro (mg/l)	Copro (mg/kg — dry wt)	Proto (mg/kg — dry wt)
Patient 1	952	3 173	2 321	2 109
Patient 2	221	1 637	446	1 589
Patient 3	2 596	648	925	603
Patient 4	807	206	31	136

Uro — uroporphyrin; Copro — coproporphyrin; Proto — protoporphyrin.

It was not possible to demonstrate ALA or porphobilinogen in the serum at any stage of the first patient's hospital stay. Likewise, neither porphyrin precursor was detectable in her CSF. Accordingly, it was decided to use the more sensitive method of Miyagi et al.[14] for the analysis of porphobilinogen in serum and CSF specimens for patients 2, 3 and 4. The highest serum level of porphobilinogen (2,77 mg/l) was found on the second day after admission, and the highest level of ALA (1,17 mg/l) on the ninth day, in patient 2. Two lumbar punctures were performed on this patient, the first on the day of admission and the second on the eighth day. The CSF concentration of ALA and porphobilinogen was found to be very much lower (33,3 and 4,5 times respectively) than in serum sampled concurrently. Similar findings were obtained in patients 3 and 4.

DISCUSSION

All 4 patients described had clear-cut attacks of acute porphyria, although the manifestations in patient 1 were somewhat different from those in the other 3. Apart from the abdominal symptoms, patient 1 had pronounced encephalopathy with minimal neuropathy, whereas the

TABLE II. PORPHYRIN PRECURSOR CONCENTRATIONS IN BODY FLUIDS OF PATIENTS WITH ACUTE PORPHYRIA

	Days after admission	Urine		Serum		CSF	
		ALA (mg/l)	PBG (mg/l)	ALA (mg/l)	PBG (mg/l)	ALA (mg/l)	PBG (mg/l)
Patient 1	7			<0,5	<0,5	<0,25	<0,25
	8	38	52	<0,5	<0,5		
	9	37	36	<0,5	<0,5		
	10	33	43	<0,5	<0,5		
	13	89	66	<0,5	<0,5		
	14	84	68	<0,5	<0,5		
	17	31	36	<0,5	<0,5		
Patient 2	1			—	1,81	—	0,25
	2	33	72	—	2,77		
	3	25	50	—	1,72		
	4	7	14	0,40	1,60		
	5	8	48	—	2,41		
	6	16	7	—	1,79		
	7	38	38	0,60	1,24		
	8	52	34	1,13	1,43	0,035	0,32
	9	31	25	1,17	1,61		
	10	7	32	0,74	0,99		
	11	17	14	0,35	0,99		
	12	14	18	0,33	0,60		
	13	8	10	0,25	0,38		
	14	3	3	0,17	0,26		
	15	1	1	0,02	0,05		
Patient 3	5	9	15	—	—		
	6	13	23	0,42	0,98	<0,016	0,07
	7	8	13	0,49	1,00		
	8	15	22	0,61	0,76		
	9	9	14	0,62	0,94		
	10	6	10	0,67	1,13		
	11	6	7	0,28	0,59		
Patient 4	3	13	26	—	—		
	4	39	175	0,63	2,12	<0,016	0,21
	5	10	49	0,23	2,06		
	6	24	95	0,38	1,40		
	7	9	91	0,60	1,99		
	9	10	56	0,70	4,09		
	10	5	39	0,64	5,66		
	11	4	42	0,65	4,71		
	12	5	44	0,47	3,71		

PBG = porphobilinogen.

main feature in the other patients was severe neuropathy without encephalopathy. It is difficult to envisage how these very different clinical presentations could result from elevated levels of porphyrin precursors in the blood. Radical differences between individuals with respect to uptake of circulating ALA and porphobilinogen into the various parts of the nervous system do not seem very likely. On the other hand, it has been suggested that encephalopathy in acute porphyria may be due to water intoxication, possibly resulting from inappropriate secretion of antidiuretic hormone and other factors, and is therefore causally unrelated to the neuropathy. This is a plausible hypothesis, but it is by no means applicable to all cases.[17] It is noteworthy that, of our 4 patients, patients 1, 2 and 4 demonstrated moderate-to-severe hyponatraemia at some stage of their illness, yet encephalopathy was a feature in patient 1 only.

