Regulation of production of δ -aminolaevulinate synthase in tissues of chick embryos

Effects of porphyrogenic agents and of haem precursors

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Studies conducted by several groups have established that porphyrogenic agents which caused elevations in chick-embryo liver δ -aminolaevulinate (ALA) synthase activity also increased the concentrations of the enzyme's RNA, and that haemin inhibited these elevations. We have determined in this study, using immune-blot analyses, that administration *in ovo* of allylisopropylacetamide (AIA) in combination with diethyl 1,4-dihydro-2,4,6-trimethyl,3,5-pyridinedicarboxylate (DDC) increased the mass of ALA synthase in intestine and kidney of chick embryos. Furthermore, the molecular mass of the subunit of the enzyme in those tissues appeared identical with that of liver ALA synthase. Using a synthetic oligonucleotide complementary to ALA synthase mRNA, we determined by solution hybridization and Northern-blot analyses that AIA and DDC also increased the concentrations of ALA synthase mRNA in intestine and kidney and that testosterone elevated the concentration of the RNA in kidney. In analyses of RNA obtained from chick-embryo liver, intestine, kidney, heart, brain and lung, the probe bound primarily in each case to a single 2.3 kb RNA. Finally, the haem precursors ALA and FeCl_3 , when injected together into the fluid surrounding embryos, inhibited both the elevations in ALA synthase mass and RNA concentration brought about by porphyrogenic agents in liver, kidney and intestine. Thus the results indicated that: (1) certain porphyrogenic agents increased ALA synthase mass and RNA in chick-embryo intestine and kidney, in addition to liver; (2) ALA and FeCl_3 inhibited the elevations; and (3) the sizes of ALA synthase's subunit as well as the enzyme's mRNA appeared identical, in each case, in all tissues examined.

INTRODUCTION

ALA synthase catalyses the condensation of glycine and succinyl-CoA to yield ALA, the first obligate intermediate in tetrapyrrole synthesis. The regulation of ALA synthase activity in the tissues of mammals and birds has been studied extensively, particularly for the liver enzymes of rat and chick embryo. In liver, the enzyme catalyses the rate-limiting step in haem production, and ALA synthase activity can become elevated within hours from an exposure to a variety of xenobiotics (reviewed by Marks, 1985). In the chick embryo, ALA synthase activity could reach 100 times the normal value after injection with a porphyrogenic agent (reviewed by Marks, 1985; Ades, 1986). For those inducers tested, the increases in ALA synthase activity were associated with elevations in the immuno-reactive mass of the enzyme (Ades & Harpe, 1981; Ades *et al.*, 1983), with higher rates of synthesis of the enzyme (Ades, 1983; Ades *et al.*, 1983), and with increases in the concentration of ALA synthase mRNA in hepatocytes (Ades & Harpe, 1981; Drew & Ades, 1986; Ades *et al.*, 1987; Zhang & Ades, 1987; Hamilton *et al.*, 1988). The changes in liver ALA synthase activity caused by porphyrogenic agents appear to result primarily from elevations in the transcription rates of ALA synthase mRNA (Drew & Ades, 1986; Ades *et al.*, 1987; Srivastava *et al.*, 1988), and the elevations can be blocked by haemin, presumably a reflection of the normal mechanism for regulating porphyrin production in hepatocytes (Ades, 1986).

The effects of porphyrogenic agents on ALA synthase activity in tissues other than liver vary. Yamamoto *et al.* (1985) have proposed that an ALA synthase gene family exists in the chicken and that the genes in the family are expressed in tissue- and developmentally-specific manners, leading to the appearance in various tissues of RNA transcripts differing significantly in size. In addition, several reports have suggested that hepatic and erythroid ALA synthase might be isoenzymes distinguishable on the basis of kinetic and immunochemical differences (Bishop *et al.*, 1981; Watanabe *et al.*, 1983; Yamamoto *et al.*, 1985). Elferink *et al.* (1987), on the other hand, have reported that a single ALA synthase gene exists per haploid genome, and that the activities of the enzyme in chicken liver and in reticulocytes were due to identical proteins.

