Pathogenesis of Neural Manifestations in Acute Porphyria

B. C. SHANLEY, V. A. PERCY, A. C. NEEFHLING

SUMMARY
At least 4 possible mechanisms may be postulated to explain the neural manifestations of acute porphyria in the hereditary hepatic porphyrias. These are: (i) excessive amounts of porphyrins or porphyrin precursors produced in the liver during acute attacks are transported to the central and peripheral nervous system, where they exert neurotoxic effects; (ii) unidentified metabolites of the aforementioned compounds may be responsible; (iii) in patients with these diseases there may be a metabolic defect in neural haem biosynthesis which is aggravated by precipitating factors, thereby leading to acute neural manifestations; and (iv) the hepatic and nervous system lesions may be metabolically quite unrelated. Each of these possibilities is considered, and evidence is adduced that a genetic defect in haem biosynthesis in the nervous system is the most plausible hypothesis.


The hereditary hepatic porphyrias, viz. acute intermittent porphyria, variegate porphyria and hereditary coproporphyria, are well recognized as diseases in which there is disordered liver haem biosynthesis and a predisposition to develop acute crises of a neurological or neuropsychiatric nature, or both. These acute attacks can be life-threatening; yet, unfortunately, virtually nothing is known of the underlying disturbance of neural function.

A number of hypotheses concerning the pathogenesis of the acute neurobiological disturbances deserve consideration. Firstly, porphyrins or porphyrin precursors which are overproduced in the liver during acute attacks may be transported to the central and peripheral nervous systems and may exert neurotoxic effects there. Alternately, unidentified metabolites of the aforementioned compounds may be responsible. Thirdly, there may be a metabolic defect in neural haem biosynthesis which is aggravated by precipitating factors, thereby leading to acute neural manifestations. Fourthly, the hepatic and nervous system lesions may be metabolically quite unrelated. These possibilities will be discussed, commencing with the least probable and proceeding ultimately to show why a genetic defect in neural cell haem biosynthesis is the most plausible theory.

UNRELATED HEPATIC AND NEURAL LESIONS
This is patently the least attractive hypothesis, since it is not readily testable, while the other 3 do afford opportunities for experimentation. Furthermore, the fact that chemical compounds such as barbiturates, which are known precipitating agents of the acute neurological disorder, also affect hepatic haem biosynthesis, suggests some metabolic link between liver and nervous system dysfunction in this group of diseases.

POSSIBLE NEUROTOXICITY OF PORPHYRINS OR PORPHYRIN PRECURSORS
The simplest and most convenient explanation for the neurological manifestations in acute porphyria is undoubtedly that one or more of the haem precursors which are overproduced in the liver during acute crises, is neurotoxic. This proposal was the first examined during the 1950s by Goldberg and Rimington and by Jarrett and co-workers. Their studies appeared to exclude 5-aminolevulinic acid (ALA), porphobilinogen (PBG) and porphyrins as possible toxic agents.

Interest in this postulate has recently been revived, following the demonstration in vivo that: (i) ALA and PBG can cause presynaptic inhibition of neurotransmitter release; (ii) ALA is an inhibitor of K+-dependent ATPase in brain; and (iii) ALA and PBG exert an inhibitory effect on monosynaptic reflex activity in the isolated frog spinal cord and can decrease the resting membrane potential of frog sartorius muscle.

No studies to date have shown, however, that ALA or PBG can penetrate the blood-brain barrier sufficiently to achieve concentrations in brain tissue or in cerebrospinal fluid (CSF) which approximate those necessary to cause the abovementioned inhibitory effects. Experiments in our laboratory have clearly shown that, in adult and weanling rats, maximal brain concentrations of ALA and PBG following intraperitoneal administration are approximately 4 - 8% of maximal blood levels.