Experimental studies have shown that the blood-brain barrier is relatively impermeable towards ALA and porphobilinogen in normal animals or in animals pretreated with phenobarbitone.[7,8,18] We have found that maximal brain concentrations of ALA and porphobilinogen in rats after intraperitoneal administration are approximately 4 - 8% of maximal blood levels.[7,8] The findings in the present

study suggest that the situation in patients with acute porphyria is comparable. In patients 2, 3 and 4 the CSF: serum ratio for ALA and porphobilinogen ranged from <0,03 to 0,22. Similar results have been reported previously.[10,11]

The concentrations of ALA and porphobilinogen found in the CSF of the 4 patients described were very low, despite the fact that they showed definite involvement of the nervous system at the time of sampling. The maximum concentration of porphobilinogen recorded (patient 2) was 0,32 mg/l (0,0014 mM). At this time the ALA level was 0,035 mg/l (0,0003 mM). These concentrations are well below those required to produce effects on neural tissue in vitro.[4,5,19] Similar findings have been reported by other authors in patients with acute intermittent porphyria.[10,11]

It may be argued that the CSF level does not reflect the level of porphyrin precursors in nervous tissue. This is naturally extremely difficult to evaluate in man, and no reliable information is available at present. Unfortunately, it was not possible to obtain fresh tissues at autopsy from the 2 patients who died. Experimental evidence has shown that ALA or porphobilinogen, when injected intraventricularly in rats, is readily taken up by brain tissue, but does not accumulate there.[7,8] If it is assumed that a partial block in neural haem biosynthesis exists in patients with acute porphyria, as we have suggested,[20] would this not lead to accumulation of ALA and porphobilinogen in the nervous system? Not necessarily! Studies in our laboratory have shown that the activity of the haem biosynthetic pathway in normal mammalian brain is very much less than in liver, and the rate-limiting enzyme, delta-aminolaevulinate synthetase, does not appear to be inducible in brain.[20] Any excess ALA or porphobilinogen produced in neural tissue or entering from the circulation would probably be slight, and if not metabolized, could be expected to diffuse into the extracellular fluid and thence into the CSF.

It must also be pointed out that the intraventricular dosage of these precursors required to produce clinically observable effects in animals is high (2 μmol or more), and the associated brain concentrations are of the order of 0,4 mmol/kg.[8] Furthermore, the effects produced bear little relation to the neuropathic syndromes seen in acute porphyria.[8]

The evidence obtained in the present study, therefore, while not conclusive, casts considerable doubt on the possibility that ALA or porphobilinogen is responsible for the production of the neurological manifestations of acute porphyria. In our view it is more probable that the symptomatology of the acute attack is due to a metabolic defect in haem biosynthesis in neural tissue, which is aggravated in some way by precipitating factors, such as barbiturate drugs. This hypothesis has been fully discussed elsewhere[20] and consequently will only be briefly recounted here. It is suggested that the metabolic, presumably enzymic, defect in the hereditary hepatic porphyrias is present within all cells, including those of the nervous system. Under certain conditions, such as administration of drugs known to precipitate acute attacks, a functional deficiency of haemoprotein in the neurons or glial cells or both develops and, if severe enough, leads to degeneration and death of cells. This would become manifest initially as disturbed neural function and ultimately as axonal degeneration with secondary demyelination, or primarily as demyelination, depending upon whether neurons or glial cells were most affected. Further studies based on the latter hypothesis promise to be most rewarding in elucidating the mysteries of the acute attack in the hereditary hepatic porphyrias.

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