In the present study, we examined various tissues of chick embryos to determine whether isoenzyme forms of ALA synthase could be detected. We also measured the effects of porphyrogenic agents and of ALA and FeCl_3 on the enzyme's activity, mass and mRNA concentration in the different tissues.

EXPERIMENTAL

Chick embryos

In these studies 18-day-old chick embryos (White Leghorn) were used. The animals were treated with the indicated agents (either 2 mg of AIA plus 4 mg of DDC per embryo or 5 mg of testosterone per embryo). The

Abbreviations used: AIA, allylisopropylacetamide; ALA, δ -aminolaevulinate; BSA, bovine serum albumin; DDC, diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate; DMSO, dimethyl sulphoxide.

agents were dissolved in 0.1 ml of DMSO and injected into the fluid surrounding each embryo. Embryos serving as controls received the vehicle only. After the injections, embryos were incubated at 38 °C for the indicated periods.

Determination of ALA synthase activity

The activity of ALA synthase in tissue homogenate was determined by the method of Sassa *et al.* (1979). The assay was modified to include succinic thiokinase as described by Whiting & Granick (1976). Protein was determined by the method of Bradford (1976), with BSA as the standard.

Isolation of RNA

RNA was isolated from tissues by minor modifications of procedures described previously (Drew & Ades, 1986). Tissues were homogenized at room temperature in a guanidine thiocyanate solution (4.0 M-guanidine thiocyanate/7% β -mercaptoethanol/10 mM-Tris/HCl, pH 7.4) to a final concentration of 7–10% (w/v). The homogenates were centrifuged at 12000 *g* for 15 min at 20 °C in a Sorvall SS34 rotor. RNA was isolated from the resulting supernatants by centrifugation, at 250000 *g* for 20 h at 20 °C in a Beckman SW41 rotor, in a CsCl step gradient (Drew & Ades, 1986).

Solution-hybridization analysis of RNA

An oligonucleotide probe complementary to bases +795 to +818 of chick-embryo liver ALA synthase mRNA was prepared and labelled at the 5' end with ^{32}P to a specific radioactivity of 2.4×10^6 c.p.m./pmol as described previously (Drew & Ades, 1986). Solution-hybridization analyses were performed as described by Drew & Ades (1986). Each reaction consisted of 40 μg of RNA and 5000–8000 c.p.m. (constant in a given set of determinations) of ^{32}P -labelled oligonucleotide probe. Basically, the reactions were incubated for 18 h at 55 °C, and after hybridization single-stranded nucleic acids in each reaction were digested with S_1 nuclease. Acid-insoluble material remaining after the treatment was precipitated in 1 M-trichloroacetic acid and collected by filtration.

Immuno-blot analyses

Mitochondrial factors from different tissues were prepared at 0–4 °C as described by Ades & Stevens (1988). Samples from the fractions were denatured by mixing with an equal volume of 10% (w/v) SDS solution and heating immediately at 95 °C for 5 min (Ades & Stevens, 1988).

The mass of ALA synthase in a mitochondrial fraction was determined by immuno-blot analysis. Proteins were first separated by electrophoresis on 1.5 mm-thick 11% polyacrylamide slab gels in the presence of SDS in the system of Laemmli (1970). After electrophoresis, gels were equilibrated with transfer buffer [192 mM-glycine/15% (v/v) methanol/25 mM-Tris, pH 8.25]. Proteins were transferred electrophoretically (225 mA for 5–6 h) to Immobilon PVDF transfer membrane (Millipore, Bedford, MA, U.S.A.) by using a Bio-Rad Laboratories Trans-Blot apparatus containing transfer buffer. Non-occupied binding sites on the membrane were blocked by overnight incubation at 37 °C in a Tris buffer (0.15 M-

