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Uroporphyria Induced by 5-Aminolaevulinic Acid Alone in *Ahr*^{d} SWR Mice

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ABSTRACT. In mice, depression of hepatic uroporphyrinogen decarboxylase (UROD) leading to porphyrin accumulation (uroporphyria) occurs with chlorinated ligands of the aryl hydrocarbon (AH) receptor especially after iron overload. However, in the absence of chlorinated ligands, iron itself will eventually cause uroporphyria, but this response is not associated with the *Ahr* genotype. These effects are potentiated by administration of the haem precursor 5-aminolaevulinate (ALA). The aim of this study was to investigate the effects of ALA alone. Prolonged administration of 2 mg ALA/mL in the drinking water to SWR mice also led to decarboxylase insufficiency (11% of control) and uroporphyria by 8 weeks, whereas DBA/2 mice did not show reduced enzyme activity. Both strains are considered AH nonresponsive and analysis of the *Ahr* gene using restriction fragment length polymorphism was consistent with SWR, like DBA/2, possessing the *Ahr*^{d} allele. Exposure of isolated hepatocytes to ALA (150–500 μM) for up to 48 hr showed a significant accumulation of both uroporphyrin and coproporphyrin in the medium, which for uroporphyrin particularly was significantly greater with SWR than with DBA/2 cells. Basal *in vivo* CYP1A2 activity, measured as microsomal methoxyresorufin dealkylation, was significantly greater in SWR than in DBA/2 mice (1.3-fold), but it was unclear whether this was sufficient to explain the marked difference in sensitivities of the two strains. Despite SWR mice being AH nonresponsive, uroporphyria and decarboxylase depression after an initial iron overload and ALA for 3 weeks were greatly potentiated by a single dose (100 mg/kg) of hexachlorobenzene (a weak AH ligand). The results demonstrate that there is a genetic difference in mice independent of the *Ahr* genotype and response to iron, which influences the susceptibility to ALA-induced uroporphyria. Thus chemicals, iron and ALA can act independently, but also together, to cause porphyria in susceptible individuals. BIOCHEM PHARMACOL 52;9:1407–1413, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. 5-aminolaevulinic acid; uroporphyria; mice; genetic variation

Human PCT§ consists of at least two types. Firstly, a familial autosomal dominant disease in which mutations of the UROD (EC 4.1.1.37) gene are expressed as reduced activity in both hepatic and erythropoietic tissues [1]. Secondly, a sporadic type in which hepatic enzyme activity is reduced by more than 50% *via* a mechanism that does not appear to be the result of inherited mutations but is due either to a change in gene expression or to inhibition of the enzyme [1, 2]. The disease is usually triggered by mild or moderate liver damage caused by a variety of agents such as alcohol, oestrogens, iron, hepatitis C and toxic chlorinated chemicals [1]. Although in sporadic PCT no familial links are usually found, it would not be surprising if genetic predispositions existed. This seems extremely likely given that only a minority of patients with liver damage develop the disease despite the variety of insults that are implicated [1]. In support of this hypothesis, some studies have suggested that heterozygosity for the haemochromatosis gene could be a factor in some patients [3–5].

Experimental models of PCT using inbred mouse strains have been extremely productive in dissecting out the genetic influences involved in the induction of hepatic uroporphyria. Potentially, the strongest influence in mice appears to be mediated via the *Ahr* locus as shown by experiments with TCDD and HCB [6, 7]. AH-responsive C57BL mice with the *Ahr*$^{b-1}$ allele [8] respond, whereas those of the AH-nonresponsive strain DBA/2 with the *Ahr*^{d} allele are resistant. This may occur through the involvement of some components of the cytochrome P450 system, although proof for this hypothesis has remained elusive [9]. However, it has become apparent that expression of other genes influence the course of this syndrome [10]. The most influential of these appears to be associated with iron metabolism. Iron overload potentiates the porphyrogenic effects of TCDD and HCB in both AH-responsive and -nonresponsive strains [7, 11, 12]. In fact, after 3–6 months, iron overload itself can cause uroporphyria, depending on a genetic

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§ *Abbreviations:* AH, aryl hydrocarbon; ALA, 5-aminolaevulinate; HCB, hexachlorobenzene; HPLC, high pressure liquid chromatography; MROD, EROD, PROD, and BROD, methoxy, ethoxy, pentoxy and benzyloxyresorufin dealkylases, respectively; PCT, porphyria cutanea tarda; RFLP, restriction fragment length polymorphism; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UROD, uroporphyrinogen decarboxylase.

