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SYNERGISTIC INDUCTION OF δ-AMINOLEVULINIC ACID SYNTHASE ACTIVITY BY *N*-ETHYLPROTOPORPHYRIN IX AND 3,5-DIETHOXYCARBONYL-1,4-DIHYDRO-2,6-DIMETHYL-4-ISOBUTYLPYRIDINE*

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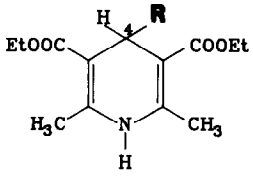
Abstract—The levels of the cellular free heme pool in chick embryo hepatocyte culture were lowered using *N*-ethylprotoporphyrin IX (*N*-ethylPP) and analogues of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC), and the effect on δ-aminolevulinic acid synthase (ALAS) was examined. *N*-EthylPP, which lowers cellular heme levels by inhibiting ferrochelatase activity, produced an induction of ALAS activity to 444% of control at 3 hr after its administration. 4-Ethyl DDC, which lowers heme levels by destroying the heme moiety of cytochrome P-450 and lowering ferrochelatase activity, caused an induction of ALAS to 565% of control at 12 hr after administration. 4-Isobutyl DDC, which lowers heme levels by destroying the heme moiety of cytochrome P-450, induced the activity of ALAS to 289% of control at 3 hr after administration. This indicates that ferrochelatase inhibition is a more important mechanism of heme lowering than alkylation of cytochrome P-450 heme when both heme-depleting mechanisms are acting in chick embryo liver cells. It was anticipated that administration of a combination of 4-isobutyl DDC plus *N*-ethylPP would mimic the effect of 4-ethyl DDC. However, this combination induced ALAS activity to levels that were much greater than those observed after 4-ethyl DDC (1257% of control at 12 hr). This synergistic induction may be attributable to lowering of free heme levels to the point where transcription, translation, and translocation of ALAS are all derepressed.

Hepatic heme synthesis is regulated by the first enzyme in the heme biosynthetic pathway, δ-aminolevulinic acid synthetase (ALAS‡) (EC 2.3.1.37), via feedback represssion by the end-product, heme [1]. It has been postulated that heme regulates ALAS activity through a free heme pool in cells; free heme is heme which is not bound by cellular components [1]. According to current ideas, as the level of free heme decreases the activity of ALAS is increased and vice versa. Heme appears to manifest this control in three ways: (1) by inhibiting transcription of mRNA for ALAS [2]; (2) by inhibiting translation of ALAS [3, 4]; and (3) by slowing the transfer of pre-ALAS in the cytosol to mature ALAS in the mitochondria [5, 6], where it participates in heme synthesis.

Some of the ways in which the free heme pool can be manipulated experimentally are as follows [7]: (1) by inactivating cytochrome P-450 through heme alkylation; the alkylated heme moiety is released from the cytochrome P-450 apo-protein, and the free apo-protein combines with a heme molecule, thereby removing heme from the free heme pool; (2) by inhibiting an enzyme in the heme biosynthetic pathway, for example ferrochelatase (FC) (EC 4.99.1.1); (3) by both alkylating the heme moiety of cytochrome P-450 and inhibiting FC activity; and (4) by inducing the synthesis of the apo-protein of cytochrome P-450 [8] which will remove heme by producing holo-cytochrome P-450.

Table 1. Structures of the 4-alkyl analogues of DDC

Name	R group
4-Ethyl DDC	$-CH_2-CH_3$
4-Isobutyl DDC	$-CH_2-CH-(CH_3)_2$



A compound which has proved useful in the study of heme biosynthesis is 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC), which lowers the activity of FC [9]. DDC causes mechanism-based inactivation of cytochrome P-450; during this metabolism, a methyl radical is released from DDC which binds to a nitrogen of the heme moiety of cytochrome P-450, producing *N*-methylprotoporphyrin IX (*N*-methylPP), which is released from cytochrome P-450 [10]. This *N*-methylPP is a potent inhibitor of FC activity [11–13] and, consequently, causes the accumulation of protoporphyrin [9, 13]. An analogue of DDC, 4-ethyl DDC (Table 1), has been shown to act in a manner similar to DDC [12, 13]. 4-Ethyl DDC causes a destruction of

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‡ Abbreviations: ALAS, δ-aminolevulinic acid synthase; DDC, 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine; *N*-ethylPP, *N*-ethylprotoporphyrin IX; and FC, ferrochelatase.