NaCl/20 mM- NaN_3 /20 mM-Tris/HCl, pH 8.2) containing 5% (w/v) BSA. Afterward, the membrane was washed three times (5 min per wash) at room temperature in Tris buffer containing 0.1% BSA. The membrane was then incubated for 2 h at room temperature in Tris buffer containing 0.1% BSA and a 1:250 dilution of rabbit serum either from a control animal (pre-immune serum) or from a rabbit immunized against purified chick-embryo liver ALA synthase (immune-serum) as described previously (Ades & Harpe, 1981). After the incubation, the membrane was washed three times (5 min per wash) at room temperature in Tris buffer containing 0.1% BSA. The membrane was then left for 2 h at room temperature in Tris buffer containing 0.1% BSA together with a 1:100 dilution of Auro Probe BL Plus gold-labelled goat anti-rabbit IgG (Janssen, Piscataway, NJ, U.S.A.) and 5% (w/v) gelatin (Janssen). The membrane was washed twice (5 min per wash) at room temperature in Tris buffer containing 0.1% BSA, followed by two washes with distilled water (1 min per wash) at room temperature. The gold-labelled antibody signal on the membrane was intensified by a Bio-Rad Laboratories gold-enhancement procedure, as described by the manufacturer. The molecular masses of proteins on the membrane were determined from the positions of marker proteins transferred to a strip of the membrane and stained with Amido Black.

Northern-blot analyses of RNA

RNA was separated on 1.2% agarose gels (20 cm $\times$ 25 cm) containing formaldehyde, by the procedure described by Ausubel *et al.* (1987). The RNA was dissolved in electrophoresis buffer [20 mM-Mops (pH 7.0)/5 mM-sodium acetate/1 mM-EDTA, pH 8.0] containing Bromophenol Blue, 48% (v/v) formamide (deionized), 2.2 M-formaldehyde and 5% (v/v) glycerol. The RNA was denatured at 95 °C for 2 min, then loaded on to the gel, and separation was carried out at 125 V for 3.5 h. After electrophoresis, the gel was rinsed several times in water and incubated for 45 min in 1.5 M-NaCl/150 mM-sodium citrate, pH 7.0. The RNA was transferred by capillary action from the gel to a nitrocellulose membrane (Bio-Rad Laboratories) in a solution of 3 M-NaCl/0.3 M-sodium citrate, pH 7.0, over a period of 12–24 h. The membrane was then air-dried and baked for 2 h at 80 °C under vacuum. Pre-hybridization and hybridization with a synthetic oligonucleotide probe were performed as described by Davis *et al.* (1986), with modifications. Non-specific DNA-binding sites on the membrane were blocked by incubating the membrane for 12 h at room temperature in a heat-sealed bag containing hybridization buffer [300 mM-NaCl/30 mM-sodium citrate (pH 7.0)/0.2% polyvinylpyrrolidone/0.2% BSA/0.2% Ficoll 400 containing 0.15 mg of salmon sperm DNA/ml of solution and 1.6 mg of yeast RNA/ml of solution]. The membrane was then incubated for 20 h at 42 °C in hybridization buffer containing the ALA synthase-specific oligonucleotide probe labelled at the 5'-end with ^{32}P (10^6 c.p.m./ml). After hybridization, the membrane was washed four times (30 min per wash) at 42 °C in 300 mM-NaCl/30 mM-sodium citrate, pH 7.0, then placed against X-ray film (Kodak XAR-5). The positions of molecular-mass markers on the agarose gel were determined by staining appropriate lanes of the gel with ethidium bromide and photographing the gel under u.v. light.

Fig. 1. Effects of porphyrogenic agents on ALA synthase activity in various tissues

Embryos were injected with the indicated agents then incubated for 18 h before ALA synthase activity was determined on tissue homogenates. Three or more organs were pooled for each determination, and three to nine independent determinations were made for each condition. Assays were run in triplicates, and the values represent means $\pm$ S.E.M. ALA synthase activity is given in nmol/h per mg of protein. * Indicates a value significantly different from its corresponding control ($P < 0.05$) as determined by Student's t test. Symbols: □, control; ▨, AIA + DDC; ▩, testosterone.