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background that is not correlated with *Ahr* genotype [10, 13]. This latter finding illustrates that uroporphyria can be viewed as a disease of iron metabolism as much as one in which iron is acting as a secondary agent.

Another factor potentiating all types of porphyria is the supply of the haem precursor ALA. Administration of ALA to mice in the drinking water will exacerbate the development of porphyria caused by chemical agents and/or iron [10, 14, 15]. In both the AH-responsive C57BL and the AH-nonresponsive SWR strains, ALA potentiates inhibition of UROD and uroporphyria induced by iron overload, whereas this does not occur with DBA/2 mice [10].

In the studies reported here, the response of the two AH-nonresponsive strains of mice, SWR and DBA/2, to treatment with iron and ALA were directly compared and then the influence of ALA alone for a prolonged period was tested *in vivo* and with isolated hepatocytes. The additional possible influences of cytochrome P450 were also investigated.

MATERIALS AND METHODS

Chemicals

Most chemicals were purchased from Sigma Chemical Co. Ltd (Poole, UK). HCB of 99.9% purity [7] was from BDH-Merck (UK). Standard porphyrins were obtained from Porphyrin Products (Logan, UT, USA). ECO 47III was obtained from Gibco Ltd. (UK).

Mice and Treatments

Male SWR and DBA/2 mice (7–10 weeks old) were bred on site or obtained from Harlan-Olac Ltd. (Bicester, UK). They were maintained at 21°C and mostly fed RM3 diet, except mice listed in Table 3, which received RM1 diet. Mice received iron-dextran solution (100 mg of Fe and 100 mg of dextran/mL) at 600 mg Fe/kg or equivalent dextran solution, by subcutaneous (s.c.) injection, except for experiments to isolate hepatocytes when, because of poor viability at this dose given intraperitoneally (i.p.), the iron was administered at 300 mg Fe/kg. Single doses of 3-methylcholanthrene and HCB (80 mg/kg) were given by i.p. injection in oil as 10-mg/ml solutions. Phenobarbital was given by i.p. injection on four occasions (60 mg/kg each). ALA was administered in the drinking water (2 mg/mL), and fresh solutions were made every 3 days.

At the end of experiments, mice were killed by cervical dislocation, and livers were frozen at −70°C until analysis.

Analyses

Total porphyrin levels in liver were estimated by spectrofluorimetry [16]. Porphyrin types were determined by the method of Grandchamp *et al.* [17].

UROD activity was estimated by HPLC by using pentacarboxyporphyrinogen I as substrate, which was generated from the porphyrin immediately prior to incubation [18].

Nonhaem iron content of liver was determined by adaptation of the method of Carthew *et al.* [19]. Hepatic cytochrome P450 levels and alkoxyresorufin dealkylations were estimated as in published methods [20].

Cell Culture

Hepatocytes were isolated from SWR and DBA/2 mice by collagenase perfusion as described by Madra and Smith [21] and then incubated with ALA for up to 48 hr in conditions as described by Brady and Lock [22] with slight modifications. Mouse hepatocytes (2×10^6 cells/dish) were seeded in Leibowitz L15 medium supplemented with fetal calf serum (5% v/v), tryptose phosphate broth (5% v/v), insulin (1 μM) and hydrocortisone (0.1 mM). The viability of the cells, as assessed by Trypan Blue exclusion, was 75–80%. Three and 24 hr after seeding the cells, the medium was aspirated and fresh medium applied. ALA was added at seeding and with each medium change to give concentrations of 150, 250 and 500 μM. At the indicated times, the medium was collected and assayed for uroporphyrin and coproporphyrin immediately or after storage at −70°C. A few experiments were done on hepatocytes isolated from mice given iron-dextran (300 mg Fe/kg) 1 week prior to perfusion. Levels of uroporphyrin and coproporphyrin were determined by reverse-phase HPLC [18].

