

Hypothesis: Metabolic targeting of 5-aminolevulinate synthase by tryptophan and inhibitors of heme utilisation by tryptophan 2,3-dioxygenase as potential therapies of acute hepatic porphyrias

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ABSTRACT

Metabolic targeting of liver 5-aminolevulinate synthase (5-ALAS) by inhibition of heme utilisation by tryptophan (Trp) 2,3-dioxygenase (TDO) or the use of tryptophan is proposed as a therapy of acute hepatic porphyrias. 5-ALAS, the rate-limiting enzyme of heme biosynthesis, is under negative feedback control by a small regulatory heme pool in the hepatic cytosol. Acute porphyric attacks, precipitated by fasting, certain hormones and some drugs, involve induction of 5-ALAS secondarily to depletion of the above pool, and the resultant elevation of 5-ALA levels initiates the abdominal and neurological symptoms of attacks. By utilising the regulatory heme, cytosolic TDO undermines the feedback control, thus allowing 5-ALAS induction to occur, e.g. upon glucocorticoid induction of TDO during fasting (starvation) and exogenous glucocorticoid administration. Currently, glucose therapy is the preferred strategy for reversing moderate attacks induced by fasting (calorie restriction), with more severe attacks being treated by intravenous heme preparations. Reversal of fasting-induced attacks by glucose is explained by the previously demonstrated reversal of increased heme utilisation by TDO. Inhibitors of this utilisation are therefore potential therapeutic targets in acute attacks and also for maintenance of a symptomless state. Existing TDO inhibitors other than glucose include allopurinol, nicotinamide and recently developed potent inhibitors such as LM10 used in cancer therapy. Based on studies in rats, the hypothesis predicts that the safety or otherwise of drugs in the hepatic porphyrias is determined by their ability to inhibit TDO utilisation of heme under basal conditions or after glucocorticoid induction or heme activation of TDO, in parallel with reciprocal changes in 5-ALAS induction. Tryptophan is also proposed as a potential therapy of acute attacks either alone or as an adjunct to the recently proposed 5-ALAS1 gene silencing. Trp increases heme biosynthesis by enhancing 5-ALA dehydratase activity and, based on a Trp-5-ALA model presented herein, Trp offers several advantages over heme therapy, namely rapid conversion of 5-ALA into heme, a greatly enhanced heme availability, a near complete inhibition of 5-ALAS induction, assumed rapid clearance of 5-ALA and hence accelerated resolution of symptoms of attacks, and finally provision of the neuroprotective metabolite kynurenic acid to neutralise the neurological symptoms. The hypothesis also addresses heme regulation in species lacking the TDO free apoenzyme and its glucocorticoid induction mechanism and proposes detailed assessment of heme biosynthesis in these species. Detailed proposals for testing the hypothesis are presented.

Introduction

The acute hepatic porphyrias are a group of genetic mutations at 4 steps in the heme-biosynthetic pathway [1]. Patients generally remain symptomless, until they suffer an attack, usually precipitated by drugs, hormones or fasting. Attacks are treated by intravenous heme preparations or by glucose. The pathway rate-limiting enzyme, 5-aminolevulinate synthase (5-ALAS) is under negative feedback control by a small pool of heme ($\sim 10^{-7}$ M) in the hepatic cytosol [2]. Attacks occur

when this “regulatory” heme pool is decreased or lost, resulting in 5-ALAS induction and accumulation of 5-ALA and some other intermediates, leading to the neurological and abdominal symptoms associated with these attacks. Loss of this heme can be induced experimentally by its destruction, synthesis inhibition, degradation by heme oxygenase, or enhancement of its utilisation by hemoproteins [3]. Under these conditions, utilisation of this pool by the liver cytosolic enzyme tryptophan (Trp) 2,3-dioxygenase (TDO; formerly Trp pyrrolyase; EC 1.13.11.11) has been amply demonstrated [3]. For such

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utilisation, TDO must exist in part as the free apoenzyme, as is the case in rats, humans and some other animal species [4]. Based on the loss of TDO heme by the experimental porphyrinogen 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), a screening test for exacerbation of hepatic porphyrias by drugs was developed [5], in which exacerbators were shown to potentiate this loss in rat liver. The use of heme utilisation by TDO as a measure of the regulatory heme pool has been adopted by various investigators.

Based on the above findings, I propose the hypothesis that inhibition of utilisation of the regulatory heme pool by TDO may form the basis of a new therapeutic approach in the treatment and management of the hepatic porphyrias. I shall also propose the use of Trp itself as a potential therapy of acute attacks with a rapid therapeutic onset. Trp or inhibitors of TDO utilisation of heme can be used as monotherapies or adjuncts to the recently reported proposed gene silencing of 5-ALAS1 synthesis [6].

In the following text, accounts of the heme-biosynthetic pathway and of heme utilisation by hepatic hemoproteins, and the biochemical status of TDO will precede a description of the hypothesis, how it applies to prevention of attacks, maintenance of a symptom-free state and therapy of attacks, and proposed means of testing it.

The heme-biosynthetic pathway

The first step in the mammalian heme-biosynthetic pathway (Fig. 1) involves condensation of glycine and succinyl CoA to form 5-aminolevulinic acid (5-ALA) and is catalysed by 5-ALA synthase (5-ALAS). This is the rate-limiting enzyme of the pathway and the site of its negative feedback control. As a pyridoxal 5'-phosphate (PLP)-dependent enzyme, 5-ALAS activity may be impaired in vitamin B₆ deficiency whether induced by malnutrition or by drugs known to inactivate the PLP cofactor. Thus, 5-ALAS inhibition has been demonstrated with the erythrocyte enzyme in patients with B₆-responsive anemia [7] and in HeLa cells expressing the human erythroid enzyme after treatment with isoniazid [8]. That conversion of 5-ALA to heme can be blocked by diversion of glycine away from the pathway by hippurate formation is suggested by the finding [9] that the substrates of glycine acyltransferase benzoate and *p*-aminobenzoate prevent the experimental porphyria induced in rats by DDC. Reversal of chemically-induced porphyria and inhibition of basal and DDC-induced 5-ALAS activity have also been demonstrated [10] with acetate.

Two molecules of 5-ALA then condense to form porphobilinogen by the action of porphobilinogen synthase, more commonly known as 5-ALA dehydratase. This Zn-dependent enzyme is activated by Zn²⁺ and Al³⁺ and inhibited by Pb²⁺ both *in vitro* and after administration [11,12]. Succinylacetone is also a potent inhibitor of the enzyme, as shown in patients with tyrosinemia [13], in whom this compound accumulates. Four molecules of porphobilinogen then undergo successive condensations with successive losses of an amino group by the action of porphobilinogen deaminase. The hydroxymethylbilane produced undergoes ring closure and flipping over one of the pyrroles to form uroporphyrinogen III by the action of uroporphyrinogen III cosynthase. Decarboxylation of uroporphyrinogen III produces coproporphyrinogen III, which is then oxidised to protoporphyrinogen IX by coproporphyrinogen oxidase. Oxidation of protoporphyrinogen IX to protoporphyrin IX then follows. The final step in heme biosynthesis involves chelation of Fe²⁺ by the latter intermediate by the action of ferrochelatase. This enzyme is also subject to inhibition by metal cations such as Cu²⁺, Zn²⁺, Co²⁺ and Ni²⁺ [14]. The antifungal agent and experimental porphyrinogen griseofulvin also inhibits ferrochelatase activity, but more strongly in mice than in rats [15,16]. Not shown in Fig. 1 is the non-enzymic oxidation of hydroxymethylbilane in conditions in which it accumulates in excess. It is converted into uroporphyrinogen I with loss of H₂O. Uroporphyrinogen I is then decarboxylated to coproporphyrinogen 1. These 2 porphyrinogens, their III isomers and protoporphyrinogen IX are further oxidised non-enzymically to

uroporphyrin I, coproporphyrin I, uroporphyrin III, coproporphyrin III and protoporphyrin IX respectively.

Fig. 1 also shows the heme-degradative pathway. Heme is first oxidised by heme oxygenase (HO) to biliverdin, which is then reduced by biliverdin reductase to bilirubin. HO is inhibited by Sn-mesoporphyrin and many metal cations [17,18].

The hepatic porphyrias: Sites of mutations and laboratory and clinical features

As shown in Fig. 1, there are 6 steps in the heme-biosynthetic pathway where mutations occur resulting in the 6 different types of the hepatic porphyrias outlined in Table 1. Of these 6 types, four are acute porphyrias (5-ALA dehydratase, acute intermittent, hereditary coproporphyrin and variegate porphyrias). As well as based on clinical features, general and differential diagnosis takes into account the pattern of urinary, plasma and stool levels of porphyrins and other heme precursors. Detailed accounts of these aspects have been published (see, e.g., [1,19,20]). In acute porphyrias, acute attacks result in a variety of symptoms including abdominal pain and neurological disturbances [1,19–21]. Various mechanisms, including the role of 5-ALA have been proposed. While a detailed discussion of these mechanisms is outside the scope of this article, a brief account may be useful in the context of the present hypothesis. It is now believed that, of the heme-biosynthetic pathway intermediates, 5-ALA is the sole initiator of abdominal pain [22], though the mechanism(s) involved remain unknown. Many studies have also implicated 5-ALA in the neurological symptoms (for details and references, see [23]). Thus, 5-ALA is neurotoxic and can undermine neuronal function by several mechanisms, including direct cytotoxicity, potent presynaptic agonism at GABA (γ -aminobutyric acid) receptors that is associated with the depersonalisation, dysphoria and visual hallucinations in porphyria, strong inhibition of presynaptic GABA release (50% at 1 μ M), and elevation of its cerebrospinal fluid (CSF) levels during acute attacks to values well above that causing this inhibition. Sweeney et al. [24] showed that CSF 5-ALA levels during porphyric attacks (21 μ M) were only 11.47% of those in plasma (183 μ M). Although brain levels have not been determined, they could generally be assumed to be higher than those in CSF [25], possibly by as much as \sim 10-fold. In this latter study [25], glutamate dysfunction has been implicated in the 5-ALA neurological effects. Thus, convulsions induced by intrastriatal injection of 5-ALA are prevented by glutamate antagonists. Inhibition by 5-ALA of glutamate uptake by astrocyte cultures has been implicated in 5-ALA-induced convulsions [26]. Thus a state of neuronal hyperexcitability exists under these conditions. The NMDA (*N*-methyl-D-aspartate) type of receptors of the excitatory amino acid glutamate are likely to be activated. The Trp metabolite quinolinic acid (QA) is the endogenous agonist of these receptors [27], but its levels in acute hepatic porphyrias have not been studied. By contrast, kynurenic acid (KA) is an NMDA receptor antagonist and neuroprotective [27]. It is generally believed that the state of neuronal excitability is determined by the balance between QA and KA. Thus, a decrease in [KA] with no change in [QA] can tilt the balance towards hyperexcitability. As will be discussed below, KA may have a role to play in preventing the neurological effects of acute porphyric attacks. It is noteworthy that depletion of the regulatory-heme undermines NMDA receptor function [28,29].