RESULTS

Induction of ALA synthase in different tissues

Injections of a mixture of the porphyrogenic agents AIA and DDC or of testosterone into the fluid surrounding chick embryos lead to elevations in liver ALA synthase activity (Sassa & Kappas, 1981; Ades, 1986) and to higher concentrations of liver ALA synthase RNA (Ades & Harpe, 1981; Drew & Ades, 1986). As a first step in the present study, we examined the effects of porphyrogenic agents on the enzyme's activities in several tissues of chick embryos. AIA and DDC significantly increased ALA synthase activity in kidney and intestine, but the agents had no measurable effects on the activities of the enzyme in heart, brain or lung (Fig. 1). In addition, by cDNA-RNA solution-hybridization analyses, we determined that AIA and DDC increased the concentrations of ALA synthase RNA in intestine and kidney, and that testosterone did so in kidney (Fig. 2). It is noteworthy that, whereas testosterone had a minimal effect on ALA synthase activities in liver and kidney (Fig. 1), the effect of the hormone on the enzyme's mRNA in those tissues was relatively more pronounced (Fig. 2). No changes in the concentrations of ALA synthase mRNA were detected in heart, lung or brain from embryos given the porphyrogenic agents.

Effects of ALA and FeCl_3 on ALA synthase mRNA

ALA and FeCl_3 when added into the medium of primary cultures of chick-embryo hepatocytes, blocked the processing of ALA synthase precursor and inhibited AIA-induced elevation of ALA synthase mRNA concentrations in the cells (Ades *et al.*, 1987; Ades & Stevens, 1988). To determine whether such a regulation might take place in tissues other than liver, we loaded embryos with ALA and FeCl_3 at the same time as the animals were given the porphyrogenic agents. The effects of the

protocol on the concentrations of ALA synthase mRNA in liver, kidney and intestine were then determined. That ALA injected into the fluid surrounding embryos could enter tissues was evident from the results in Fig. 3. The amount of ALA measured in liver varied almost linearly with the amount of ALA injected through the air-sac membrane, such that the concentration of ALA in livers of embryos given 20 mg of ALA was 6-fold greater than the control (Fig. 3). The results of the RNA analyses clearly indicated that loading embryos with the haem precursors inhibited the inducing effects of porphyrogenic agents in liver, kidney and intestine. As seen in Fig. 4, the concentrations of ALA synthase mRNA were consistently lower in the tissues of embryos loaded with ALA and FeCl_3 compared with those embryos treated with the inducers only.

Sizes of ALA synthase in different tissues

Several investigators have suggested that ALA synthase activity in different organs of rodents and chickens might be due to tissue-specific isoenzymes that can be distinguished on the basis of structural and immunochemical differences (Bishop *et al.*, 1981; Watanabe *et al.*, 1983; Yamamoto *et al.*, 1985). We have examined such a possibility by analysing, with immuno-blots, mitochondrial proteins from different tissues of embryos subjected to various experimental protocols (Fig. 5). A polyclonal antibody prepared against liver ALA synthase (Ades & Harpe, 1981) bound to the enzyme's subunit (65000 Da) in liver mitochondria from embryos treated with AIA plus DDC or with testosterone (Fig. 5a). The weakly cross-reacting lower-molecular-mass proteins (Fig. 5a, lanes 4–8) were mitochondrial proteins unrelated to ALA synthase (Ades *et al.*, 1983). The antibody also cross-reacted with 65000 Da proteins in fractions from kidney and from intestine (Fig. 5b), and the concentrations of the 65000 Da proteins were higher in fractions

Fig. 2. Effects of porphyrogenic agents on ALA synthase RNA

Embryos were treated with porphyrogenic agents (symbols as in Fig. 1) and incubated for 14 h. RNA was then isolated from the indicated tissues. Solution-hybridization analyses were performed with 40 μ g of RNA per reaction, and each determination was run in triplicate. Each value represents the average c.p.m. ($\pm$ S.E.M.) of acid-insoluble ³²P-labelled nucleic acids remaining after treatment of the hybridization reaction with S₁ nuclease. Three or more organs were pooled for each determination, and three to six independent determinations were made for each condition. *Indicates a value significantly different from its corresponding control ($P < 0.05$) as determined by Student's test.

from embryos treated with porphyrogenic agents (Fig. 5). That the 65000 Da intestinal and kidney proteins were indeed ALA synthase was supported by the facts that (a) the concentrations of the proteins were lower in mitochondria from embryos injected with testosterone

compared with those given AIA and DDC, and (b) the concentrations of the proteins fell in fractions from embryos loaded with ALA plus FeCl₃ (Fig. 5). Thus the molecular masses of the subunits of ALA synthase in liver, intestine and kidney appeared identical; in addition, the haem precursors ALA and FeCl₃ blocked the elevation in the enzyme's mass brought about by porphyrogenic agents in each tissue, paralleling the observations on ALA synthase mRNA concentrations (Fig. 4).