Gene Restriction Fragment Length Polymorphism

The RFLP in the *Ahr* gene, an ECO47III restriction site in exon 7, was performed as described by Schmidt *et al.* [23] by using SWR and DBA/2 genomic DNA. C57BL/6J DNA was used as a positive control.

Statistics

Results were assessed by analysis of variance, with significance set at $P < 0.05$.

RESULTS

Comparison of SWR and DBA/2 Mice in Their Response to ALA

SWR and DBA/2 mice were dosed with dextran or iron-dextran and then after 3 days given water or ALA in their water for 5 weeks. Livers were examined for total porphyrin levels and UROD activity. At this time following iron dosing alone, there was little effect on porphyrin levels or UROD activity. However, when combined with ALA treatment, a significant depression of UROD activity was detected, with a rise in the levels of total porphyrins (Table 1). No such depression of UROD activity or marked porphyrin accumulation were observed with DBA/2 mice. In this experiment, a small rise in porphyrin levels was also observed in SWR mice that had only been given ALA.

TABLE 1. Comparison of the effects of iron and ALA in SWR and DBA/2 mice

Strain	N	ALA	Iron	Hepatic UROD (pmol/min/mg)	Hepatic Porphyrins (nmol/g)	Hepatic Nonhaem iron (μg/g)
SWR	3	−	−	37.6 ± 2.0	0.5 ± 0.1	170 ± 15
	3	−	+	37.1 ± 1.7	0.6 ± 0.1	2667 ± 148
	4	+	−	32.4 ± 2.2	30 ± 18*	130 ± 15
	4	+	+	6.7 ± 3.0*	154 ± 117*	2776 ± 125
DBA/2	3	−	−	40.5 ± 1.0	0.5 ± 0.1	86 ± 2
	3	−	+	46.0 ± 4.6	0.7 ± 0.1	2673 ± 261
	3	+	−	44.6 ± 4.0	1.7 ± 0.4	187 ± 3
	3	+	+	54.1 ± 1.3	0.9 ± 0.3	2166 ± 238

Mice received iron-dextran or dextran (600 mg Fe/kg) and after 1 week were administered ALA for 5 weeks. Results are means ± SEM. *Significantly different from other groups.

Reexamination of previous data revealed a small rise had also been detected but had not been considered further [10].

To see whether a longer administration of ALA would have a greater effect, SWR mice were given ALA for 8 weeks without prior loading with iron. Analysis of the urine using HPLC showed that a rise in the excretion of both uroporphyrin I and III isomers could be detected by 2 weeks with SWR mice. Investigation of the livers after 8 weeks showed that a significant accumulation of total porphyrins in the liver had occurred, with an accompanying decrease in the activity of UROD (Table 2).

Interestingly, a smaller but significant rise in total porphyrins occurred in the livers of DBA/2 mice treated with ALA in an identical manner, but in this case no decreased UROD activity was detected (Table 2). In another experiment, SWR and DBA/2 mice were again administered ALA for 8 weeks and the porphyrins analysed by the spectrofluorometric method of Grandchamp *et al.* [17]. This method distinguishes between uroporphyrin, coproporphyrin and protoporphyrin types. The response was less than that observed in the previous experiment but showed that both uroporphyrin and protoporphyrin accumulation had occurred in the livers of SWR mice (controls: 0.02 ± 0.01, ALA: 3.34 ± 1.78 nmol uroporphyrin/g; controls: 0.22 ± 0.01, ALA: 5.39 ± 1.73 nmol protoporphyrin/g), but only protoporphyrin was detected in the livers from mice of the DBA/2 strain (controls: 0.53 ± 0.02, ALA: 13.87 ± 4.31 nmol protoporphyrin/g). UROD activity was depressed significantly again in the livers from SWR mice administered ALA but not in those from DBA/2 mice treated similarly (SWR: 58 ± 2%, DBA/2: 88 ± 3% of control values). These results suggest that there is a difference between SWR and DBA/2 mice in the response to ALA alone, which is potentiated by iron and contributes to UROD inhibition and uroporphyrin accumulation.

