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Lack of effect of phenobarbitone treatment on metabolism and brain uptake of delta-aminolaevulinic acid in rats

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The hereditary hepatic porphyrias, acute intermittent porphyria (AIP), variegate porphyria (VP) and hereditary coproporphyria (HC) are characterized by acute attacks of a neuropsychiatric nature. The aetiology of the neural dysfunction in these attacks which can be life-threatening is unknown. Association with the administration of a variety of drugs, including barbiturates, sulphonamides and some steroids which affect hepatic haem biosynthesis has, however, been well established [1].

One of the current hypotheses linking disordered hepatic haem biosynthesis with neurological or psychiatric disturbances is that one or other of the haem precursors which are overproduced in these diseases in neurotoxic [2]. The obvious candidates are delta-aminolaevulinic acid (ALA) and porphobilinogen (PBG) which are the only known metabolites overproduced in all three conditions. *In vitro* experiments, both with ALA and PBG [3, 4], have shown that these compounds can influence neural function but considerable doubt exists with regard to the physiological or pathological significance of these findings. Studies *in vivo* on experimental animals indicate that ALA and PBG penetrate the blood brain barrier only to a limited extent [5, 6] and no marked behavioural, neurological or electroencephalographic disturbances are produced [5–8].

An alternative suggestion is that drugs such as barbiturates may either facilitate neural uptake of ALA and or PBG, or may possibly promote the formation of neurotoxic metabolites from these compounds. In this connection it is notable that substances such as the anaesthetic, fluoroxene, which are normally non-toxic give rise to highly noxious metabolites in animals pre-treated with barbiturate, as a result of induction of hepatic cytochrome P-450 [9, 10]. This study was designed to test some of these possibilities.

Two groups comprising three female Wistar rats (100–130 g) each were used. The animals were kept individually in metabolic cages to facilitate urine collection. Both groups were starved for the full duration of the experiment. Drinking water was supplied *ad lib*. One group was injected with phenobarbitone (50 mg/kg) subcutaneously and the other with an equivalent vol of saline once daily for 5 days. ALA (500 mg/kg)(pH 6.7–7.4) was administered to both groups intraperitoneally twice daily for 3 days, beginning on the second day of phenobarbitone or saline administration. On the fifth day one dose of ALA, together with phenobarbitone or saline, was given at 8 a.m. and the animals were sacrificed 30 min later by decapitation. Blood was collected and the livers and brains were immediately removed for determination of the substances indicated in Table 1. Urine samples were stored at −20° until analysis. The ALA content of serum [11], brain and liver [12] and the ALA and PBG content of the urine [12] were determined spectrophotometrically. Microsomes were isolated from liver according to Fry and Bridges [13] and the cytochrome P-450 content was determined by the method of Omura and Sato [14].

No gross behavioural effects were noted in either group of animals at any stage of the study, other than those attributable to phenobaritone alone. On the morning of the fifth day prior to termination of the experiment all the animals appeared to be quite normal. The results of the ALA, PBG and cytochrome P-450 determinations are shown in Table 1. It is evident that phenobarbitone treatment did not increase the uptake of ALA from the blood into brain or liver. Hepatic microsomal P-450 concentration was, on the other hand, markedly and highly significantly increased in the phenobarbitone-treated animals. However, no clinical differences between the two groups were noted as mentioned above, and no significant differences in serum concentration or in urinary excretion of ALA or PBG were found.

Previous *in vivo* studies involving administration of ALA or PBG to animals have failed to reveal evidence of neurotoxicity comparable to the neuropathic manifestations of acute porphyria [5–8]. Moore, McGillion and Goldberg [15] have reported that ALA has a hypotensive effect in pithed, anaesthetized rats and that acute and chronic administration of this compound produces behavioural effects in these animals, including alteration in spontaneous locomotor activity and rearing times and decreased excitability. These findings do not correspond with the clinical manifestations of acute porphyria [16] and may simply reflect pharmacological effects of relatively large doses of ALA on systems other than the nervous system. Furthermore, no evidence has been produced that ALA can cause pathological lesions in neural tissue such as have been described by a number of authors [17–20] in patients with acute porphyria.