Fig. 3. Concentration of ALA in livers of embryos pre-loaded with the amino acid

The indicated amount of ALA was dissolved in 0.1 ml of DMSO and injected into the fluid surrounding each embryo. Embryos serving as controls received the vehicle only. The embryos were then incubated for 18 h, and, after the incubation, the amounts of ALA in liver homogenates were determined. Livers from at least three embryos were pooled for each determination. The assays were performed in triplicates, and the values represent means $\pm$ S.E.M. Liver ALA is expressed in nmol/mg of protein.

Sizes of ALA synthase mRNA in different tissues

The results of the immuno-blot analyses suggested that ALA synthase activities in liver, intestine and kidney were due to an identical protein whose production was modulated by a similar mechanism in each tissue. In order to determine the sizes of the enzyme's mRNA in different organs, we performed Northern-blot analyses on RNA isolated from liver, intestine, kidney, heart, brain and lung. As seen in Fig. 6(a), the probe bound in each case primarily to a 2.3 kb RNA, which agreed with the sizes previously reported for chicken liver and erythroid ALA synthase mRNA (Elferink *et al.*, 1987) and ALA synthase mRNA from tissues of mouse (Schoenhaut & Curtis, 1986) and rat (Srivastava *et al.*, 1988). In addition, the Northern-blot analyses of RNA isolated from tissues of embryos pre-treated with haem precursors and/or porphyrogenic agents (Fig. 6b) supported the results of the solution-hybridization assays (Figs. 2 and 4). In responsive tissues, the porphyrogenic agents increased the tissue content of ALA synthase mRNA (Fig. 6b), and loading embryos with ALA and FeCl₃ counteracted the process. Thus, together with data from the immuno-blots, the results of the Northern-blot analyses

Fig. 4. Effect of loading embryos with ALA and FeCl_3 on tissue concentrations of ALA synthase mRNA

Embryos were administered either AIA plus DDC or testosterone and incubated for 14 h. Where indicated, the embryos were also given 20 mg of ALA plus 0.2 mg of FeCl_3 (in 0.1 ml of DMSO) together with the porphyrogenic agent(s). Control embryos received the vehicle only. At the end of the incubation, RNA was isolated from selected tissues and analysed by solution hybridization for ALA synthase mRNA sequences. Hybridization reactions were run in triplicate, with 40 μg of RNA used in each reaction. Organs were pooled from three or more embryos for each determination, and three independent determinations were made for each condition. The values represent the radioactivity (c.p.m.) of acid-insoluble ^{32}P -labelled nucleic acids remaining after treatment of the hybridization reaction mixtures with S_1 nuclease. Symbols: ■, control; □, AIA + DDC; ▨, AIA + DDC + ALA + FeCl_3 ; ▩, testosterone; ▤, testosterone + ALA + FeCl_3 .

Fig. 5. Effects of porphyrogenic agents on the immuno-reactive mass of ALA synthase