Control of hepatic heme biosynthesis

As stated above, hepatic heme biosynthesis is controlled by a feedback mechanism exerted at the rate-limiting enzyme of the pathway, 5-ALAS, by heme in chick embryos [2] and mammals [30], including humans [31]. Heme is thought to exert this negative feedback by three mechanisms: repression of 5-ALAS synthesis at the post-transcriptional level [2], regulation of translocation of newly synthesized 5-ALAS from cytosol into mitochondria [32] and inhibition of 5-ALAS

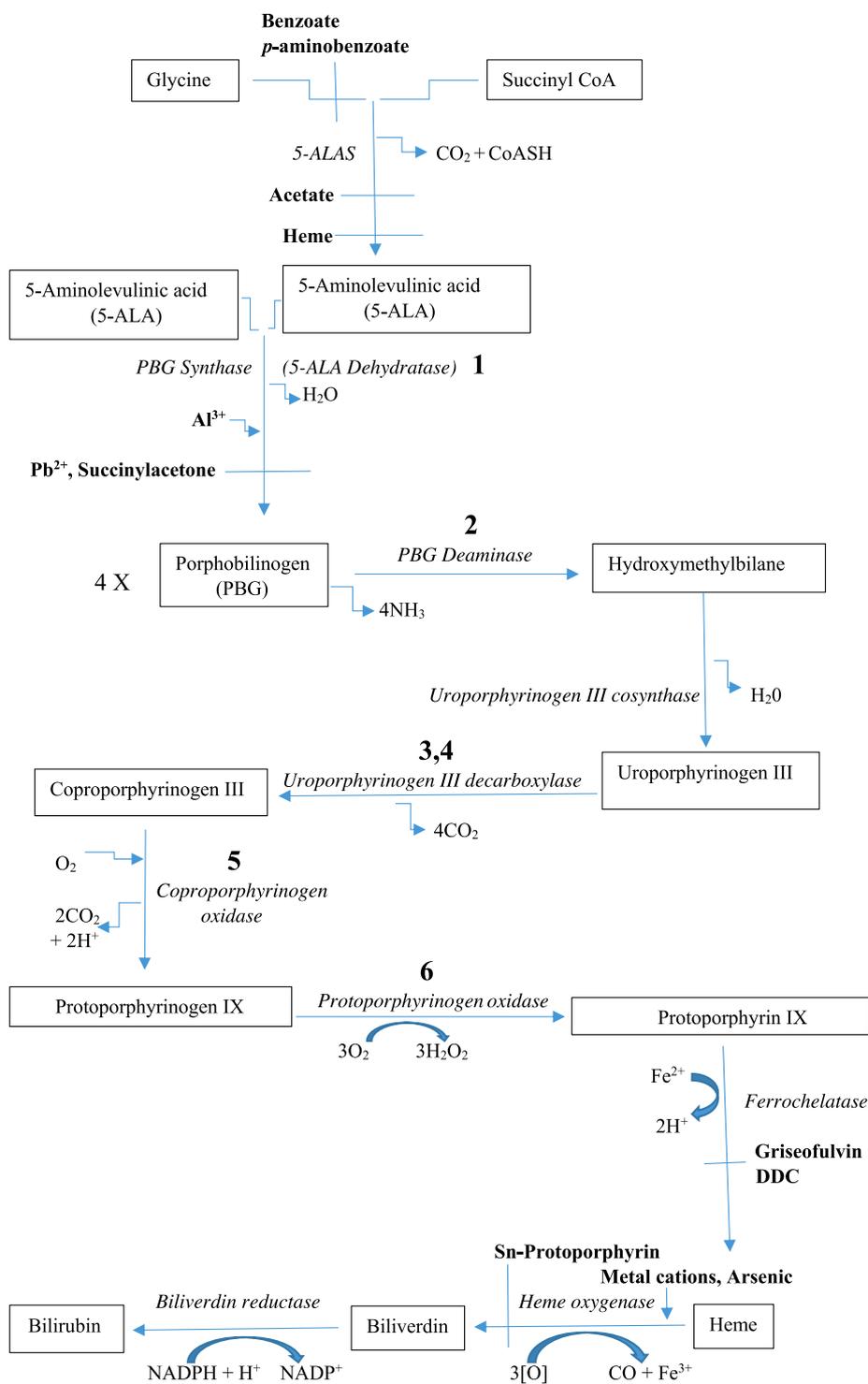


Figure 1. The heme-biosynthetic and degradative pathways

Fig. 1. The heme-biosynthetic and degradative pathways. Enzymes are in italic and inhibitors or stimulators are in bold letters. The bold numbers denote the enzyme defect in the following hepatic porphyrias: 1 (5-ALA dehydratase porphyria); 2 (acute intermittent porphyria); 3 (porphyria cutanea tarda); 4 (hepato-erythropoietic porphyria); 5 (hereditary coproporphyria); 6 (variegate porphyria).

activity [33]. This feedback concept has gained credence from previous studies demonstrating 5-ALAS induction and elevation of liver porphyrins by chemicals capable of depleting hepatic heme either by its destruction to green and other pigments, e.g., by 2-allyl-2-isopropylacetamide (AIA) or DDC, or by inhibition of heme synthesis at the ferrochelatase step by DDC or griseofulvin [34]. By contrast, another group of chemicals were found to enhance 5-ALAS activity moderately on their own and to potentiate its induction by the above

porphyrins. Phenobarbitone, phenylbutazone and other lipid-soluble drugs are among these potentiators and it has been suggested [34,35] that this may explain the exacerbation of hepatic porphyrias by certain drugs and other chemicals. As stated above, potentiation of the loss of heme utilised by the cytosolic hemoprotein TDO formed the basis of a screening test [5] for drug exacerbation of porphyrias. It has subsequently become logical to establish if the depletion of liver heme by these and other mechanisms influences levels or activities of

Table 1
The hepatic porphyrias: sites of defects and laboratory features.

No.	Porphyria (P)	Enzyme defect	Laboratory features		
			Urine	Plasma	Stools
1	5-ALA Dehydratase P	5-ALA dehydratase	↑ 5-ALA	↑ 5-ALA	–
2	Acute intermittent P	PBG deaminase	↑ 5-ALA, PBG	↑ 5-ALA, PBG	–
3	P Cutanea tarda	Uroporphyrinogen III decarboxylase	↑ Uroporphyrin III	↑ Uroporphyrin III	↑ Isocoproporphyrin III
4	Hepato-erythropoietic P	Uroporphyrinogen III decarboxylase	↑ Uroporphyrin III	↑ Uroporphyrin III	↑ Isocoproporphyrin III
5	Hereditary copro P	Coproporphyrinogen III oxidase	↑ 5-ALA, PBG	↑ Coproporphyrin III	↑ Coproporphyrin III
6	Variegate P	Protoporphyrinogen III oxidase	↑ Coproporphyrin III		↑ Protoporphyrin IX
			↑ 5-ALA, PBG	↑ Protoporphyrin IX	↑ Protoporphyrin IX
			↑ Coproporphyrin III	↑ Coproporphyrin III	↑ Coproporphyrin III

Abbreviations used: 5-ALA (5-aminolevulinic acid); PBG (porphobilinogen).

hemoproteins.

Heme utilisation by hepatic hemoproteins

An intense period of research ensued during which the potential roles of hepatic hemoproteins in utilisation of heme in general and the “regulatory-heme” pool in particular were extensively studied. Heme is the prosthetic group of a wide range of hemoproteins, including the mitochondrial cytochromes, microsomal cytochromes *P*-450 and *b*₅, catalase, peroxidases and cytosolic TDO, in addition to hemoglobin, myoglobin and NO synthases [36]. About 85% of synthesized heme is utilised for hemoglobin synthesis in bone marrow, with the remaining 15% being synthesised in the liver, where it is utilized mainly by cytochromes and catalase [37]. The major hepatic heme-utilising protein is the microsomal *P*-450 (66%), followed by catalase (16%), whereas TDO utilises only 1.4% of hepatic heme [36]. The remainder of hepatic heme is used by mitochondrial and other cytochromes. Of special importance in the context of this hypothesis are the turnover of the hemoproteins and the readily-exchangeable nature of the regulatory-heme pool. This pool has been suggested to be rapidly turning-over [2,30]. To utilise this heme, the hemoprotein should have a relatively short half-life. That of *P*-450 is 7–10 h and 24–48 h [28] and that of catalase is 29 h [38] or 2.5–3.6 days in various hepatocyte compartments [39]. Mitochondrial cytochromes have even-longer half-lives and do not respond to rapid changes in liver heme. By contrast, as will be shown below, TDO responds exquisitely to rapid changes in liver heme under various experimental conditions. The study of heme utilisation by hepatic hemoproteins is further complicated by the observations that decreased heme availability limits the synthesis of *P*-450 [40] and catalase [39], but not that of TDO [41]. By contrast, response to rapidly turning-over heme must occur early in the sequence of events of the heme utilisation process. In the following section, evidence against utilisation of the regulatory-heme pool by cytochrome *P*-450 and catalase will be described.

Evidence against utilisation of the regulatory-heme pool by cytochrome *P*-450 and catalase

During the 1970s and early 1980s, the initial prevailing view was that hepatic heme biosynthesis is controlled by its utilisation by other hemoproteins, notably cytochrome *P*-450. While this concept led to development of experimental models of the hepatic porphyrias, contrary evidence from various research groups was, however, already emerging. The following are examples.

(1) The marked 5-ALAS enhancement by AIA caused by loss of heme is not potentiated by phenobarbitone, despite the further loss of *P*-450 heme [42]. (2) Potentiation by phenylbutazone of the 5-ALAS enhancement by DDC occurs without a further early loss of *P*-450 heme [43]. (3) Cytochrome *P*-450 and other hepatic cytochromes, but not TDO, are insensitive to exogenous 5-ALA despite 5-ALAS repression [44]. (4) The decreased ability of AIA to induce 5-ALAS in

adrenalectomized (and/or ovariectomised) rats and restoration of full induction by cortisol occur in the absence of changes in *P*-450 heme [45]. (5) 5-ALAS activity is enhanced by acute hexachlorophenyls [46] and by chronic fenitrothion [47] without loss of *P*-450. (6) Small AIA doses enhance 5-ALAS activity without altering *P*-450 [48]. (7) No consistent correlation exists between the decrease in *P*-450 concentration and 5-ALAS enhancement in iron-dextran-treated rats [49]. (8) Induction of 5-ALAS activity in chick-embryo liver by propyl isopropylacetamide does not involve destruction of cytochrome *P*-450 [50]. (9) Impaired induction of 5-ALAS in pregnant rats is not associated with impaired destruction of *P*-450 heme [51]. (10) In chick-embryo *in ovo* and in cultured hepatocytes, phenobarbitone causes prompt induction of 5-ALAS without any measurable initial destruction of *P*-450 heme [52]. (11) Induction of 5-ALAS and increased heme saturation of TDO by carbamazepine occur in the absence of changes in cytochrome *P*-450 [53]. (12) With catalase, little is known about any potential early changes in its levels or activity following rapid depletion of the regulatory-heme pool, perhaps because of its relatively long half-life. In one study [54], catalase activity was shown to be decreased after 3–5 days of daily treatment of rats with AIA and related acetamides or barbiturate derivatives. The relevance of these late changes to the early loss of the regulatory-heme pool is, however, questionable.

In conclusion, it is clear that neither cytochrome *P*-450 nor catalase utilises the regulatory-heme pool. By contrast, it will be shown conclusively below that this pool is utilised by TDO. Before describing this utilisation, it may be helpful to outline briefly the pathway of Trp degradation, the kynurenine (Kyn) pathway (KP), of which TDO is the first and rate-limiting enzyme. A review of the functions and regulation of the KP can be consulted for details [55].

The hepatic kynurenine pathway of tryptophan degradation

The hepatic KP accounts for ~90% of dietary Trp disposal [55]. As shown in Fig. 2, Trp is first oxidized by TDO to *N*-formylkynurenine. The latter is rapidly hydrolysed to Kyn by the abundant *N*-formylkynurenine formamidase. Kyn is metabolised mainly by oxidation to 3-hydroxykynurenine (3-HK) by the action of Kyn monooxygenase (KMO) (also known as kynurenine hydroxylase). 3-HK is then hydrolysed by kynureninase to 3-hydroxyanthranilic acid (3-HAA). Kynureninase can also hydrolyse Kyn to Anthranilic acid (AA). 3-HAA 3,4-dioxygenase (3-HAAO) converts 3-HAA to ACMS (2-amino-3-carboxymuconic acid-6-semialdehyde; also known as acroleyl aminofumarate). This intermediate occupies a central position at 2 branches of the KP. It undergoes non-enzymic cyclisation to quinolinic acid (QA) and eventually to NAD⁺, or further metabolism, first by decarboxylation by ACMS decarboxylase (also known as picolinic acid carboxylase) to AMS, which either undergoes non-enzymic cyclisation to picolinic acid (PA) or further metabolism to acetyl CoA. The KP also includes a minor transamination reaction involving the conversion of Kyn to KA and 3-HK to xanthurenic acid (XA). These aminotransferase reactions are normally minimal under normal conditions, because of the relatively