TABLE 2. Comparison of UROD activity in SWR and DBA/2 mice after 8 weeks of ALA treatment

Strain	N	ALA	Hepatic UROD (pmol/min per mg)	Hepatic Total porphyrins (nmol/g)
SWR	4	−	38.6 ± 0.4	0.9 ± 0.1
	5	+	4.1 ± 1.0*	106 ± 14*
DBA/2	3	−	39.7 ± 0.4	1.0 ± 0.2
	3	+	39.4 ± 0.9	38.0 ± 3.1*

SWR and DBA/2 mice were administered ALA in the drinking water (2 mg/ml) for 8 weeks without any other treatment. Results are means ± SEM. *Significantly different from the group not receiving ALA.

Previous studies have shown that both SWR and DBA/2 strains are relatively nonresponsive to induction of CYP1A1 activity by β-naphthoflavone [25], confirming their status as AH nonresponsive and consistent with their possessing the Ahr^d allele as assessed by receptor size [8]. To check the *Ahr* genotype of these mice, DNA samples from both strains were probed by restriction fragment length polymorphism for the *Ahr* gene. The fragment for SWR mice was identical to that for the DBA/2 strain in showing lack of cleavage, whereas with C57BL/6J mice two smaller fragments were observed, indicating enzymic cleavage at the restriction susceptible site (Fig. 1). This result was consistent with SWR and DBA/2 both possessing the Ahr^d allele.

Exposure of SWR and DBA/2 Mouse Hepatocytes to ALA

Some attempts to duplicate *in vitro* chemically induced uroporphyria produced *in vivo* in C57BL mice have not proven easy even by using freshly isolated hepatocytes cultured in the presence of ALA and grown on matrigel [21, 24]. However, another study has reported accumulation of octacarboxylic porphyrins after exposure of hepatocytes from outbred Swiss mice to ALA alone [22]. To reproduce these latter findings and to see whether a strain difference in uroporphyrin accumulation could be detected *in vitro*, hepatocytes were isolated from SWR and DBA/2 mice and cultured in the presence of ALA for up to 48 hr on a medium used by Brady and Lock [22]. An accumulation of uroporphyrin in the medium after 24 and 48 hr was ob-

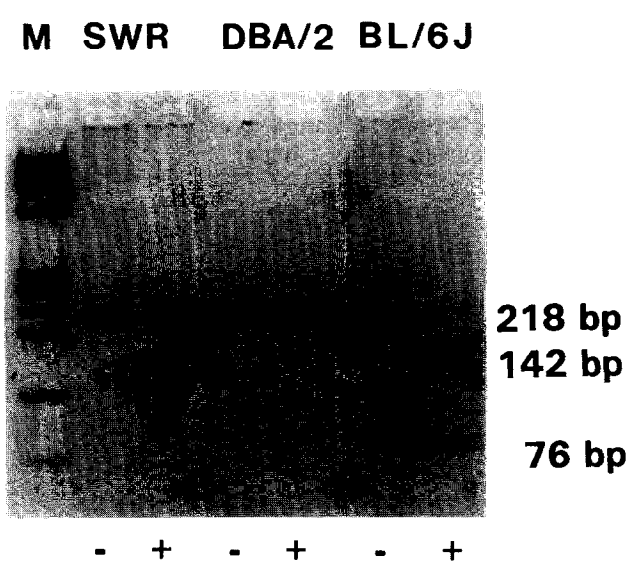

FIG. 1. Restriction fragment length polymorphism analysis for the *Ahr* gene in SWR, DBA/2 and C57BL/6J mice. DNA from the liver of the three mouse strains was amplified by polymerase chain reaction (PCR) by using primers for the *Ahr* gene across the RFLP sequence as described elsewhere [23]. The products were digested with *Eco*47III and analysed on 3% agarose gels. M = mol. wt standards. Minus and plus represent incubations of PCR products without and with *ECO*47III. Visualization by ethidium bromide. Only the sequence from C57BL/6J (218 bp) was cleaved (142- and 76-bp fragments).