These findings are consistent with the fact that no marked behavioural, neurological or electrophysiological disturbances have been noted following intraperitoneal injection of ALA or PBG in rats and mice. There are few reports of porphyrin precursor concentrations in CSF of patients with acute porphyric attacks. Available data are summarized in Table I. It is noteworthy that CSF levels of porphyrin precursors during acute attacks have been found to be much lower (0 - 22%) than those in blood sampled concurrently. The highest value recorded for ALA in CSF is 280 μg/100 ml and for PBG it is 79 μg/100
TABLE I. CONCENTRATIONS OF PORPHYRIN PRECURSORS IN BLOOD AND CSF OF PATIENTS WITH NEUROLOGICAL AND/OR PSYCHIATRIC MANIFESTATIONS OF ACUTE PORPHYRIA

<table>
<thead>
<tr>
<th>Authors</th>
<th>Diagnosis</th>
<th>ALA (µg/100 ml) Blood</th>
<th>PBG (µg/100 ml) Blood</th>
<th>ALA (µg/100 ml) CSF</th>
<th>PBG (µg/100 ml) CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldberg²</td>
<td>AIP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sweeney et al.²⁵</td>
<td>AIP</td>
<td>2 400</td>
<td>-</td>
<td>280</td>
<td>14</td>
</tr>
<tr>
<td>Bonkowsky et al.¹</td>
<td>AIP</td>
<td>91</td>
<td>363</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>Bonkowsky et al.¹</td>
<td>AIP</td>
<td>38</td>
<td>393</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Percy and Shanley³</td>
<td>VP</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;25</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Percy and Shanley³</td>
<td>VP</td>
<td>113</td>
<td>143</td>
<td>3.5</td>
<td>32</td>
</tr>
</tbody>
</table>

ND = not detectable; AIP = acute intermittent porphyria; VP = variegate porphyria.

These CSF concentrations are below those required to produce the inhibitory effects on neural tissue in vitro.²⁰,²¹,²²

ALA and PBG, when introduced into the nervous system in animals by intraventricular injection, are taken up by brain tissue and are metabolized.²²,²⁶ There is no evidence that porphyrin precursors, once having entered neural tissue, accumulate there. Behavioural effects follow intraventricular injection of 393-655 µg ALA or 452 µg PBG in adult rats.²⁵ These effects are excitatory and transient in nature and cannot be equated with the clinical manifestations of acute porphyria.

**METABOLITES OF HAEM PRECURSORS**

If haem precursors themselves are innocuous, could not one or other of these compounds give rise to toxic metabolites under conditions prevailing during acute attacks of porphyria? It has been well shown that certain compounds, such as fluroxene, which are otherwise innocuous, give rise to highly toxic derivatives following metabolism by hepatic microsomal cytochrome P-450.²⁶ Synthesis of this microsomal enzyme in the liver is greatly enhanced by many drugs, particularly those such as barbiturates, which are known to play a role in the precipitation of acute porphyria. It is just conceivable that induction of hepatic microsomal cytochrome P-450 in patients with the hereditary hepatic porphyrias may lead to the production of neurotoxic metabolites from ALA or PBG.

However, recent studies in our laboratory²³ in which young adult rats were treated with two intraperitoneal injections per day of ALA (250 mg/kg) with or without phenobarbitalone (50 mg/kg) over a period of 3 days, revealed no observable behavioural effects other than those attributable to phenobarbitalone alone.

**DEFECT IN NEURAL HAEM BIOSYNTHESIS**

The porphyrias have long been considered to be divisible into two major groups, viz. the erythropoietic and the hepatic porphyrias, according to the site of disordered haem biosynthesis. It is now realized that this classification is inadequate. Erythropoietic protoporphyria, for example, is currently looked upon as an erythrohepatic disease as opposed to a purely erythropoietic disorder. Similarly, it has been found that patients with acute intermittent porphyria have decreased levels of uroporphyrinogen I (URO I) synthetase, not only in hepatocytes²¹ but also in erythrocytes²² and fibroblasts.¹ It is therefore quite likely that a deficiency of this enzyme exists in other tissues, including the cells of the central and peripheral nervous systems in patients with acute intermittent porphyria. No definitive enzymatic deficiency in the haem biosynthetic pathway has yet been described in the other two hereditary hepatic porphyrias. But it is quite reasonable to expect that such a defect does underlie the marked increase in haem precursor excretion accompanying acute attacks. If this is so, then it is also reasonable to suggest that the postulated enzymatic deficiency is common to the cells of the liver, nervous system and probably other tissues of the body.