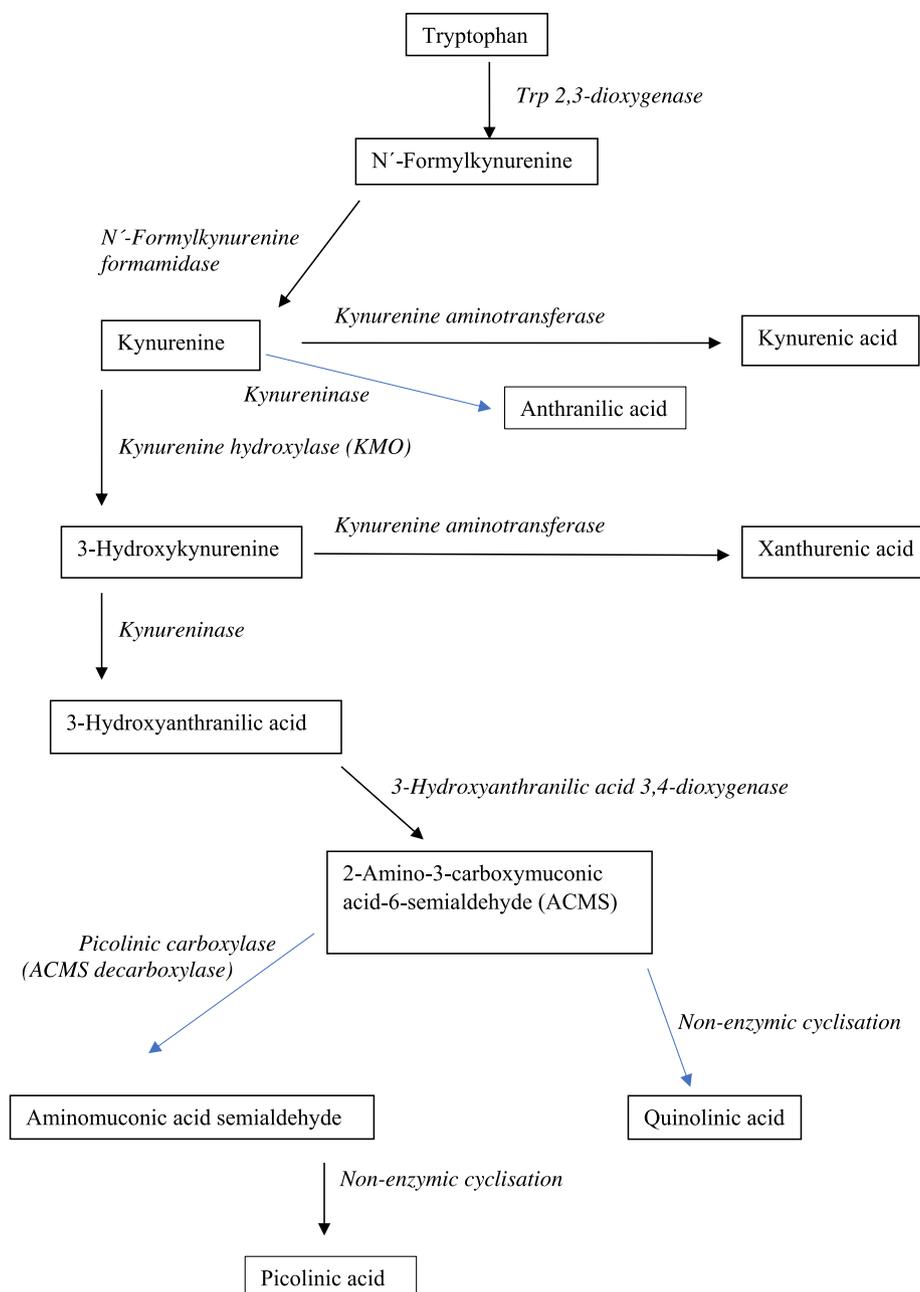


Fig. 2. The hepatic kynurenine pathway up to the quinolinic acid and picolinic acid steps. Adapted from Fig. 1 in Ref. [23] (Badawy AA-B. Pellagra and alcoholism: a biochemical perspective. Alcohol Alcohol 2014; 49: 238–250).

higher K_m values of its 2 substrates Kyn and 3-HK, compared with the corresponding K_m values for KMO and kynureninase respectively [55]. Transamination becomes more significant when concentrations of the substrates are increased, e.g. after acute Trp or Kyn loading or KMO or kynureninase inhibition.

Tryptophan 2,3-dioxygenase (TDO) and hepatic heme

The KP also exists extrahepatically and is controlled by another hemoprotein, indoleamine 2,3-dioxygenase (IDO). Liver TDO in rats, mice, humans and some other, but not all, animal species exists in two forms: the active heme-containing holoenzyme and the inactive heme-free apoenzyme [4]. The latter becomes active after its conjugation *in vitro* with a source of heme, e.g. hemin (hematin) or methemoglobin or *in vivo* after administration of hematin, 5-ALA or Trp (see below). By contrast, IDO exists as the heme-containing holoenzyme: it cannot be

activated by addition of heme *in vitro* and shows a moderate enhancement after administration of a large Trp dose [56]. These differences and the fact that very little IDO exists in liver suggest that, of these two Trp-degrading hemoproteins, only TDO plays a role in hepatic heme utilisation. The existence of Liver TDO only as the heme-containing holoenzyme in some animal species [4] also suggests that heme utilisation in these species may be subject to control mechanisms different from those in humans, rats and mice. This will be discussed later in this article.

To appreciate heme utilisation by TDO, it is important to discuss briefly here the TDO control mechanisms [55,57]. In rats, mice or humans, TDO is inducible by glucocorticoids and activated by Trp and by heme. Glucocorticoid induction of TDO involves enhanced *de novo* synthesis of new enzyme protein, whereas substrate activation by Trp involves promotion of conjugation of the apoenzyme with heme and stabilisation of pre-existing apoenzyme in the presence of the normal

Table 2
Heme utilisation by and saturation of liver tryptophan 2,3-dioxygenase under various conditions

Condition	Holoenzyme	Total enzyme	Apoenzyme	% HS	HSR	Refs.
<i>(a) No change</i>						
Nil (fed state)	2	4	2	50	1.00	[59]
Starvation (24–48h)	4	8	4	50	1.00	[59]
Cortisol	9	20	11	45	0.82	[57]
<i>(b) Increase</i>						
5-ALA	7	8	1	87	7.00	[57]
Hematin	11	12	1	92	11.00	[57]
Trp	15	22	7	68	2.14	[57]
<i>(c) Decrease</i>						
CoCl ₂	1	7	6	14	0.17	[60]
DDC	2	8	6	25	0.33	[41]
AIA	2	11	9	18	0.22	[41]
GF	2	12	10	17	0.20	[41]

Abbreviations used: AIA (2-allyl-2-isopropylacetamide); 5-ALA (5-aminolevulinic acid); DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine); GF (griseofulvin).

rate of its synthesis. It has been suggested that the Trp promotion of heme conjugation may involve enhanced heme biosynthesis, perhaps at the 5-ALA dehydratase step [57] (see further below). Heme simply activates the TDO apoenzyme by conjugation. A 4th regulatory mechanism is that of feedback or end-product allosteric inhibition by NAD (P)H. Agents that increase the hepatic concentrations of these dinucleotides inhibit TDO activity, e.g. nicotinamide and glucose [58]. As will be discussed below, the TDO inhibitory activity of these 2 agents may have important applications in therapy of the hepatic porphyrias.

Heme utilisation by tryptophan 2,3-dioxygenase (TDO)

The heme conjugation with apo-TDO can best be defined by 2 phenomena: (1) heme utilisation without any increase in saturation; and (2) altered saturation determined by heme availability. The former is true heme utilisation that plays the major role in control of heme biosynthesis, whereas the latter is a reflection or an expression of heme availability. Both phenomena are exemplified by the data in Table 2 based on referenced [41,57–60] observations. Utilisation is exemplified by glucocorticoid induction of TDO, where a half of the newly synthesised apoenzyme becomes conjugated (saturated) with heme. Glucocorticoid induction is mediated by corticosterone in rats starved for 24–48 h, but not longer, or if they receive exogenous cortisol. The saturation of TDO with heme can be expressed as either the percentage heme saturation ($100 \times$ holoenzyme activity/total enzyme activity) or the heme-saturation ratio (holoenzyme activity/apoenzyme activity). Average values of these two expressions in fed rats are $\sim 50\%$ or ~ 1.00 respectively. Glucocorticoid induction does not alter these heme saturation parameters, and the increase in holoenzyme activity represents increased heme utilisation. Reflection of heme availability involves altered TDO saturation with heme. Increased saturation is observed with the TDO heme cofactor and Trp substrate. With the heme cofactor, the TDO apoenzyme becomes strongly heme-saturated such that the enzyme exists almost completely in the active holoenzyme form, with a % heme saturation of 87–92% and a HSR 7–11-fold the basal value. With Trp, saturation also occurs, but to a lower extent than that by heme (68% and 2.14 respectively). Decreased heme saturation is observed when there is a decrease in heme availability. There are many examples of this decrease (see below), but a few are listed in Table 2, namely induction of heme oxygenase by CoCl₂, destruction of heme by AIA and DDC and inhibition of ferrochelatase by DDC and griseofulvin.

Response of TDO to changes in heme availability: Altered heme saturation

The response of TDO to changes in heme availability is wide-ranging and exquisitely sensitive. The various conditions in Fig. 1 under which heme synthesis and degradation are altered are reflected in the TDO saturation with heme. These conditions are:

(1) Substrates of glycine acyltransferase, such as benzoate and *p*-aminobenzoate, which inhibit heme biosynthesis by diverting glycine away from the heme-biosynthetic pathway [9], decrease the TDO heme saturation in fed rats and in those treated with cortisol, Trp and agents acting via Trp (salicylate and ethanol), but do not alter the heme saturation induced by administration of 5-ALA or hematin [60]. (2) Acetate, which inhibits heme biosynthesis by inhibition of 5-ALAS activity [10], inhibits the TDO heme saturation under basal conditions and after administration of cortisol, but not that by 5-ALA, hematin or Trp [60]. The failure of acetate to inhibit the Trp-induced increase in the heme saturation is consistent with the notion that Trp enhances heme biosynthesis at a step beyond 5-ALAS. (3) Enhancement of 5-ALA dehydratase activity by Al³⁺ and its prevention by Pb²⁺ [10,11] are reflected in the increased TDO heme saturation by the former, and its prevention by the latter, cation [60]. (4) Destruction of heme by AIA or DDC to green and other pigments or its inhibition at the ferrochelatase step by DDC or griseofulvin [26] is reflected in the decreased heme saturation of TDO [41,60]. (5) Induction of heme oxygenase by CoCl₂ has been shown to lower microsomal heme levels and those of cytochrome *P*-450 and b₅ [61] and to inhibit catalase synthesis [31]. The loss of heme by this metal cation is reflected in decreased TDO heme saturation [60]. These changes occur a later time point (24 h). CoCl₂ also decreases the heme saturation of TDO observed after administration of cortisol, Trp, agents acting via Trp, hematin and 5-ALA, thus suggesting that both newly formed and endogenous heme is degraded by this metal cation [60]. However, CoCl₂ is also known to cause early inhibition of heme biosynthesis at and beyond the 5-ALAS step [62]. Many other metal cations, except Zn²⁺, also decrease the TDO heme saturation at 24 h [60]. (6) The increase in heme availability after inhibition of heme oxygenase by Sn-protoporphyrin is reflected in increased heme saturation of TDO [63]. (7) Acute arsenic administration, which increases heme oxygenase activity, decreases TDO saturation with heme [64].

Monitoring the regulatory-heme pool by TDO saturation with heme

Rapid changes in the TDO saturation with heme are associated with reciprocal changes in 5-ALAS activity. In studies from the author's laboratory in which both 5-ALAS and TDO activities were measured simultaneously, a robust inverse correlation always existed between 5-ALAS activity and TDO heme saturation, irrespective of whether the latter is expressed as the % HS or the HSR [65–68]. This is summarised in Table 3. 5-ALAS was also correlated negatively with the TDO holoenzyme activity, but positively with those of the total enzyme and apoenzyme. This is not surprising, as the holoenzyme is the heme-saturated form. One of many examples of the above reciprocal

Table 3

Correlations between 5-aminolevulinic synthase activity and parameters of heme saturation of tryptophan 2,3-dioxygenase.

5-ALAS activity vs:	Correlation coefficient (r)	Significance (P)	Number
% Heme saturation	−0.829	0.00000	30
Heme saturation ratio	−0.749	0.000	20
TDO holoenzyme activity	−0.571	0.0085	20
TDO total enzyme activity	0.619	0.0036	20
TDO apoenzyme activity	0.857	0.00000	20

Pearson Product Moment correlations were derived from data reported by the author in references [65–68].