served with SWR hepatocytes in an approximately dose-dependent manner (150–500 μM), although levels were lower than those reported with the outbred mice. Accumulation was significantly less with hepatocytes from DBA/2 mice at all concentrations of ALA (Fig. 2). ALA also caused an elevation of coproporphyrin, but in this case no significant difference between the strains was detected except at the highest dose level. Efforts to measure UROD activity in these systems was unsuccessful. The addition of ALA at the lowest concentration (150 μM) to hepatocytes isolated from iron-treated SWR mice increased the level of uroporphyrin 1.5 times further at 48 hr as opposed to hepatocytes from mice not given iron as observed in the *in vivo* studies, but no effects of iron treatment were observed with hepatocytes from DBA/2 mice (results not shown). In other studies using Williams E medium, a more marked synergistic interaction between iron and ALA was observed with C57BL/10ScSn hepatocytes (A.G. Smith and S. Madra unpublished data).

Comparison of Cytochrome P450 Activities in SWR and DBA/2 Mice

The development of uroporphyria in rodents has long been proposed as being associated with induction of the CYP1A subfamily of cytochrome P450 [9, 14, 26], in particular CYP1A2. Because SWR and DBA/2 mouse strains are apparently AH nonresponsive, it was thought possible that constitutive cytochrome CYP1A2 expression could be a factor in the different responses of the two Ahr^d strains to ALA. To examine this possibility, microsomal total cytochrome P450 and MROD, EROD, PROD, and BROD were measured in untreated SWR and DBA/2 mice. Higher levels of EROD and MROD were found in SWR than in DBA/2 mice (Table 3). In induced animals, these are thought to represent CYP1A1 and CYP1A2 activities, re-

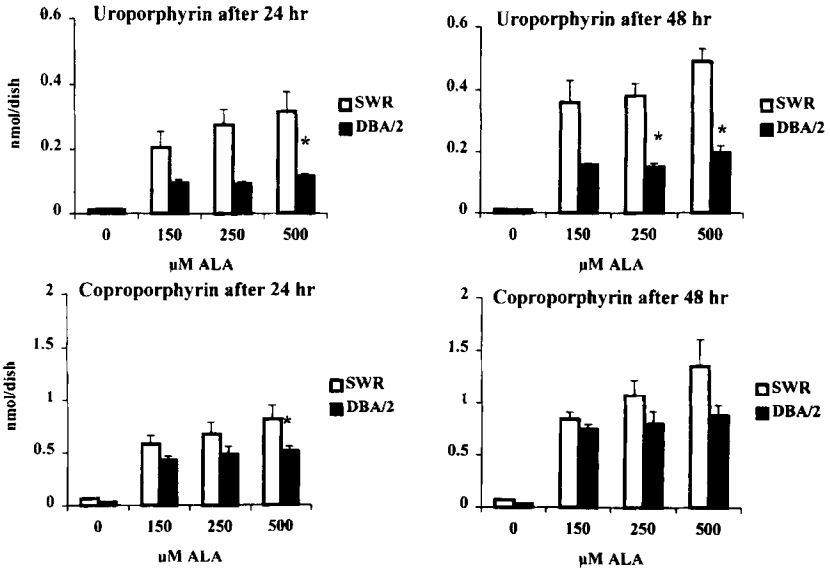

FIG. 2. The effect of ALA on isolated SWR and DBA/2 mouse hepatocytes. Cells were isolated and cultured as described in the Materials and Methods section. ALA was added to cells at seeding and with each medium change to concentrations of 150, 250 and 500 μM. Uroporphyrin and coproporphyrin in medium samples were estimated by HPLC with detection by absorbance at 400 nm. Amounts are expressed per culture dish. Results are means ± SE of four experiments, and asterisk indicates a significant difference between the two strains ($P < 0.05$).

TABLE 3. Comparison of cytochrome P450-catalysed dealkylations in SWR and DBA/2 mice

Strain	N =	N = Cytochrome P450 (nmol/mg protein)	Microsomal dealkylations (pmol/min per mg protein) MROD	EROD	PROD	BROD
SWR	7	0.78 ± 0.02	96 ± 6	101 ± 9	5 ± 1	44 ± 6
DBA/2	5	0.68 ± 0.02*	75 ± 5*	88 ± 8	5 ± 1	44 ± 9

Cytochrome P450-catalysed dealkylations of alkoxyresorufins were measured with hepatic microsomes as described in the Materials and Methods Section. Results are means ± SEM. *Significantly different from SWR mice.

spectively, although in the uninduced state it is unlikely that EROD truely represents CYP1A1 and is probably reflecting CYP1A2 status [27]. The differences between the strains were small, and it was not clear that these were sufficient to prove that CYP1A2 variability was a factor for the significantly greater response of the SWR strain to ALA alone or in combination with iron. Studies on P450 activities after prolonged treatment with ALA were not performed. No major difference between the strains was detected by Western blotting of microsomes (results not shown) using an antibody against CYP1A2 [20].