Embryos were administered either AIA plus DDC or testosterone, then incubated for 18 h. Where indicated, the embryos were also given 20 mg of ALA plus 0.2 mg of FeCl_3 together with the porphyrogenic agent(s). At the end of the incubation, the mitochondrial fractions were prepared from the indicated tissues and analysed for ALA synthase by immuno-blots. In each case, 7.5 μg of protein from the mitochondrial fraction was loaded on to the polyacrylamide gel used in the electrophoresis. The arrow indicates the position of the liver ALA synthase subunits (65000 Da). (a) Lanes 1–3 show a blot incubated with pre-immune serum. The slots on the gel used had been loaded with proteins from embryos treated with AIA + DDC from (1) kidney, (2) intestine and (3) liver. Lanes 4–8 show a blot incubated with immune serum. Only fractions from liver were analysed on the blot, and the slots contained samples from embryos treated as follows: (4) testosterone and ALA + FeCl_3 ; (5) AIA + DDC and ALA + FeCl_3 ; (6) testosterone; (7) AIA + DDC; (8) control. (b) The blot was processed with immune serum. Lanes 1–5 contained fractions from the kidney of embryos treated as follows: (1) testosterone and ALA + FeCl_3 ; (2) AIA + DDC and ALA + FeCl_3 ; (3) testosterone; (4) AIA + DDC; (5) control. Lanes 6–8 contained fractions from intestine of embryos treated as follows: (6) AIA + DDC and ALA + FeCl_3 ; (7) AIA + DDC; (8) control.

suggested that ALA synthase activities in the tissues examined were due most likely to identical proteins synthesized from identical mRNA molecules.

DISCUSSION

Porphyrogenic agents cause substantial increases in liver ALA synthase activity, especially in chicken embryos

(reviewed by Sassa & Kappas, 1981; Marks, 1985). The elevations in activity of the enzyme in hepatocytes have been associated with selective increases in the rate of production of the enzyme (reviewed by Ades, 1986) and with higher concentrations of the enzyme's mRNA (Ades & Harpe, 1981; Drew & Ades, 1986; Ades *et al.*, 1987; Zhang & Ades, 1987; Hamilton *et al.*, 1988). Early studies have indicated that actinomycin D blocks the

Fig. 6. Effects of porphyrogenic agents on ALA synthase mRNA

Embryos were treated with the various agents, and RNA was isolated from the tissues under the conditions described in the legend of Fig. 4. The RNA was analysed for ALA synthase-specific sequences by Northern blots. The positions of the RNA molecules (18S, large; sm, small) are indicated on the Figure. The arrow indicated the position of a 2.3 kb RNA corresponding to ALA synthase mRNA. (a) RNA (40 μ g) isolated from tissues of control embryos: lane 1, intestine; lane 2, heart; lane 3, brain; lane 4, kidney; lane 5, lung. (b) RNA (10 μ g) isolated from tissues of embryos treated with the agents indicated. Lanes 1-5, liver RNA from: (1) control; (2) treated with AIA + DDC; (3) treated with testosterone; (4) treated with AIA + DDC + ALA + FeCl_3 ; (5) treated with testosterone and ALA + FeCl_3 . Lanes 6-8, intestine RNA from: (6) control; (7) treated with AIA + DDC; (8) treated with AIA + DDC + ALA + FeCl_3 . Lanes 9-13, kidney RNA from: (9) control; (10) treated with AIA + DDC; (11) treated with testosterone; (12) treated with AIA + DDC + ALA + FeCl_3 ; (13) treated with testosterone and ALA + FeCl_3 .

induction of ALA synthase activity by xenobiotics (Sassa & Granick, 1970; Strand *et al.*, 1972; Tyrrell & Marks, 1972), and so porphyrogenic agents probably act in part by elevating the transcription rate of ALA synthase mRNA. It is also clear now that haem, either provided to hepatocytes or generated by the cells from ALA and iron, regulates the production of ALA synthase by arresting the maturation of the precursor form of the enzyme (Ades, 1983; Ades *et al.*, 1983; Ades & Stevens, 1988) and by lowering the cellular concentration of the enzyme's mRNA (Drew & Ades, 1986; Ades *et al.*, 1987; Zhang & Ades, 1987; Hamilton *et al.*, 1988).

The results in the present paper indicated that, in addition to their effects on liver ALA synthase, AIA and DDC increased the enzyme's activity, mass and mRNA in the intestine and kidney, and that testosterone did so in the kidney. The agents did not detectably alter the enzyme's activity or mRNA levels in the heart, brain and lung. In addition, the analyses did not indicate that ALA synthase activities in the tissues examined resulted from tissue-specific isoenzymes. Of course, the interpretation is limited to the enzymes in the tissues examined and to the developmental stage of the animals. The possibility of the appearance of development-specific or even tissue-specific isoenzyme forms of ALA synthase after hatching remains to be examined. Finally, ALA and FeCl_3 inhibited the inductions of the enzyme in tissues responsive to the porphyrogenic agents. In related work, Srivastava *et al.* (1988) reported that ALA does decrease the constitutive levels of ALA synthase mRNA in several rat tissues.