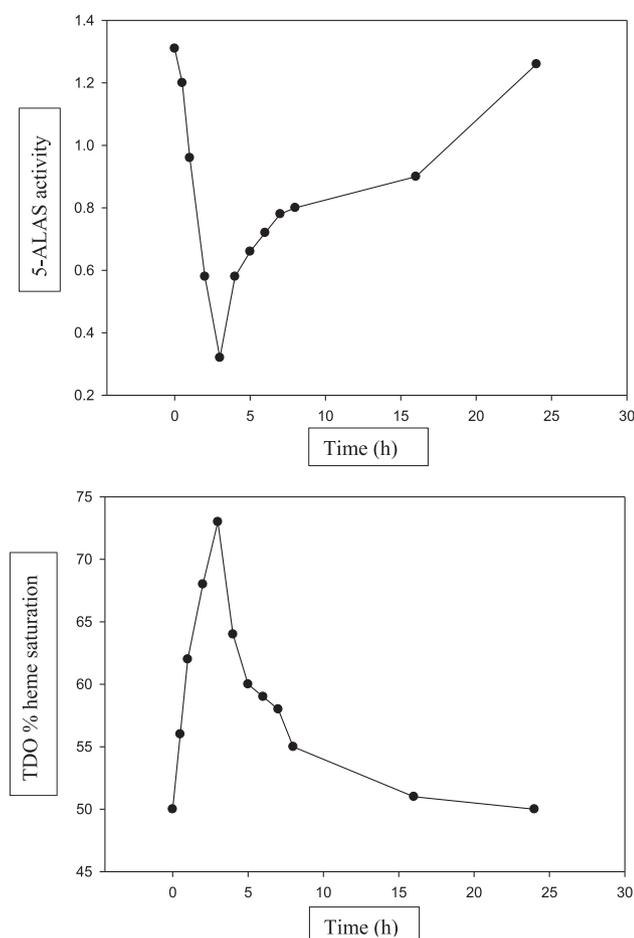


Fig. 3. Time-course of effects of hematin HCl (1 mg/kg body wt) on 5-aminolevulinic acid synthase (5-ALAS) activity (top) and the % heme saturation of tryptophan 2,3-dioxygenase (TDO) in rat liver. 5-ALAS is expressed in nmol of 5-ALA formed/min per g wet wt of liver, whereas the TDO % heme saturation is = $100 \times$ holoenzyme activity/total enzyme activity. Values are means of each group of 4 rats and have been calculated from Fig. 6 in Ref. [68] (Welch AN, Badawy AA-B. Tryptophan pyrrolase in haem regulation – experiments with administered haematin and the relationship between the haem saturation of tryptophan pyrrolase and the activity of 5-aminolaevulinic acid synthase in rat liver. *Biochem J* 1980; 192: 403–410).

relationship between 5-ALAS activity and the TDO % heme saturation is illustrated in the time-course of effects of administration of a small dose of hematin HCl [68] shown in Fig. 3.

Utilisation of the regulatory-heme pool by TDO

True utilisation of the regulatory-heme pool by TDO has been amply demonstrated in many studies in which increased utilisation is associated with 5-ALAS induction and blockade or prevention of this increase reversing the 5-ALAS induction. The following are examples.

(1) In 48 h-starved adrenalectomized rats, AIA does not influence TDO activity or alter the % heme saturation and its 5-ALAS induction is relatively impaired, but cortisol administration restores the 5-ALAS induction and the decrease in the TDO % HS to values observed in sham controls [69]. This is made possible by cortisol inducing TDO apoenzyme synthesis thereby enabling the newly synthesized apoenzyme to utilise the regulatory-heme pool. (2) Starvation of rats for 24–48 h enhances TDO activity by a corticosterone-mediated glucocorticoid induction mechanism [70]. Utilisation of the regulatory-heme pool by the newly synthesized apoenzyme leads to induction of 5-ALAS activity [59]. Both effects are blocked by glucose, fructose and sucrose and by

allopurinol [59]. Sugars inhibit TDO activity by increasing [NAD(P)H] [58], whereas allopurinol acts by preventing the conjugation of the apoenzyme with heme [71]. At these periods of starvation, the TDO % HS is unaltered (44–46% vs 46% in fed rats). However, starvation for 72–96 h increases the % HS to 63–66% and causes a return of 5-ALAS activity to levels observed in fed rats [59], suggesting increased heme availability by prolonged starvation, presumably mediated by Trp following its tissue protein breakdown (see below for the Trp effect on 5-ALAS). (3) By contrast, the hematin-induced decrease in 5-ALAS activity in fed or 48 h-starved rats is potentiated by allopurinol, by virtue of prevention of apoenzyme conjugation with heme, thereby increasing availability of the latter for 5-ALAS repression [68]. (4) The increased heme saturation of TDO by Trp is associated with decreased 5-ALAS activity [65]. As stated above, Trp may cause these changes by enhancing heme biosynthesis. Although Trp does not induce apo-TDO synthesis, its activation of the enzyme has long been known to be sensitive to blockade by translational inhibitors [57] and it was suggested that Trp may act by stimulating heme biosynthesis. As Trp decreases 5-ALAS activity, the potential enhancement of heme biosynthesis may occur at a subsequent step, possibly that of 5-ALA dehydratase. Evidence in support of this possibility is twofold. First, the 5-ALA dehydratase inhibitor Pb^{2+} prevents the Trp-induced increase in TDO heme saturation and the decrease in 5-ALAS activity [65]. Second, joint administration of Trp plus 5-ALA leads to a full (100%) TDO saturation with heme and a near complete (96%) abolition of 5-ALAS activity, whereas joint administration of Trp plus hematin does not influence the hematin effects on either parameter [65]. As will be discussed below, these effects of Trp may form the basis of a potential Trp therapy of acute attacks in patients with hepatic porphyrias. (5) Agents that increase plasma free and hence liver [Trp] either by direct displacement from albumin-binding sites (e.g. palmitate and salicylate) or via non-esterified fatty acids (NEFA) (e.g. endotoxin, morphine and theophylline) also lower 5-ALAS activity and increase the TDO heme saturation [66]. Inhibition of lipolysis by the β -adrenoceptor blocker propranolol or the opioid antagonist naloxone prevents the changes in 5-ALAS and TDO heme saturation by the above lipolytic agents, but not by Trp or direct displacers [66]. A small dose of Trp, which does not alter 5-ALAS or TDO heme saturation is rendered effective in both respects when given with a small dose of salicylate [66]. From these observations, several important correlations other than those listed in Table 3 have been established [66], most importantly the negative correlation between liver 5-ALAS activity and free serum and liver [Trp]. Overall, these results suggest that 5-ALAS synthesis may be subject to regulation by changes in lipid metabolism and by pharmacological agents that affect Trp disposition.

The regulatory-heme pool

From the above accounts, it is reasonable to conclude that TDO utilises the regulatory-heme pool in the hepatic cytosol. As stated in the Introduction, the size of this pool in liver cytosols of chick embryos was estimated by Granick et al. [2] from repression studies to be $\sim 10^{-7}$ M. A close value (0.093 μ M) in rat liver was calculated based on the largest depletion of TDO heme after joint administration of DDC plus phenylbutazone [72]. This concentration represents relatively low affinity, compared with those of cytochrome P-450 and catalase [2] and is therefore most suitable for rapid changes in heme binding to occur in response to those in heme metabolism [3]. So far, only TDO has been demonstrated to fulfil these criteria.

The hypothesis: Metabolic targeting of 5-aminolevulinic acid synthase by tryptophan and inhibitors of heme utilisation by tryptophan 2,3-dioxygenase as potential therapies of acute hepatic porphyrias

I hypothesise that, by utilising the regulatory-heme pool, liver tryptophan 2,3-dioxygenase (TDO) controls hepatic heme biosynthesis

by determining availability of this pool to exert its feedback control of the pathway at the 5-ALAS step. Acute porphyric attacks involve induction of 5-ALAS synthesis, as a result of which the elevated levels of 5-ALA and possibly also other intermediates cause the neurological and other symptoms of the attacks. It follows therefore that targeting 5-ALAS with a view to lowering 5-ALA levels is the logical approach to preventing and reversing the attacks. This is already the mechanism of therapies with glucose, heme preparations and, more recently, 5-ALAS 1 gene silencing. The proposed hypothesis uses a metabolic approach involving either tryptophan itself or pharmacological inhibition of utilisation of the regulatory-heme pool by TDO. From the above accounts, four simple elements of the proposed hypothesis are identified: the rapid response of TDO to changes in heme synthesis and degradation; the preferential utilisation of the regulatory-heme pool by TDO; the inverse relationship between this utilisation and 5-ALAS activity; the ability of Trp to inhibit 5-ALAS via rapid enhancement of heme synthesis. Targeting heme utilisation by TDO as a proposed therapy can be applied to 3 settings: acute attacks by fasting, maintenance of a symptom-free state by avoidance of precipitants, and during heme therapy. Targeting 5-ALAS by Trp is also proposed as a monotherapy or as an adjunct to gene silencing. The following sections address each of these settings.

Fasting-induced acute attacks

As stated above, fasting (starvation) induces 5-ALAS activity by enhancing TDO utilisation of the regulatory-heme pool as a result of glucocorticoid induction of the TDO apoenzyme [59]. Accordingly, patients with acute hepatic porphyrias should avoid exposure to glucocorticoids and stressful situations associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis. Patients should also ensure adequate carbohydrate (glucose) nutrition to prevent development of a fasting state. Currently, fasting-induced attacks are treated with glucose and the fact that this and related sugars prevent the fasting-induced increases in 5-ALAS activity and heme utilisation by TDO provides support to the present hypothesis. Many drugs are known to inhibit TDO activity by preventing its conjugation with heme. These include allopurinol [71] and antidepressants [73–77]. Nicotinamide, like glucose, inhibits apo-TDO activity by an NAD(P)H-mediated allosteric mechanism [58]. The safety of drugs, including antidepressants, in porphyrias will be considered below.

Role of PGC-1 α in fasting-induced porphyric attacks

More recently, the effects of fasting and glucose on 5-ALAS have been explained in terms of changes in the peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α). Expression of the latter and of 5-ALAS mRNA is increased by fasting and diminished by feeding [78]. It is thought that this role of PGC-1 α is limited to the effects of fasting and glucose intake on porphyrin metabolism in the hepatic porphyrias. To what extent, if any, does this mechanism relate to changes in the regulatory heme pool and its utilisation by TDO is not clear. Evidence, however, is emerging to justify exploration of a possible relationship and the following relevant questions are worthy of addressing. As PGC-1 α induces heme oxygenase 1 (HO1) [78], does the resulting decrease in heme trigger 5-ALAS induction? As far as I could ascertain, the time course of the effect of PGC-1 α on HO1 has not been reported, but a 2.5–3.0-fold increase in 5-ALAS mRNA expression is observed in mice fasted for 6 and 24 h [78]. mRNA expression of PGC-1 α is however decreased from 6- to 3-fold at these time intervals respectively [78]. Starvation for longer time intervals was not examined and it is therefore important to establish if levels of PGC-1 α continue to be elevated beyond 24 h. In our study in rats [79], we reported a twofold induction of 5-ALAS activity by starvation for 24 and 48 h, coinciding with depletion of heme through its utilisation by TDO secondarily to glucocorticoid induction of the apoenzyme. However, 5-

ALAS activity returned to levels observed in fed rats at 72 and 96 h, when heme availability was increased by Trp released by protein breakdown, as assessed by an increased heme saturation of TDO. We also observed that HO activity was enhanced by 109% and 115% at 24 and 48 h of starvation respectively, but activity subsided to only +33% at 72 h before a final return to fed levels at 96 h. A study of the sequence of events in a fuller time-course of the effects of starvation of rats or mice on PGC-1 α , 5-ALAS, HO1 and TDO would be informative. Under the above conditions, P-450 levels were increased over the entire 96 h starvation period, by 63, 50, 55 and 50% respectively [79]. Another point to address is that of establishing if utilisation of the regulatory heme pool by TDO during starvation plays a role in the increased expression of PGC-1 α . This can be tested by using TDO inhibitors other than glucose, e.g., allopurinol, to block this utilisation. Alternatively, PGC-1 α levels could be measured to see if they are elevated in 24 h-starved guinea pigs, despite the absence at this starvation time point of 5-ALAS induction or heme utilisation by TDO in this species (see below the further discussion of species differences).

Maintenance of a symptom-free state

As well as avoiding fasting through dieting or calorie restriction, patients with acute hepatic porphyrias should avoid drugs and chemicals that precipitate acute attacks. Lists of safe and unsafe drugs have been published (see, e.g., [21,80]). Whereas, in experimental settings, drugs that exacerbate the hepatic porphyria potentiate the 5-ALAS induction by porphyrogens by causing a further depletion of heme, more recent evidence suggests that these drugs cause a direct induction of 5-ALAS that is independent of increased P-450 synthesis [81]. Examples include carbamazepine [53], estrogens [82], ethanol in doses of up to 2 g/kg (in rats) [83], sulphonamides [84] and phenobarbitone [81].