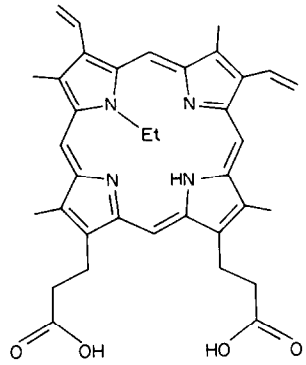

Fig. 1. Structure of *N*-ethylprotoporphyrin IX.

cytochrome P-450 heme along with the formation of *N*-ethylPP (Fig. 1), and this *N*-ethylPP causes the inhibition of FC and the accumulation of protoporphyrin IX. A series of analogues of DDC act in this way [12, 13]; however, it has been found that all the 4-alkyl analogues of DDC do not affect heme biosynthesis in the same manner [14]. 4-Isobutyl DDC (Table 1) decreases P-450 levels through mechanism-based destruction of cytochrome P-450 heme but the *N*-isobutylPP produced does not inhibit FC; coproporphyrin is the major porphyrin to accumulate in this case [15].

DDC also produces an induction of ALAS activity [12]. De Matteis and Marks [16] have suggested that this induction can be attributed primarily to the inhibition of FC caused by the *N*-methylPP produced during metabolism of DDC. The goal of this work was to determine the contribution of FC inhibition by *N*-ethylPP to the induction of ALAS activity caused by 4-ethyl DDC [12] in chick embryo hepatocyte culture. The specific objectives of this study were: (i) to determine the effect of inhibiting FC activity with *N*-ethylPP on ALAS activity over 24 hr; (ii) to compare this to the effect of 4-ethyl DDC on ALAS activity; (iii) to compare this to the effect of 4-isobutyl DDC on ALAS activity, as it does not inhibit FC. A 4 μM concentration of the DDC compounds and a 2 μM concentration of *N*-ethylPP were chosen for the determination of ALAS activity, as these concentrations have been shown previously to produce maximal inhibition of ferrochelatase activity [15, 17].

MATERIALS AND METHODS

Materials. [2,3-^{14}C]Succinic acid (40–80 mCi/mmol) was purchased from New England Nuclear (Dupont, Boston, MA). The DDC analogues were synthesized as previously described [14, 15]. *N*-Ethylprotoporphyrin IX was synthesized as described by DeMatteis *et al.* [18]. White Leghorn chick embryos obtained from Archer's Poultry Farm, Brighton, Ontario, were used at 18 days after fertilization.

Determination of δ-aminolevulinic acid synthetase activity. Hepatocytes of 18-day-old chick embryos were obtained as previously described [19] and maintained in 10-cm dishes containing 15.0 ml of Waymouth 705/1 medium. The medium was changed 24 and 42 hr after plating. Drugs were added in 95% EtOH, along with an ethanol solvent control, at 42 hr, with the ethanol not exceeding 0.064% of the final volume, and the cells were incubated for 0, 3, 6, 12, or 24 hr. The activity of δ-aminolevulinic acid synthase was measured as previously described [20, 21]. Briefly, a homogenate of the hepatocytes was incubated with [2,3-^{14}C]succinic acid, pyridoxal phosphate, glycine, and ATP. The labelled ALA formed during that time was isolated by cation exchange chromatography and by reaction of the ALA with acetylacetone to form a pyrrole. The pyrrole was extracted and quantitated using a liquid scintillation counter. The activity was expressed as picomoles ALA formed per milligram of protein per 30 minutes.

Protein determination. The amount of protein was determined by the method of Lowry *et al.* [22].

Statistical analysis. An analysis of variance test was used to determine whether two means differed significantly from each other ($P \leq 0.05$) in the assay of ALAS activity. If a significant F ratio at the 0.05 level was obtained, a Newman–Keuls test was used to indicate the means which differed significantly from each other ($P \leq 0.05$).