We had determined in previous studies that haemin, when added into the medium of primary cultures of

chick-embryo hepatocytes, inhibited the elevations in ALA synthase activity and mass brought about by AIA (Ades, 1983; Ades *et al.*, 1983). This probably reflects an end-product modulation of porphyrin production in liver, since ALA synthase catalyses the rate-limiting step of the process. We wished to determine whether such a regulation by haemin might also take place in tissues other than liver. Our attempts to load embryos with haemin were not successful, however, because both haemin and haem arginate (Tokola, 1987) formed aggregates upon injection into the fluid surrounding chick embryos. Thus the less direct approach of loading embryos with ALA and FeCl_3 was used. Anderson *et al.* (1981) had determined that ALA, when administered *in ovo* to chicken embryos, formed haem in liver. In addition, ALA and FeCl_3 blocked the processing of ALA synthase precursor and inhibited the effect of AIA on ALA synthase mRNA concentrations in primary cultures of hepatocytes (Ades *et al.*, 1987; Ades & Stevens, 1988); in those studies, neither ALA or FeCl_3 alone was as effective as the combination in limiting the effect of the porphyrogenic agent. We had also determined that protoporphyrin IX, when added into the medium of hepatocytes in culture, was considerably less effective than haemin (Lorber & Ades, 1989). On the basis of the observations with hepatocytes in culture, it is our working hypothesis at present that haem concentration, rather than that of a haem precursor, regulates the production of ALA synthase in liver, intestine and kidney.

It is interesting that porphyrogenic agents also elevate the levels of cytochrome P-450 in liver, intestine and kidney (Anderson *et al.*, 1982; Adesnik & Atchison,

1986; Traber *et al.*, 1988). Increased production of cytochrome *P*-450 in a tissue could lead to a fall in intracellular free haem concentration, as free haem becomes associated with apo-cytochrome *P*-450. Thus porphyrinogenic agents, by inducing the production of cytochrome *P*-450, may indirectly cause a de-repression of the gene coding for ALA synthase, owing to lower intracellular haem concentration. Inducers may affect free haem concentrations by other mechanisms, such as inhibiting haem production (Ades, 1986; Marks *et al.*, 1988) or causing the suicidal inactivation of cytochrome *P*-450 through the formation of adducts with the enzyme's haem moiety (reviewed by Marks *et al.*, 1988). Of course, porphyrinogenic agents may also stimulate ALA synthase mRNA transcription in responsive tissues by mechanisms independent of haem. This possibility is supported by the studies by Hamilton *et al.* (1988). They measured the time course of the inductions of the mRNAs for apo-cytochrome *P*-450 and ALA synthase in chick-embryo hepatocytes. The concentrations of the mRNA molecules for both enzymes rose rapidly and with similar rates, suggesting simultaneous inductions of the two messengers. Given the relatively large number of porphyrinogenic agents and the structural dissimilarities among the classes of inducers, it would not be surprising if xenobiotics induce ALA synthase by more than one mechanism.

We can only speculate at present about the process by which haem affects ALA synthase mRNA levels. Nuclear transcriptional run-off measurements on rat liver nuclei have suggested that either haem or one of its precursors negatively regulates the transcription of ALA synthase mRNA (Srivastava *et al.*, 1988). However, the possibility that haem might also affect the stability of ALA synthase mRNA has not been investigated. It is conceivable that the effect of haem on the concentration of ALA synthase mRNA might be in part the result of haem-induced degradation of the messenger.

Note added in proof (Received 19 July 1989)

Recently we determined that haem affects the stability of ALA synthase such that the half-life of the messenger is shortened considerably in hepatocyte cultures when haemin is added to the medium (Drew & Ades, 1989).

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