Drugs and chemicals thought to be safe or unsafe in the porphyrias and whose effects on TDO activity have been reported are listed in Table 4. According to the present hypothesis, safe drugs are those which in the main inhibit TDO utilisation of the regulatory-heme pool, thereby allowing this pool to repress 5-ALAS synthesis, whereas, by contrast, unsafe drugs will induce 5-ALAS either directly or secondarily to facilitating heme utilisation by TDO, and their 5-ALAS induction will be reflected in increased TDO saturation with heme. Not all drugs in both categories, however, can be expected to follow these strict criteria and so there will be some exceptions. These and further explanatory comments will be made below in conjunction with the information in Table 4.

Safe drugs

Adrenaline, allopurinol, aspirin, benserazide, chlorpromazine and noradrenaline: Adrenaline and noradrenaline increase the TDO heme saturation (not utilisation) by acting via Trp [85]. With catecholamines, release of albumin-bound Trp is achieved by elevation of NEFA secondarily to enhancement of lipolysis by a c-AMP-dependent mechanism. AMP also causes direct inhibition *in vitro* of 5-ALAS [86]. Trp itself, which inhibits 5-ALAS activity and increases the heme saturation of TDO by enhancing heme biosynthesis beyond the 5-ALAS step [65,66], has not been used clinically in hepatic porphyrias, but could be a potential candidate (see below). Allopurinol inhibits TDO apoenzyme conjugation with heme under both utilisation and saturation conditions, hence reversing 5-ALAS induction by starvation [59] and potentiating 5-ALAS inhibition by Trp [65]. The active moiety of aspirin, salicylate, inhibits TDO directly *in vitro* [87] and after administration [87,88]. By inhibiting the holoenzyme directly and by preventing the apoenzyme conjugation with heme, salicylate is almost certain to inhibit heme utilisation by TDO. However, its 5-ALAS inhibition is likely to be mediated by Trp [66]. In an earlier study [89] in which 5-ALAS induction in chick embryo liver cell cultures was tested by measuring intensity of fluorescence, neither salicylate nor aspirin caused 5-ALAS

Table 4
Effects of safe and unsafe drugs in acute hepatic porphyrias on activity and heme utilisation or saturation of rat liver tryptophan 2,3-dioxygenase.

Drug	TDO-enzyme				Mechanism	Refs.
	Holo	Total	Apo	Heme U or S		
<i>Safe drugs:</i>						
Adrenaline	↑↑	↑	↓	↑↑S	via Trp (↑NEFA)	[85]
Noradrenaline	↑↑	↑	↓	↑↑S	via Trp (↑NEFA)	[85]
Allopurinol	-	↓↓	↓↓	↓↓U	↓ conjugation	[59,71]
Aspirin	↓↓	↓↓	↓↓	↓↓U	↓ conjugation, direct	[87,88]
Benserazide	↓↓	↓↓	↓↓	↓↓U	↓ conjugation	[65]
Chlorpromazine	↓	↓	↓	↓U	↓ conjugation, direct?	[73]
Corticosteroids ¹	-	-	-	-	-	[70]
Estrogens ²	↓	↓	↓	↓U	↓ conjugation, direct	[95]
Fluoxetine	-	↓	↓↓	↓↓U	↓ conjugation, direct	[104]
Li ⁺	-	↓	↓↓	↓↓U	↓ conjugation	[105]
Lofepamine	-	↓	↓↓	↓↓U	↓ conjugation, direct	[76]
Morphine	↑↑	-	↑↑	↑↑S	via Trp (↑NEFA)	[66]
Naloxone*	-	-	-	-	↓NEFA	[66]
Paroxetine	-	↓	↓↓	↓↓U	↓ conjugation, direct	[75]
Progestogens ²	↓	↓	↓	↓U	↓ conjugation, direct	[95]
Propranolol*	-	-	-	-	↓NEFA	[66]
<i>Unsafe drugs:</i>						
Amitriptyline ⁷	-	↓↓	↓↓	↓↓U	↓ conjugation, direct	[73]
Barbiturates	↑	-	↓	↑U	↑conjugation	[60]
Carbamazepine	↑↑	↑	-	↑↑U	↑conjugation	[53]
Chloramphenicol ³	?	↓	↓	↓U	↓conjugation?	[117]
Estrogens ⁴	↑	↑	↑	↑U	↑conjugation	[110]
Ethanol	↑↑	↑	↑	↑↑U	↑conjugation	[83]
Fasting	↑	↑	↑	↑U	↑conjugation	[59]
Glucocorticoids ⁵	↑↑	↑↑	↑↑	↑U	↑conjugation	[57]
Griseofulvin ⁶	↑,↑↑	↑↑, -	↑↑, -	↓,↑	↓, ↑ saturation	[41]
Imipramine ⁷	-	↓↓	↓↓	↓↓U	↓ conjugation, direct	[73]
Phenylbutazone	-	-	-	?	?	[72,127]
Phenytol	-	-	-	?	?	[74]
Progestogens ⁴	↑	↑	↑	↑U	↑conjugation	[112]
Tranlycypromine ⁷	-	↓↓	↓↓	↓↓U	↓conjugation	[73,74]

↓: moderate decrease (≤40%); ↓↓: strong decrease (≥50%); ↑: moderate increase (≤40%); ↑↑: strong increase (≥300%).

¹ Denotes corticosteroids that do not induce TDO, exemplified here by deoxycorticosterone.

² Denotes safety when used in small doses, e.g. as contraceptives.

³ Denotes indirect effects involving cortisol induction.

⁴ denotes not safe in larger doses.

⁵ Denotes several TDO-inducing corticosteroids, exemplified here by cortisol and are discussed in the text.

⁶ Denotes early (4 h) and late (24 h) effects.

⁷ Denotes differences from safe antidepressants as discussed in the text.

* Denotes ability to prevent changes induced by lipolytic agents, e.g. morphine.

induction. Benserazide inhibits 5-ALAS in starved rats and potentiates the inhibition induced by Trp most likely by preventing heme conjugation to apo-TDO [65]. It is also possible that benserazide may directly inhibit 5-ALAS activity by inactivating its pyridoxal 5'-phosphate (PLP) cofactor by virtue of being a hydrazine compound. Other PLP-dependent enzymes are inhibited by benserazide, e.g. aromatic L-amino acid decarboxylase, kynureninase and kynurenine aminotransferase [90,91]. Chlorpromazine inhibits TDO activity moderately (by 23–28%) [73] and decreases 5-ALAS activity after acute administration [92]. Its chronic administration has been reported [93] to inhibit 5-ALA

dehydratase activity.

Corticosteroids: Corticosteroids are generally referred to in the literature as safe in acute hepatic porphyrias [94]. This generalisation may be based on the little information available on 5-ALAS induction. However, I consider that glucocorticoids, which are TDO inducers, are likely to be unsafe. The mineralocorticoid deoxycorticosterone does not induce TDO [70]. As far as I could ascertain, the effects of deoxycorticosterone and the other (main) mineralocorticoid aldosterone on 5-ALAS and those of the latter steroid on TDO activity have not been reported. The potential unsafe status of glucocorticoids will be discussed below.

Estrogens and progestogens in small doses: When used in small doses, as contraceptives, estrogens and progestogens may prevent cyclic monthly attacks of acute porphyria in some women [80]. Estradiol administration inhibits rat liver TDO activities and Trp metabolism in women [95–98]. Progesterone [95] and the synthetic progestogen medroxyprogesterone [99] also inhibit TDO activity. As inhibition involves heme utilisation, the likelihood of estradiol and progesterone inhibiting 5-ALAS activity is strong. Estradiol exhibits an oscillatory behavior towards 5-ALAS [100]. A 4 µg intravenous dose produces a series of oscillations in 5-ALAS activity lasting over several days, with a rebound rise following an initial inhibition. The latter has a rapid onset, with inhibition at 1, 2, 3 and 4 h being ~40%, 48%, 60% and 80% respectively [100]. The authors suggested that the oscillatory changes in 5-ALAS are limited to changes in enzyme amount, rather than involving inhibition or activation and that endocrine changes may be among determinants of these changes. The rebound phenomenon is not without precedent. TDO inhibition by chronic administration to rats of a range of drugs of dependence (ethanol, morphine, nicotine and phenobarbitone) [101] or of glucose or nicotinamide [58] results upon withdrawal in rebound glucocorticoid induction mediated by corticosterone elevation. Whether estradiol elicits a rise in corticosterone under the experimental conditions of the above paper [100] is unknown. The potential effect of progesterone or its derivatives on 5-ALAS is less clear. Progesterone induces a minimal fluorescence in cultured chick embryo liver cells [89] and exerts no effect on liver 5-ALAS activity in estrogen-primed chicks [102]. However, in these 2 studies, measurements were made at relatively late time points: 22 h [89] and 8 h [102], thus missing possible changes in 5-ALAS at earlier time intervals of exposure. As will be discussed below, relatively large doses of progesterone induce 5-ALAS.

Whereas the female sex hormones estrogens and progestogens can in small doses prevent monthly cyclical porphyric attacks in women, they are thought to be the worst offenders [100]. It is therefore surprising that their effects on 5-ALAS have not been studied in greater detail. Administration of the synthetic estrogens ethinylestradiol, norethindrone and norethandrolone has, however, been shown to enhance rat liver 5-ALAS activity and to inhibit TDO conjugation with heme, presumably by heme destruction (Abstract in Ref. [103], unpublished work).

The antidepressants fluoxetine, lofepramine and paroxetine and the mood stabiliser Li⁺: These 4 drugs are also safe in porphyria. The 3 antidepressants inhibit apo-TDO conjugation with heme, thereby preventing heme utilisation by TDO [75,76,104]. With Li⁺, the TDO inhibition is transient (reaching a maximum at 1 h) [105], in contrast with the antidepressants 2 h maximum. Relative TDO inhibitory potencies are illustrated in Ref. [106]. 5-ALAS activity has not been measured with the above 3 antidepressants, but urinary excretion of 5-ALA and porphobilinogen was not significantly altered by lofepramine [107] or fluoxetine or paroxetine [108], thus suggesting that these antidepressants do not induce 5-ALAS. Whether they can inhibit 5-ALAS in other liver systems remains to be assessed.

Morphine, naloxone and propranolol: Morphine, propranolol and naloxone are also safe in the acute hepatic porphyrias. Morphine inhibits 5-ALAS activity in 24 h-starved rats and increases TDO saturation with heme [66]. This suggests that morphine acts via Trp, most likely by

enhancing lipolysis, thereby increasing free serum and liver [Trp] [66]. Accordingly, the β -adrenoceptor blocker propranolol blocks the morphine inhibition of 5-ALAS activity, the increased TDO saturation with heme, the NEFA elevation and the increased free serum and liver [Trp] [66]. The opioid antagonist naloxone also blocks the morphine effects on 5-ALAS and TDO heme saturation [66]. By contrast, the effects on both parameters of direct provision of Trp by its administration or that of its direct displacers from albumin-binding sites, such as palmitate and salicylate, are insensitive to propranolol [66]. It may therefore be concluded that the morphine effects, though mediated by Trp, are initiated by lipolysis. Propranolol also blocks the starvation-induced increase in heme utilisation by TDO and the elevation of serum free and liver Trp [70] thereby preventing 5-ALAS induction.

Unsafe drugs

As stated above, unsafe drugs are likely to induce 5-ALAS activity. Such induction has been demonstrated with carbamazepine [53], estrogens and progesterone in larger doses than those used in birth control or after elevation during the menstrual cycle [82,89,106], ethanol in doses of up to 1.6 g/kg (in rats) [83], phenobarbitone [81] and sulphonamides [84].

Carbamazepine: With carbamazepine, the 5-ALAS induction is secondary to increased heme utilisation by TDO, because the increased heme saturation precedes 5-ALAS induction [53]. Effects of other anticonvulsant drugs are described below.

Estrogens and progestogens (large doses): With larger doses of estrogens and progestogens, 5-ALAS induction may involve their metabolites, which are stronger inducers than the parent compounds [109]. A more direct effect could also be involved. For example, a time-course study in chick embryo liver *in ovo* of effects of progesterone [110] showed that 5-ALAS activity is doubled at 1 h and reaches 5-fold at 10 h. Whereas TDO induction by estrogens is likely to be mediated by glucocorticoids [111–113], potential TDO induction by progesterone is a less certain phenomenon. Various reports suggest that this progesterone inhibits TDO, though it may induce a modest increase associated with elevation of corticosterone in non-stressed rats [114]. As far as I could ascertain, the potential effects of progesterone and its metabolites on TDO activity have not been adequately explored.