Influence of Chemicals on the Response of SWR Mice to Iron and ALA

Multiple doses of 3-methylcholanthrene, an inducer of the CYP1A subfamily, or one dose in combination with ALA treatment, cause uroporphyria in iron-loaded AH-responsive C57BL mice [14, 28]. To compare the effect of 3-methylcholanthrene in the AH-nonresponsive SWR strain, mice received iron-dextran and then after 1 week, all but one group were administered ALA in the water for another 3 weeks. At the beginning of the ALA treatment (day 0), groups of mice also received single doses of 3-methylcholanthrene or HCB. Another group received four doses of phenobarbital (a CYP2B inducer) on days 0, 7, 12 and 18, and an additional group were treated with two extra doses of iron on days 0 and 12. The single dose of 3-methylcholanthrene had no additional effects on SWR mice treated with both iron and ALA, unlike findings reported in C57BL/6J mice [14]. In contrast, a single dose of HCB had a marked potentiating influence on enzyme inhibition and development of uroporphyria (Table 4). After multiple doses of phenobarbital, a small but significant potentiating effect was also observed. HPLC showed that with both HCB and phenobarbital the marked increases in porphyrin levels were due to uroporphyrin. As a in a previous study [10], additional iron loading had an inhibitory effect on the development of uroporphyria, which is consistent with the possibility that a critical maximum iron level is necessary for the development of the effect.

DISCUSSION

In previous studies, we have shown that iron overload will induce uroporphyria and associated UROD insufficiency in susceptible mice after 6 months [10]. This type of experimental porphyria when induced by chemicals was assumed originally to be associated exclusively with the *Ahr* locus [6], but subsequent evidence including genetic cross studies of the response induced by TCDD [12, 29] and the response of *Ahr* congenic strains to HCB [30] demonstrated that genes besides *Ahr* influence uroporphyria development. In fact, uroporphyria caused by iron showed no correlation with the apparent *Ahr* genotype of the mouse strains examined [10]. Furthermore, development of the porphyria after iron overload was rapidly advanced by the administration of the haem precursor ALA to mice in the drinking water [10, 14]. In these circumstances, the SWR strain was the most susceptible, whereas mice of the DBA/2 strain were still very resistant. Both strains are AH nonresponsive [8, 25]. The present work has demonstrated that a genetic difference in response to ALA in the absence of additional iron administration can also account for some of the differences observed in the variability of the uroporphyric response of mouse strains. The difference between the Ahr^d SWR and DBA/2 strains was observed (to a lesser extent) *in vitro* as well as *in vivo*.

The administration of ALA is presumed to enhance haem biosynthesis by producing increased pools of uroporphyrinogen, thus potentiating any initiated restriction occurring at the UROD stage. We have no evidence that there is any constitutive difference in UROD activity between these strains, which becomes evident after treatment with ALA [10]. One could suppose that some unknown

TABLE 4. Influence of selcted chemicals on uroporphyria induced in SWR mice

Chemical treatment	ALA	UROD (pmol/min per mg)	Porphyrins (nmol/g)
Oil	–	36.7 ± 1.0	1.5 ± 0.3
Oil	+	12.1 ± 4.6*	75.6 ± 37.7*
3-MC	+	11.4 ± 0.7	37.2 ± 8.8
Phenobarbital	+	5.7 ± 1.2**	106 ± 23**
HCB	+	0.9 ± 0.3**	531 ± 31**
Extra iron	+	25.8 ± 3.8**	4.8 ± 0.3**