The results of the present study have again demonstrated that neither acute nor chronic administration of ALA to rats produces significant neuropathic effects.

We have previously reported that ALA and PBG penetrate the blood brain barrier only to a limited extent [5, 6] and that having gained entry to the brain, neither of these compounds accumulate there [5, 6] as has been previously suggested [2].

Phenobarbitone administration did not apparently affect the rate of uptake of ALA from plasma into brain tissue or liver in the present study. It may be argued that observation at a single point in time could give an erroneous impression. On the other hand, we have found repeatedly that brain concentrations of ALA are maximal at 30 min after intraperitoneal administration of a dose of 500 mg/kg [6]. Furthermore, had there been accumulation of ALA in the brain over the 5-day period this should have been reflected in our results. The fact that the values reported are not higher than those previously found, following a single injection of ALA [6], is good evidence against this possibility.

Induction of cytochrome P-450 in the livers of the phenobarbitone-treated animals undoubtedly occurred. However, this did not apparently influence the metabolism of ALA in these animals. Urinary ALA and PBG excretion was not significantly different in the two groups, suggesting that hepatic uptake and metabolism of ALA was not affected. These findings do not, of course, exclude the possibility of the production of other unidentified metabolites from ALA under the influence of phenobarbitone. But the lack of any distinctive clinical effect of combined treatment with phenobarbitone and ALA does not appear to warrant pursuit of this line of investigation.

Various drugs, including barbiturates, sulphonamides

Table 1. Effect of phenobarbitone on tissue uptake, metabolism and urinary excretion of administered delta-aminolaevolinic acid in the rat*

Determination	Controls†	Phenobarbitone-† treated	Significance
Hepatic microsomal cytochrome P-450 (nmole/g liver)	14 ± 2.4	32 ± 1.4	$P < 0.001$
Serum ALA (μmole/ml)	2.3 ± 0.45	2.5 ± 0.22	$P > 0.1$
Brain ALA (nmole/g)	39 ± 5.2	33 ± 4.5	$P > 0.1$
Liver ALA (μmole/g)	3.6 ± 0.58	3.4 ± 0.23	$P > 0.1$
Urinary ALA (μmole/24 hr)			
day 2	390 ± 56	490 ± 48	$P > 0.1$
day 3	390 ± 11	390 ± 31	$P > 0.1$
day 4	330 ± 26	340 ± 154	$P > 0.1$
Urinary PBG (μmole/24 hr)			
day 2	22 ± 4.7	27 ± 2.4	$P > 0.1$
day 3	20 ± 2.3	24 ± 3.8	$P > 0.1$
day 4	17 ± 1.2	24 ± 5.8	$P > 0.1$

* All values represent mean ± S.D. for three rats.

† Control animals received saline. Phenobarbitone dosage was 50 mg/kg daily. Both groups received ALA intraperitoneally (500 mg/kg) twice daily from day 2. Animals were sacrificed by decapitation on day 5, 30 min after final doses of saline, phenobarbitone and ALA.

and some steroids, have been implicated in the precipitation of the acute neuropsychiatric symptomatology of the hereditary hepatic porphyrias. This study was designed to test the proposal that the mechanism of action of such drugs might be (i) to promote neural uptake of porphyrin precursors which have been claimed to be potentially neurotoxic or (ii) to alter the metabolism of porphyrin precursors with resultant formation of neurotoxic products. Rats were treated daily with phenobarbitone and delta-aminolaevulinic acid (ALA) for 5 days. Phenobarbitone treatment had no significant effect on ALA metabolism and excretion or on ALA uptake into brain tissue. No significant behavioural effects other than those attributable to phenobarbitone alone were observed. These results do not support the suggestion that porphyrinogenic drugs may precipitate acute neuropathic effects in the hereditary hepatic porphyrias through altering porphyrin precursor metabolism or uptake of these compounds by neural tissue.

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