Ethanol: With ethanol, the 5-ALAS induction in rat liver is biphasic regarding dosage. Doses of 0.8 and 1.6 g/kg body weight produce a linear increase, but enzyme activity begins to decline with larger doses [83]. With the largest 5-ALAS-inducing dose of ethanol (1.6 g/kg), the % heme saturation of TDO is only moderately increased. 5-ALAS induction by larger ethanol doses is blocked by the alcohol dehydrogenase inhibitor pyrazole and the aldehyde dehydrogenase inhibitor disulfiram, but is potentiated by propranolol [83]. It may therefore be concluded that ethanol induces 5-ALAS by virtue of its own metabolism, but without mediation by acetaldehyde or acetate and that, as the propranolol results suggest, heme utilisation by TDO may be responsible.

Fasting: The effects of fasting on 5-ALAS and TDO utilisation of heme have already been discussed above and an entry for fasting is inserted in Table 4 among unsafe “drugs”.

Phenobarbitone: With phenobarbitone, increased heme utilisation is the most likely cause of the 5-ALAS induction. Thus, phenobarbitone increases the TDO holoenzyme activity by 73–100% at 24 h after administration in drinking water and by 48% and 150% at 2 and 3 days after administration to starved rats [60]. In fed rats, the increase in the TDO holoenzyme activity induced by cortisol, Trp and 5-ALA, but not that by heme, is further potentiated by phenobarbitone [60].

Chloramphenicol and griseofulvin: Chloramphenicol inhibits heme biosynthesis at the ferrochelatase step [115,116]. Its inhibition of Fe^{2+} incorporation into protoporphyrin IX in bone marrow cultures is rapid (within hours) [115]. However, it was reported [117] to inhibit 5-ALAS activity in rabbit reticulocyte mitochondria after chronic treatment. As

inhibition of heme biosynthesis removes the negative feedback control, 5-ALAS should be induced as a consequence. Whether it induces 5-ALAS at certain time intervals remains to be established from detailed time-course experiments. Another ferrochelatase inhibitor is griseofulvin, which has been reported to induce 5-ALAS mRNA expression in mouse liver significantly after 2 days of treatment [118]. That effects on TDO activity and heme saturation after griseofulvin administration are time-dependent is illustrated by the findings [41] that, at 4 h after griseofulvin, TDO saturation with heme is decreased, whereas the opposite is true at 24 h. The early heme depletion can be attributed to ferrochelatase inhibition, whereas the later increase in heme saturation reflects the rebound enhancement of heme synthesis. The effects of chloramphenicol on TDO activity and heme saturation have not been thoroughly investigated. Although it inhibits protein synthesis in microbial organisms, it does so moderately in mammals. It has been shown [111] to cause a moderate decrease (38%) of the net induction of TDO by cortisol. Thus, whereas TDO activity is 2.52-fold higher after cortisol administration alone, this value is decreased to 1.94-fold in the presence of chloramphenicol. Only the total TDO activity was determined in this study [119]. As will be discussed below with unsafe antidepressants, the weak inhibition by chloramphenicol of TDO induction by cortisol renders chloramphenicol incapable of exerting a significant inhibition of heme utilisation by TDO during starvation.

Glucocorticoids: Glucocorticoids that induce TDO synthesis and hence utilisation of the regulatory-heme pool should be considered unsafe or usable with caution in acute hepatic porphyrias. TDO induction has been demonstrated with the natural glucocorticoids corticosterone [70], cortisone [120] and cortisol [121] and the synthetic ones prednisolone [122], dexamethasone [123] and triamcinolone [124]. The permissive effect of glucocorticoids on 5-ALAS induction by the porphyrogen AIA has been documented. Thus, in adrenalectomized female Sprague-Dawley rats, the poor ability of AIA to induce liver 5-ALAS is corrected by cortisol administration, although cortisol alone does not induce 5-ALAS [125]. The authors could not propose an explanation of this permissive effect of cortisol other than enhancement of RNA synthesis. Our explanation of the permissive effect is based on TDO utilisation of the regulatory-heme pool [69]. Thus, in adrenalectomized male Wistar rats, 5-ALAS induction by AIA is impaired by 78%, but cortisol reverses this deficit and restores induction to 14% above the AIA value in sham controls [69]. We have confirmed [69] the lack of 5-ALAS induction by cortisol in controls or adrenalectomized rats [125]. The poor induction of 5-ALAS by AIA in adrenalectomized rats is most likely due to the absence of utilisation of the regulatory-heme pool by TDO which occurs normally with starvation. By inducing TDO synthesis, cortisol enables this latter enzyme to utilise the heme pool, thereby initiating derepression of, and restoring the AIA ability to fully induce, 5-ALAS [69].

Another example of the permissive effect of glucocorticoids on 5-ALAS induction is provided by a study in the isolated perfused rat liver [126]. In this system, 5-ALAS activity declines rapidly from the steady-state value observed *in vivo* and AIA causes a moderate induction of the enzyme during perfusion. However, addition of dexamethasone restores 5-ALAS activity to the *in vivo* value and enhances markedly the AIA induction [126]. This permissive effect of dexamethasone can also be explained by TDO induction leading to increased utilisation of the regulatory-heme pool.

Phenylbutazone: Phenylbutazone does not induce 5-ALAS, but potentiates induction by DDC [43]. This potentiation was observed at time intervals of ≥ 17 h after administration, but not at 5 h. A potential early effect of phenylbutazone on 5-ALAS activity is currently unknown. The drug is also ineffective against TDO. It does not inhibit the basal enzyme activity nor the cortisol induction [127]. It, however, potentiates the early loss of heme by TDO in DDC-treated rats, in common with other exacerbators of porphyrias [72], irrespective of their ability to induce 5-ALAS.

The anticonvulsants phenytoin and valproate: 5-ALAS activity is

Table 5
Classification of safe and unsafe drugs in hepatic porphyrias according to the proposed hypothesis

Safe drugs (↓ 5-ALAS induction)		Unsafe drugs (↑ 5-ALAS induction)	
↓ TDO heme utilisation	↑ TDO heme saturation via Trp	↑ TDO heme utilisation	Failure to block heme utilisation
Allopurinol	Trp	Barbiturates	Amitriptyline
Aspirin	Adrenaline (↑ NEFA)	Carbamazepine	Imipramine
Corticosteroids	Noradrenaline (↑ NEFA)	Estrogens (large dose)	Tranlycypromine
Estrogens (low dose)	Morphine(↑ NEFA)	Ethanol	
Fluoxetine		Fasting	
Li ⁺		Glucocorticoids	
Lofepamine		Griseofulvin (late effect)	
Naloxone (↓ NEFA)		Progestogens (large dose)	
Paroxetine			
Propranolol (↓ NEFA)			
Progestogens (low doses)			
			Mechanism unknown
			Chloramphenicol
			Phenylbutazone, Benserazide
			Phenytoin, Chlorpromazine

Abbreviations used: NEFA (non-esterified fatty acids); 5-ALAS (5-aminolevulinate synthase); Trp (tryptophan); TDO (Trp 2,3-dioxygenase: formerly Trp pyrrolase).

enhanced by the anticonvulsants phenytoin and valproate [128–130]. In these studies, induction was observed after repeated daily treatments, with the first significant induction occurring after 1 day [129]. TDO activity is not altered at 2 h after acute administration of phenytoin [74]. As far as I could ascertain. A potential effect of valproate on TDO activity has not been reported. Further work is clearly required to assess the effects of these two anticonvulsant drugs on TDO over a detailed and longer time-course.

The antidepressants amitriptyline, imipramine and tranlycypromine: Whereas some antidepressants are safe in acute hepatic porphyrias, others are not. Unsafe antidepressants listed in Table 4 are amitriptyline, imipramine and tranlycypromine. Dothiepin, fluvoxamine and flupentixol are also unsafe, but are not listed as they have not been tested in the context of the present hypothesis. As far as I could ascertain, only amitriptyline has been tested for induction of 5-ALAS and was found to cause a 2.19–2.34-fold induction at 12 and 24 h respectively following administration to rats [131]. A large number (23) of antidepressants prevent apo-TDO conjugation with heme [73–77]. How to distinguish between safe and unsafe antidepressants in the context of heme utilisation by TDO can be attempted by examining the effects of both types of antidepressants on the TDO response to induction by glucocorticoids and activation by the heme cofactor. The first of these responses occurs in fasting, whereas the second occurs when heme synthesis is already established or during heme therapy. In our previous studies, we observed that, although most antidepressants tested at a 10 mg/kg body wt dose inhibited rat liver TDO activity induced by prior cortisol, or activated by prior hematin, administration, inhibition by a small dose (0.5 mg/kg: corresponding to that within the therapeutic dose range in humans) was observed only with safe, but not with unsafe, antidepressants. Thus, at a 10 mg/kg dose, amitriptyline fails to inhibit the cortisol-induced enzyme and causes only a 15% decrease in the heme saturation of TDO activated by prior treatment with hematin [74]. A similar dose of imipramine fails to inhibit the hematin-activated enzyme and causes little change in the apoenzyme activity (8%) of the cortisol-induced enzyme [74]. The small dose of tranlycypromine fails to inhibit the hematin-activated TDO and causes a modest decrease (12–19%) in the holoenzyme and total enzyme activities induced by cortisol [74]. By contrast, antidepressants that are safe in porphyria are more effective as TDO inhibitors under these conditions. Thus, paroxetine [75] at a 0.5 mg/kg dose inhibits activities of the cortisol-induced holoenzyme, total enzyme and apoenzyme by 28%, 40% and 52% respectively and those of the hematin-activated enzyme by 37%, 19% and 23% respectively. Lofepamine [76] is even better, with corresponding inhibition of 41%, 51% and 61% for the cortisol-induced enzyme and 43% and 37% for the holoenzyme and total enzyme activities of the hematin-activated TDO. Desmethylinipramine, a major metabolite of lofepramine, is broadly as effective as its parent compound (19%, 51% and 82% for the cortisol-induced, and 36% and 18% for the hematin-activated, enzyme) when given in a similarly small dose [76].

Another important question regarding the efficacy of antidepressant drugs as TDO inhibitors is their ability to decrease circulating glucocorticoids: a potentially useful effect in fasting by porphyric patients. Previous studies have demonstrated the ability of some antidepressants to significantly lower rat serum corticosterone (acute citalopram, lofepramine, mianserin, paroxetine and viloxazine and chronic desmethylinipramine), but not other antidepressants such as tranlycypromine, moclobemide, sertraline and acute desmethylinipramine (for a discussion and references, see Ref. [106]). It is noteworthy that pretreatment with antidepressants does not prevent glucocorticoid induction of TDO [75,76]. This account therefore provides a reasonable explanation of the differential safety of antidepressants in the hepatic porphyrias. More potent TDO inhibitors have been and/or are being developed to overcome immune escape by tumors and there is no reason why their use in the hepatic porphyrias could not be explored.

The safe and unsafe drugs listed in Table 4 can now be classified under 2–3 headings each, according to the proposed hypothesis. As shown in Table 5, the 17 safe drugs inhibit 5-ALAS activity either by inhibiting TDO utilisation of the regulatory-heme pool or by acting via Trp either directly (Trp itself) or by a NEFA-mediated release of albumin-bound Trp. Of the 14 unsafe drugs, 11 induce 5-ALAS by increasing heme utilisation by TDO or failing to prevent this increase. Only 3 cannot be assigned. Future studies assessing the effects of safe and unsafe drugs on 5-ALAS activity and heme utilisation by TDO will establish more clearly the validity or otherwise of the present hypothesis.