Mice received iron and then after 1 week, they received 3 weeks of ALA in the water. 3-MC and HCB were given as a single dose of 80 mg/kg on day 0 of ALA. Phenobarbital was given as four doses of 60 mg/kg on days 0, 7, 12 and 18 of the ALA treatment. Additional iron was given to one group on days 0 and 12. Results are means ± SE (N = 3). *Significantly different from mice not given ALA. **Significantly different from mice given ALA alone.

process leading to uroporphyrinogen oxidation occurs more readily in SWR than in DBA/2 mice, giving uroporphyrin (and in isolated cells coproporphyrin) and other oxidation products that inhibit UROD [9, 26, 31, 32]. An alternative possibility to ALA acting in its capacity as a haem precursor is that it acts as an agent of free radical production [33]. In this hypothesis, iron overload would potentiate any free radical mechanism. Of course, this still leaves the fundamental problems as to how such a process is initiated, why it differs between strains, and how this causes UROD insufficiency. During studies of prolonged ALA administration in mice, we have observed that hepatic total nonhaem iron status can be changed significantly (see Table 1). Although we did not observe any consistent changes or correlation with onset of porphyria, a subtle aspect of iron metabolism may be involved in the differing responses of the two strains. The development of uroporphyria in SWR but not DBA/2 mice after ALA-alone administration may be related to that observed when ALA is combined with prior iron treatment, that is to say, a genetic difference in overall sensitivity to development of uroporphyria by some unknown mechanism rather than just in the responses to ALA.

A number of studies have shown that cytochrome P450 1A2 can oxidise uroporphyrinogen to uroporphyrin, and it has been suggested that this may be the mechanism by which the latter tetrapyrrole can accumulate [26, 32, 34]. *In vivo*, this process seems to be suppressed by ascorbate [35]. CYP1A2-mediated activity (MROD) was slightly greater in SWR than in DBA/2 mice, but it is unclear whether the difference was sufficient to account for the distinct sensitivities of the two strains to induction of uroporphyria and how this would result in inhibition of UROD. However, no studies were conducted after prolonged ALA treatment. We observed a significant, but again small, difference in hepatic ascorbate levels between the strains (unpublished data). In previous comparisons of the sensitivities of six stains of mice to iron-induced porphyria, no correlation with microsomal cytochrome P450 activity or uroporphyrinogen oxidation could be detected [10]. Dietary or multiple gavage administration of nonchlorinated polycyclic aromatic hydrocarbon inducers of the CYP1A subfamily will enhance uroporphyria in iron-loaded C57BL mice and even a single dose will suffice if combined with ALA [14, 28]. The fact that a single dose of one of these chemicals, 3-methylcholanthrene, had no effect in Ahr^d SWR mice under similar circumstances is consistent with the *Ahr* locus playing a role in uroporphyria development but one that maximises the response without necessarily being a dominant influence. It is curious that, in contrast, HCB, which is also an AH-receptor ligand [36], had a marked influence. This finding may reflect the slow metabolism of this chemical when compared with 3-methylcholanthrene, thus sustaining occupation of the receptor from the Ahr^d allele to cause significant effects. However, HCB may act by additional mechanisms. For instance, it might be acting as a phenobarbital-type inducer. Phenobarbital will act weakly in conjunction with iron to cause porphyria in C57BL/105cSn mice [28], and similar findings have been observed with cultures of chick embryo liver cells [32, 37, 38]. It is also pertinent to note that TCDD will induce uroporphyria in iron-loaded SWR and AKR (also Ahr^d) mice, albeit to a lesser extent than in C57 strains, but not in DBA/2 [12, 39].

In summary, the UROD insufficiency and uroporphyria that can be produced in mice as models for human PCT appears to be under a number of genetic influences: firstly, *Ahr* or a related gene, perhaps controlling *cyp1a2* expression, which accounts for the maximum responses with polychlorinated aromatic hydrocarbons such as TCDD and HCB; secondly, there seems to be a gene(s) associated with iron metabolism and having no correlation with the *Ahr* genotype; thirdly, genes that are associated with haem metabolism. The interaction and relative importance of these genes will depend on the precise experimental circumstances, i.e. the strain used and the chemicals involved. Further dissection and identity of these genes will involve techniques of molecular genetic analysis and manipulation. Studies of this nature are in progress.

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