The available evidence strongly suggests that a profound disturbance of neuronal or glial metabolism, or of both, underlies the observed pathological lesions. Some authors have described patchy but widespread demyelination affecting predominantly peripheral nerves but also involving the central nervous system.²²,²³ They regard the observed axonal degeneration as secondary to loss of the myelin sheath. Other studies have shown axonal degeneration to be the main histological feature.²² Either pathological picture would be compatible with the hypothesis outlined above, depending on whether neurons or glial cells were most affected by the relative block in haem biosynthesis. It is particularly interesting that asymptomatic patients with acute intermittent porphyria and variegate porphyria, some of whom have never experienced an acute attack, demonstrate electromyographic evidence of peripheral neuropathy.²² In other words, there is a latent genetic defect present in the cells of the nervous system which may be "activated" by a precipitating agent or circumstance.

Table II shows the results of preliminary studies on haem biosynthesis in mammalian brain. Animals were fasted 48 hours prior to administration of phenobarbitalone (100 mg/kg), allylisopropylacetamide (AlA) (400 mg/kg) or phenobarbitalone 50 mg/kg plus AlA (400 mg/kg) by intraperitoneal route and sacrificed 18 hours later. Brains were perfused with cold saline in situ prior to removal. As might be expected, the values for ALA synthetase and total haem are considerably lower than the corresponding results for liver.²² More important, perhaps, is the apparent
lack of any significant effect of phenobarbitone or AIA treatment, alone or combined, on brain haem biosynthesis. This suggests that neural tissue is unable to alter its rate of haemoprotein production according to demand.

In the case where one of the enzymes other than ALA synthetase becomes rate-limiting, e.g. URO I synthetase as postulated for acute intermittent porphyria, a state of relative haem deficiency may ensue in the cells of the nervous system. The condition would, however, remain asymptomatic until one or other precipitating agent or factor further reduced haemoprotein function and seriously interfered with intracellular energy transfer, thereby disrupting normal neural activity and leading, if prolonged, to cell degeneration and necrosis. In this connection barbiturates and certain steroids are well known for their inhibitory effect on mitochondrial electron transport through inhibition of NADH oxidase.6,6

Recently, haematin infusions have been employed therapeutically in a number of patients with acute porphyria, apparently to good effect.5,6,15 Watson and co-workers have attempted to explain their results in terms of feedback repression of hepatic ALA synthetase with consequent decreased production of ALA and PBG. While the haematin infusions may well be exerting such an effect on the liver, this is unconvincing as an explanation for the haematin infusions may well be exerting such an effect on mitochondrial electron transport, thereby disrupting normal neural activity and leading, if prolonged, to cell degeneration and necrosis. In this connection barbiturates and certain steroids are well known for their inhibitory effect on mitochondrial electron transport through inhibition of NADH oxidase.5,6

We wish to thank the South African Medical Research Council for financial assistance, and the University of Stellenbosch and the Cape Provincial Administration for the use of facilities.

REFERENCES


### TABLE II. EFFECT OF PHENOUBARBITONE AND ALLYISOPROPYLACETAMIDE ON BRAIN HAEM BIOSYNTHESIS IN YOUNG ADULT WISTAR RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Phenobarbitone</th>
<th>AIA</th>
<th>AIA + Phenobarbitone</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA synthetase (nmol/g/h)</td>
<td>0.87</td>
<td>0.95</td>
<td>1.03</td>
<td>0.88</td>
</tr>
<tr>
<td>Total haem (nmol/g wet wt)</td>
<td>19</td>
<td>24</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Cytochrome P-450 (pmol/mg protein)</td>
<td>37</td>
<td>41</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>