Therapy of acute attacks by heme preparations

Therapy of acute porphyric attacks by hematin or heme arginate should result in cofactor activation of TDO. This is likely to result in increased hepatic Trp degradation and a consequent decrease in plasma Trp availability for cerebral serotonin synthesis. These changes are, however, expected to occur temporarily only during heme therapy and are therefore unlikely to precipitate episodes of dysphoria or depression, neither of which has been reported in heme therapy studies. The occurrence of depression in the hepatic porphyria cannot be explained by a defective availability of Trp for brain serotonin synthesis, because there is always sufficient heme synthesis in symptomless patients to maintain TDO at normal levels. Thus, plasma total (free + albumin-bound) [Trp] (which is controlled by TDO [55]) immediately before heme arginate administration appears to be normal ($69 \pm 9 \mu\text{M}$: mean \pm SEM) but is promptly decreased to $44 \pm 5 \mu\text{M}$ upon heme arginate treatment [132]. It is therefore unlikely that serotonin synthesis will be impaired in symptomless patients. This is unlike the situation in experimental porphyria models [133], wherein decreased TDO activity due to heme destruction by experimental porphyrogens leads to decreased hepatic Trp degradation and a consequent increase in plasma Trp availability for brain serotonin synthesis. Regarding experimental porphyria models, it should be pointed out that these models may

possess a biphasic mode of action: the above effects [133], which occur rapidly (within a few hours) and later effects of the opposite nature, which are observed at some ~16 h after administration of experimental porphrogens, namely TDO activation and decreased hepatic [Trp] and [serotonin] [134]. A situation of this nature in patients can explain the incidence of depression. Other than the above decrease in plasma [Trp] [132], very little work on Trp metabolism and disposition in the human hepatic porphyrias has been reported. Increased urinary excretion of Trp metabolites of the kynurenine pathway (Kyn, KA and XA), especially after an oral Trp load, has been reported in acute porphyric patients, but not in those in remission or in chronic porphyria [135]. The pattern of metabolite excretion after Trp loading of acute porphyric patients is consistent with enhancement of TDO and inhibition of kynurenine aminotransferase, kynurenine monooxygenase and kynureninase. Although the pattern of urinary Kyn metabolite excretion is consistent with functional vitamin B₆ deficiency, it is not corrected by B₆ supplementation. A more recent study [136] concluded that acute intermittent porphyric patients in a symptomless phase exhibit decreased Kyn aminotransferase activity and induction of IDO. This latter conclusion is based on an increase in the urinary [Kyn]/[Trp] ratio: a marker not specific to IDO, but applies equally well, among others, to TDO (for a review, see Ref. [137]). Also, urinary metabolite excretion encompasses whole body Trp metabolism and while the liver is the major site of Kyn production, subsequent degradation of Kyn also occurs elsewhere in the body, notably the kidney. Further studies are therefore required to clearly define the Trp status in acute hepatic porphyrias preferably through analysis of plasma Trp and Kyn metabolites and other relevant measurements.

The potential role of Trp during heme therapy of acute porphyric attacks is worthy of further comment and is the subject of an account later on in this text.

Heme regulation in mammals lacking the free apo-TDO

An important question to address in the context of the current hypothesis is the status of the regulatory heme pool in mammalian species lacking the free apo-TDO and its glucocorticoid induction mechanism. Of these species, the guinea pig and to a lesser extent the golden (Syrian) hamster have been the most studied [4,138]. The Guinea pig exhibits cortisol resistance, characterised by high circulating and urinary cortisol levels and a glucocorticoid receptor with a 20-fold lower affinity for dexamethasone than that of the mouse [139]. Unlike the rat, starvation of guinea pigs for 24 h fails to enhance TDO activity and hence heme utilisation by the apoenzyme [59] and also fails to alter significantly liver 5-ALAS [59,140], presumably because of the absence of glucocorticoid induction of apo-TDO synthesis. The guinea pig TDO does not exhibit activation by 5-ALA administration or by hematin *in vitro* [138]. Only Trp administration enhances TDO activity, which is expressed totally as the active holoenzyme, but agents which activate the rat liver enzyme by the release of albumin-bound Trp, such as salicylate and ethanol, fail to activate the guinea pig enzyme [138]. Similar responses are also observed with 2 other species lacking the free apo-TDO and its glucocorticoid induction mechanism, the golden hamster and frog [4]. Other differences in TDO activation by Trp are observed between rat on the one hand and guinea pig and golden hamster on the other, notably rapid TDO activation by small doses of Trp in the latter 2 species, but not in rats [4,138].

TDO activation by Trp in rat [57] and guinea pig [138] is not blocked by the transcriptional inhibitor actinomycin D. The translational inhibitor cycloheximide completely blocks the TDO activation in rats, but only partially (by 34%) in guinea pig [138]. In the rat, it was suggested that Trp activates TDO by enhancing heme biosynthesis, most probably at the 5-ALA dehydratase step [57,65]. It is possible that this mechanism may play a partial role in the Trp activation of TDO in guinea pigs. The rapidity with which this activation occurs in guinea pigs and hamsters was also suggested to be due to release of a latent

form of the TDO apoenzyme by Trp, rather than enhanced apoenzyme synthesis [138]. In the rat, a 1–2 h period is required for Trp to activate TDO. Also, as allopurinol blocks the Trp activation of the rat TDO by preventing its conjugation with heme, synthesis can reasonably be excluded. It is possible that Trp may play multiple roles involving in part control of the regulatory heme pool in these species. Further work is therefore required to assess these possibilities, which may yield important information on potential mechanisms of heme regulation in various mammalian species.

Other therapies targeting 5-ALAS

Gene silencing

Both glucose and heme preparations target 5-ALAS in acute hepatic porphyrias. Glucose therapy is aimed mainly at fasting-induced and other mild attacks, whereas heme therapy is reserved for stronger ones. A more recent development in 5-ALAS targeting is the use of gene silencing in the form of 5-ALAS1-siRNA. This has been shown in a pre-clinical study in a mouse model of an acute porphyric attack to lower plasma 5-ALA and porphobilinogen [141] and a Phase I trial of the ALAS1-siRNA fraction givosiran™ [6] resulted in reductions in induced ALAS1 mRNA levels, a near normal return of 5-ALA and porphobilinogen levels and a lower attack rate than that observed with placebo. This new gene therapy is indeed promising at this early stage of its clinical development and despite the small numbers of study subjects. A number of issues are noteworthy: (1) the occurrence of serious or severe adverse events in 12–18% of patients; (2) a 79% success rate in prevention of recurring attacks; (3) the need to use concomitant hemin therapy to reduce frequency of attacks; (4) the speed of lowering of plasma 5-ALA and porphobilinogen levels. In the clinical trial [6], the first measurements of urinary 5-ALA and porphobilinogen were performed 1 day after a single injection of siRNA, whereas in the mouse study [141], the first significant decreases in plasma levels of these 2 intermediates were observed at 8 h. A rapid response (i.e. within a few hours) is therefore unlikely, but could be assessed. With hemin treatment, no significant effects are observed up to 16 h [141]. A rapid response in terms of 5-ALAS inhibition occurs when Trp is given jointly with 5-ALA. This and several other advantages render Trp a potential monotherapy or adjunctive therapy of acute porphyric attacks as detailed below.

The case for tryptophan as a potential therapy of acute hepatic porphyric attacks

The data described in Table 6 represent a model of, and provide a case for, the use of Trp in therapy of acute porphyric attacks. All current therapies are aimed at targeting 5-ALAS induction through repression of its synthesis by glucose, heme or the proposed gene silencing in order to lower levels of 5-ALA and other intermediates of the pathway. In the present model, raised levels of 5-ALA are (presumably) replicated by its exogenous administration [142,143] and the increased conversion of 5-ALA into heme is reflected in the 39% increase in the heme saturation of TDO and in the simultaneous 73% decrease in 5-ALAS activity. These changes are rapid, occurring at 1 h after a single 5-ALA dose. What is more striking is the near complete (96%) inhibition of 5-ALAS activity when Trp is administered even 0.5 h after 5-ALA. Under these conditions, TDO becomes fully (100%) heme-saturated. The Trp effects on the TDO holoenzyme activity and the % heme saturation in the presence of 5-ALA are not simply additive, but reflect potentiation: a 48% higher holoenzyme and a 59% higher % heme saturation over the sum of the corresponding separate effects. This suggests that Trp promotes the conversion of 5-ALA into heme, possibly by enhancing 5-ALA dehydratase activity. This possibility is supported by the ability of the dehydratase inhibitor Pb²⁺ to block the Trp-induced inhibition of 5-ALAS activity and the associated increase in the TDO heme saturation

Table 6

Near complete inhibition of rat liver 5-aminolevulinatase synthase activity by joint administration of tryptophan and 5-aminolevulinatase: a model for tryptophan therapy of acute hepatic porphyrias

Treatment	5-ALAS activity (nmol 5-ALA/ min/g liver)	TDO activity (μmol kynurenine/g liver/h)		TDO heme saturation %
		Holoenzyme	Total enzyme	
<i>Experiment 1</i>				
Control	0.88 \pm 0.08	4.1 \pm 0.33	10.0 \pm 0.24	41 \pm 3
Trp	0.66 \pm 0.02	6.6 \pm 0.39	10.7 \pm 0.83	62 \pm 2
5-ALA	0.24 \pm 0.04	5.8 \pm 0.25	10.2 \pm 0.66	57 \pm 3
Trp + 5-ALA	0.04 \pm 0.02	10.3 \pm 0.61	10.3 \pm 0.60	100 \pm 1
<i>Experiment 2</i>				
Control	0.80 \pm 0.07	3.9 \pm 0.22	9.5 \pm 0.35	41 \pm 3
Trp	0.27 \pm 0.02	6.3 \pm 0.24	10.3 \pm 0.47	61 \pm 2
Hematin	0.46 \pm 0.02	7.6 \pm 0.33	10.9 \pm 0.44	70 \pm 3
Trp + hematin	0.51 \pm 0.04	6.7 \pm 0.69	9.7 \pm 0.54	69 \pm 5

Adapted from Table 2 in Ref. [65]: (Badawy et al. Biochem J 1981; 198: 309-314: Tryptophan pyrrolase in haem regulation – the mechanism of the opposite effects of tryptophan on rat liver 5-aminolevulinatase synthase activity and the haem saturation of tryptophan pyrrolase). Trp (75 mg/kg), 5-ALA (15 mg/kg) and hematin (2 mg/kg) were administered intraperitoneally to 48 h-starved male Wistar rats either separately or in combination. Rats were killed at 0.5, 1 and 2 h respectively after their administration. Values are means \pm SEM for each group of 4 rats. Compared with the respective controls, the effects of all treatments on 5-ALAS activity, TDO holoenzyme activity and the % heme saturation were statistically significant. With the total TDO activity, only the value with hematin was significantly different from the group control. Significance (*P*) values and other details are in Ref. [65]. Abbreviations used: 5-ALA (5-aminolevulinatase); 5-ALAS (5-aminolevulinatase synthase); Trp (tryptophan); TDO (tryptophan 2,3-dioxygenase, formerly Trp pyrrolase).

[65]. The Trp-induced increase in hepatic heme biosynthesis is likely to be considerable, given that the amino acid at a 92 mg/kg dose causes at 2 h a 25% increase in microsomal heme [144], which represents at least two-thirds of the total liver heme content. The data in Table 6 also show that no potentiation of the decrease in 5-ALAS activity or the increase in TDO heme saturation occurs when Trp is given to rats previously treated with hematin, thus suggesting that both Trp and hematin do not act by different mechanisms.

Advantages of Trp over heme therapy are several-fold: (1) a strong and rapid inhibition of 5-ALAS synthesis resulting from (2) rapid induction of heme biosynthesis, presumably involving (3) rapid conversion of 5-ALA into heme and (4) a potential consequential rapid lowering of 5-ALA levels which should result in (5) rapid resolution of the neurological and bodily symptoms and (6) avoidance of the side effects of hematin or heme arginate injections at the injection sites and internally; (7) provision of a potentially specific therapy of 5-ALA dehydratase porphyria and its Pb^{2+} counterpart. Trp can be used alone or as an adjunct to gene silencing in preference to heme infusions.

Another area wherein Trp can provide a further advantage is production of its neuroprotective metabolite kynurenic acid (KA). As stated above, acute porphyric attacks are likely to be associated with neurotoxicity, in particular hyperexcitability caused by activation of NMDA receptors, and that this could occur if QA levels are increased or if the QA:KA balance is shifted in favour of QA if KA levels are decreased. Evidence in favour of this latter possibility is implied by the suggested decrease in Kyn aminotransferase based on the urinary Kyn metabolite patterns described above [135,136]. However, compared with control subjects in whom a 2 g Trp load increases urinary KA by 5.5-fold (from 11 to 60 $\mu\text{mol}/24\text{ h}$), patients with acute porphyria excrete greatly increased amounts of KA (16.8-fold: from 12 to 202 $\mu\text{mol}/24\text{ h}$) [135]. In these 2 studies [135,136], QA was not measured. Plasma Kyn metabolite measurements in normal subjects receiving an oral Trp load [145] confirm the enhancement of the Kyn aminotransferase (KAT) reaction of Kyn \rightarrow KA. Additionally, Trp loading inhibits the

kynureninase reaction of 3-HK \rightarrow 3-HAA. This latter effect is important, because it suggests decreased formation of QA by virtue of decreased availability of its precursor. Thus, Trp administration may provide the necessary neuronal protection during acute porphyric attacks by a dual mechanism: increased KA and decreased QA.