RESULTS AND DISCUSSION

De Matteis and Marks [16] have postulated that the induction of ALAS caused by DDC is primarily a result of the inhibition of FC caused by the *N*-methylPP which accumulates following the administration of DDC. This study was designed to determine the contribution of FC inhibition by *N*-ethylPP to the induction of ALAS caused by 4-ethyl DDC in chick embryo hepatocyte culture. To do this, the effect of *N*-ethylPP, the metabolic product of 4-ethyl DDC, on the activity of ALAS was examined over a 24-hr period. *N*-EthylPP produced an induction of ALAS activity to 444% of control levels at 3 hr after administration (Fig. 2a); activity returned to control levels by 24 hr, despite the fact that FC activity remained maximally inhibited (data not shown). This increase in ALAS activity is attributed to the inhibition of FC by *N*-ethylPP [16], which will decrease the levels of the cellular free heme pool of the hepatocytes. Previous experiments in this laboratory revealed no induction of ALAS activity when enzyme activity was measured 12 hr after the administration of *N*-methylPP [23]. Subsequently it was shown in our laboratory that an increase in ALAS activity may be demonstrated when the activity is assessed 5 hr after the administration of *N*-methylPP [16]. The results of the present study are in general agreement with the previous observations; ALAS activity was maximal 3 hr after *N*-ethylPP administration and dropped thereafter (Fig. 2a).

The effect of 4-ethyl DDC on ALAS activity was measured and compared to the effect of *N*-ethylPP on ALAS activity. 4-Ethyl DDC (Fig. 2b) caused an induction of ALAS to 565% of control levels at 12 hr after administration; activity returned to control levels by 24 hr, despite the fact that FC activity remained maximally inhibited (data not shown). 4-Ethyl DDC did not cause maximal induction of

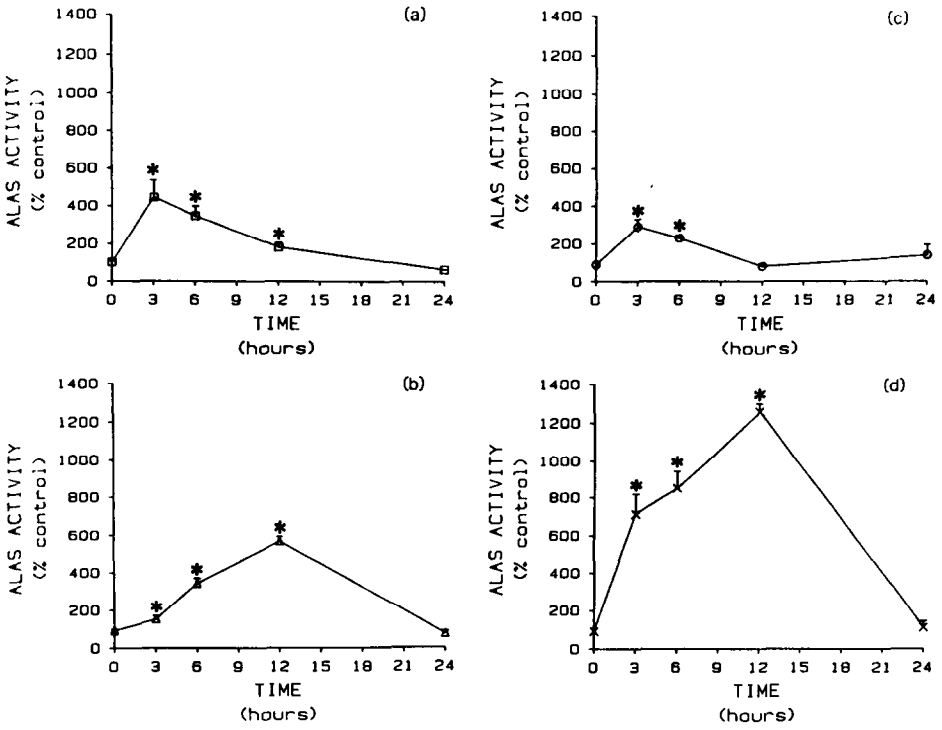

Fig. 2. Activity of δ-aminolevulinic acid synthase expressed as percent of a 95% ethanol (0.064%) solvent control in chick embryo liver cell culture at various times following the administration of: (a) *N*-ethylPP; (b) 4-ethyl DDC; (c) 4-isobutyl DDC; and (d) 4-isobutyl DDC + *N*-ethylPP. All DDC compounds were given at a concentration of 4 μM, and *N*-ethylPP was given at a 2 μM concentration. Each point is the mean (±SD) of three determinations. Results were confirmed in an additional experiment run with all compounds simultaneously. An asterisk (*) indicates an increase over control levels at $P \leq 0.05$. Control levels of ALAS activity were 216.5 ± 22.6 pmol/mg protein/30 min.