Choice of the tryptophan dosage

Trp has been used in a number of clinical conditions, mainly depression and related mood disorders [146]. Dosage is dependent on factors such as safety, drug interactions, physiological modulators, disease severity and metabolic considerations.

Safety: The withdrawal of Trp from sale outlets in the USA in the late 1980s was based on the incidence of the eosinophilia-myalgia syndrome (EMS), which was due to contaminants that have been traced to a single manufacturer in Japan following their modification of the manufacturing and purification conditions [147]. In particular, 3 changes led to the formation of contaminants: (1) the use of a new strain (Strain V) of *Bacillus amyloliquefaciens*, which increased the synthesis of two intermediates in the Trp-biosynthetic pathway, namely serine and 5-phosphoribosyl-1-pyrophosphate; (2) the amount of charcoal used in the purification process was halved; (3) a filtration step was replaced by a reverse osmosis filter to remove chemicals with molecular weights of more than 1000 [147]. Of these 3 factors, the use of the above strain was significantly associated with the incidence of EMS in batches prepared with it [147]. The majority of EMS cases (>1500) were in the USA, though several cases were reported in the UK, Germany, Canada, Belgium, France, Israel and Japan. No cases of EMS were reported in subjects taking Trp from other sources. Trp was subsequently reintroduced under named prescriptions with monitoring for EMS. Clinicians will be aware of contraindications for Trp, mainly related to drug-drug interactions and, in particular, in relation to the serotonin syndrome if Trp is prescribed jointly with a range of antidepressant and other drugs. Trp is not contraindicated in patients with the porphyrias and there is therefore no barrier to its use in these conditions.

Clinical use and side effects: In the 1960s, Trp was used as antidepressant in doses of as much as 16 g daily. Currently, its use is indicated in the UK for the treatment of resistant depression in doses of 1 g thrice daily or a 6 g daily maximum [148]. Similar or lower doses have been used in anxiety and sleep disorders, but there are currently no official clinical indications for its use in these conditions. Though frequency of their incidence is unknown, side effects of Trp include asthenia, dizziness, drowsiness, headache, light-headedness and nausea. EMS, and related symptoms in subjects previously affected and suicidal tendencies in (depressed) subjects with previous history of suicidal ideation could also occur. These side effects apply more to long-term therapy with Trp. As the use of Trp is suggested during the 3–5 day duration of therapy of acute attacks, any side effects experienced by patients should resolve following discontinuation of Trp. The intention is also to use small, rather than large, doses of Trp, which may minimise side effects. The LD_{50} for Trp is 1.634 g/kg (intraperitoneally) and >16 g/kg (orally) in rats and 4.8 g/kg (orally) in mice.

Metabolic considerations in choice of dosage: The ideal Trp dose is the smallest dose that can maximally convert 5-ALA into heme, maximally enhance 5-ALA dehydratase activity, produce significant amounts of kynurenine to promote its transamination to kynurenic acid and undergo flux down the kynurenine pathway without strong TDO activation. This can be established initially in studies in rats. In rats, the Trp dose used in the Trp-5-ALA model in Table 6 was 75 mg/kg. This and larger doses activate rat liver TDO, whereas a 50 mg/kg dose does not, though it stabilises the enzyme against degradation [57]. While stabilisation is not remarkable after a single dose and is limited to the inactive apoenzyme [57], repeated administration of 50 mg/kg twice daily for 7 days shows a greater TDO activation after a single 50 mg/kg dose administration [149]. Thus both baseline plasma [Kyn] and that

elevated by the above single Trp dose are the highest in rats repeatedly treated with Trp. Doses of Trp of ≤ 50 mg/kg still undergo metabolism down the KP by their flux through TDO. Thus, plasma [Kyn] is elevated dose-dependently by doses of Trp of 10–50 mg/kg [149].

In humans, there is evidence that doses of Trp of 25–50 mg/kg elicit a moderate activation of TDO, whereas maximum activation is observed with a 74 mg/kg dose (5.15 g in a 70 kg adult) [145]. This dose in humans is similar to that (75 mg/kg) used in rats jointly with 5-ALA in the above model to maximally inhibit 5-ALAS activity. I suggest that smaller doses are used based on the preclinical studies with Trp + 5-ALA suggested above. Initially, single doses of Trp of 0.5 g, 1 g and 1.5 g per 70 kg adult patient (equivalent to 7, 14 and 21 mg/kg respectively) could be used either once or twice daily during acute attacks. Ethical approval is likely to be granted if Trp is initially given along with hematin or heme arginate, with occurrence of a rapid clinical response and rapid normalisation of blood 5ALA and PBG by the addition of Trp being one end-point. The final Trp dose is likely to be better defined following these exploratory studies.

Testing the hypothesis

From the above accounts, many aspects of the proposed hypothesis are testable at the experimental, preclinical and clinical levels. The following are summary accounts.

Fasting-induced attacks

1. Use of TDO inhibitors other than glucose to block 5-ALAS induction by starvation in rats and in starved rats undergoing experimental porphyria induced by AIA, DDC and/or griseofulvin. Proposed TDO inhibitors include allopurinol, nicotinamide or LM10 (a 6-fluoroindole substituted in the 3-position by a tetrazolyl-vinyl side chain), which displays a stronger TDO inhibition ($K_i = 5.6 \mu\text{M}$) with a competitive inhibition profile [150]. Nicotinamide and LM10 should also be tested for the effects of starvation in the absence of experimental porphyrogens. Measurements should also include activity and heme saturation of TDO, plasma and liver porphyrins and PGC-1 α expression in control and porphyric rats.
2. A time-course study of the effects of daily starvation of rats for up to 4 days on TDO activity and heme saturation, 5-ALAS activity, heme oxygenase activity, plasma Trp (free and total) and PGC-1 α expression. This study may establish the relationship, if any, between PGC-1 α and the above parameters as a function of starvation.
3. Studies in guinea pigs and golden hamsters, as examples of species lacking the TDO free apoenzyme and its glucocorticoid induction mechanism. PGC-1 α levels along with liver 5-ALAS, heme oxygenase and TDO activities and cytochrome *P*-450 levels should be measured over a 1–4 day starvation period.

Safety status of drugs and chemicals based on changes in 5-ALAS activity and heme utilisation by or saturation of TDO

1. Known safe and unsafe drugs can be tested for their effects on the above parameters. In the main and according to the proposed classification in Table 5, safe drugs are expected to inhibit 5-ALAS activity and utilisation of the regulatory-heme pool by TDO, whereas unsafe drugs can be expected to cause the reverse effects. Experiments should be performed preferentially in starved (24–48 h) male rats and, if necessary, in fed controls.
2. Further distinction between safe and unsafe drugs can be attempted by examining their ability to inhibit significantly heme utilisation by TDO already induced by cortisol or heme saturation already induced by hematin.
3. Ability of safe and unsafe drugs to elevate circulating corticosterone in rats can be a further discriminative test.
4. Some information on effects of existing and potential safe and

unsafe drugs on plasma cortisol in human subjects may already be available in the literature.

Therapy of acute attacks

1. A detailed assessment of Trp metabolism and disposition including the kynurenine pathway should be performed during acute attacks (precipitated by fasting or drugs) and in remission through measurements of plasma free and albumin-bound Trp, Kyn and its metabolites KA, 3-HK, XA, 3-HAA and QA, cortisol, albumin, non-esterified fatty acids and neutral amino acids which compete with Trp for entry into the brain (Val, Leu, Ile, Phe and Tyr). This will establish the Trp disposition status, the state of flux of plasma free Trp into the liver, activities of kynurenine pathway enzymes, levels of neuroactive kynurenine metabolites and Trp availability to the brain for serotonin synthesis. Details of background information on these various aspects have been described [55,106,145]. Parameters of heme metabolism should also be measured (in particular plasma 5-ALA and PBG levels) and attempts to correlate changes in these with Trp-metabolic parameters.
2. The effects of glucose therapy and hematin infusions on the above measures should be assessed.
3. PGC-1 α levels and expression should be assessed under the conditions in 1 above.

Potential therapeutic role of tryptophan

1. Effects of administration of various doses of Trp on plasma or serum 5-ALA and PBG in control rats and in those treated with 5-ALA should be assessed to establish the ability of Trp to accelerate the removal of 5-ALA by promoting its conversion to heme. The proposed Trp stimulation of 5-ALA dehydratase activity should be tested directly and its modulation by Pb^{2+} and Al^{3+} assessed. From the results, an ideal dose of Trp can be defined for further studies.
2. Similar experiments to those suggested in 1 above should be performed in guinea pigs and/or golden hamsters to establish the 5-ALA pharmacokinetic status and the role of Trp in heme metabolism in these species.
3. Trp metabolism and disposition should be examined during experimental porphyria in rats, at both the early and late phases of disease model, i.e. loss of heme and the subsequent increase. Plasma or serum free and albumin-bound Trp, Kyn and its metabolites and 5-ALA and PBG should be determined. Enzymes of the Kyn pathway could be assessed by direct assays and compared with assumed activities from product/substrate ratios in plasma.
4. Effects of Trp administration on behavior in behavioural models of acute hepatic porphyrias should be assessed to establish if Trp can modify behavior in parallel with its proposed acceleration of 5-ALA's conversion to heme. Measurements of serum kynurenine and its metabolites, notably KA and QA and serum and brain Trp and 5-hydroxyindoles could be informative.
5. Pilot studies with doses of Trp of 7, 14 or 21 mg/kg one or twice daily in patients with acute porphyric attacks could be initiated. Initially, Trp can be given as an adjunct to heme infusions to see if relief of symptoms occurs faster with Trp. The effect of Trp on 5-ALA and PBG levels can be assessed simultaneously. Plasma levels of Kyn and its metabolites, including up to QA could also be measured. Hourly blood sampling should be performed for all measurements for at least the first 7–8 h after Trp administration.
6. In patients with moderate acute attacks due to fasting, Trp can also be administered along with glucose in a similar pilot study. 5-ALA, PBG measurements could be accompanied with PGC-1 α determination and all the other measurements proposed in 5 above.
7. A pilot study with gene silencing can be performed using Trp as an adjunct to establish if a rapid response occurs in comparison with that observed with heme therapy.

Conclusions and comments

This account has provided evidence consistent with a role of the mammalian hepatic cytosolic enzyme Tryptophan 2,3-dioxygenase (TDO) in the exclusive utilisation of the regulatory heme pool. On this basis, it is reasonable to suggest that TDO exerts an important role in the regulation of hepatic heme biosynthesis. Therapy of acute porphyric attacks targets the rate-limiting enzyme of heme biosynthesis, 5-aminolevulinic acid synthase (5-ALAS) and has so far been limited to the use of glucose in fasting-induced moderate attacks and of heme preparations otherwise. A more recent therapy development is the proposed use of 5-ALAS1 gene silencing and preliminary results are encouraging. The present hypothesis aims at metabolic targeting of 5-ALAS focusing on prevention of utilisation of the regulatory-heme pool by TDO, thus presenting a new therapeutic approach. TDO inhibitors preventing this utilisation are the most likely candidates. Existing TDO inhibitors in this regard include glucose, allopurinol and nicotinamide, but more potent inhibitors, such as LM10 and others developed to combat tumoral immune escape are also potential candidates for treatment of porphyric attacks. Another therapeutic approach is the use of Trp itself and the model presented herein suggests that Trp in the presence of elevated 5-ALA levels, as would be the case during acute attacks, elicits a rapid and near complete inhibition of 5-ALAS and a high level of heme availability. In addition to its proposed enhancement of 5-ALA dehydratase and consequently its particular usefulness in the dehydratase porphyria, Trp possesses several other therapeutic advantages that make its use an attractive prospect. The proposed hypothesis also suggests new ways of exploring the control of hepatic heme biosynthesis in certain mammalian species lacking the regulatory mechanisms operating in rats and humans. It is hoped that this account will stimulate newer interests in understanding the control of mammalian heme biosynthesis and exploring new strategies in the therapy of the hepatic porphyrias.

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.109314>.

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