ALAS activity until 12 hr, a period considerably later than the 3-hr peak caused by *N*-ethylPP. This delay can be explained, at least in part, by the time required for formation of adequate levels of *N*-ethylPP. The total induction, seen as the area under the curve, caused by 4-ethyl DDC was greater than that caused by *N*-ethylPP (1.9 times greater). It is apparent that the induction of ALAS caused by 4-ethyl DDC cannot be accounted for solely by the inhibition of FC caused by *N*-ethylPP, as the two curves are not identical. Another important mechanism(s) must be involved.

One possible mechanism by which 4-ethyl DDC could be lowering the cellular free heme pool involves the mechanism-based inactivation of cytochrome P-450. *N*-EthylPP is formed during the metabolism of 4-ethyl DDC [9]; an ethyl radical is released from the position 4 group of 4-ethyl DDC which binds to one of the nitrogens of the heme moiety of cytochrome P-450. The loss of the heme from cytochrome P-450 leaves the apo-protein moiety. If this apo-protein is unchanged, then it could combine with a free heme molecule, regenerating holo-cytochrome P-450 and lowering the levels of the free heme pool. Support for this idea comes from work done in several laboratories with the potent porphyrinogenic compound allyisopropylacetamide (AIA) [24–30]. To study the contribution of the destruction of cytochrome P-450 by analogues of DDC in their induction of ALAS activity, the effect of 4-isobutyl DDC on ALAS activity was determined. This compound causes the formation of *N*-isobutylPP through the destruction of cytochrome P-450 heme, but FC activity is not affected by the *N*-alkylPP [31]. The amount of cytochrome P-450 which is destroyed over a 30-min period in a chick embryo liver microsomal preparation is similar for 4-isobutyl DDC and 4-ethyl DDC [14, 15]. 4-Isobutyl DDC (Fig. 2c) caused an increase in ALAS activity to 289% of control levels at 3 hr after administration to chick embryo hepatocytes; activity returned to control levels by 12 hr. This result, along with the results obtained earlier, suggests that the destruction of cytochrome P-450 is involved in the induction of ALAS seen with DDC analogues like 4-ethyl DDC, but that the inhibition of FC is more important.

Additional mechanisms have been considered whereby porphyrin-inducing drugs might induce an elevation of ALAS activity [32]. These additional mechanisms, which are independent of inhibition of heme biosynthesis or of stimulation of heme degradation, are the following: (1) a direct action on the nucleus to increase the amount of mRNA for ALAS; and (2) increased synthesis of the mRNA for apocytochrome P-450, leading to increased synthesis of

apo-cytochrome P-450 with increased utilization of heme from the "regulatory free heme pool" for the synthesis of cytochrome P-450. Propylisopropylacetamide (PIA), the saturated analogue of AIA, induces ALAS activity in chick embryo hepatocytes without destroying the heme moiety of cytochrome P-450 or inhibiting any step in heme biosynthesis [33]. Recent studies of the action of PIA in chick embryo hepatocytes shows that PIA increases the steady-state levels of the mRNA for ALAS concurrently with the mRNA for cytochrome P-450 [34]. Moreover, the addition of heme to the cultures does not cause the mRNA for ALAS to return to control levels. These results suggest a direct action of PIA on the nucleus to increase the amount of mRNA for ALAS. It is possible that one component of the action of dihydropyridines on ALAS activity may be attributable to a direct action on the nucleus to increase the steady-state levels of mRNA for ALAS.

It was anticipated that the combination of *N*-ethylPP plus 4-isobutyl DDC would produce a curve similar to the one seen with 4-ethyl DDC, since this represents a combination of an agent inhibiting FC with an agent causing the destruction of cytochrome P-450 heme. To confirm this, the effect of a combination of *N*-ethylPP and 4-isobutyl DDC on ALAS activity was determined. The results from this work (Fig. 2d) show that the combination of *N*-ethylPP and 4-isobutyl DDC produced a considerably larger induction of ALAS activity than was anticipated. The levels of ALAS activity reached a peak of 1257% of control at 12 hr after administration; activity returned to control levels by 24 hr. It is obvious that the area under the curve for this figure is much greater than for any other treatment. The most interesting result was that obtained at the 12-hr point. At this point, *N*-ethylPP (Fig. 2a) caused only a slight increase and 4-isobutyl DDC (Fig. 2c) caused no significant increase in the activity of ALAS; however, the combination of the two produced over a 1200% increase in the activity of ALAS.

There are several possible explanations for the synergistic effect of these two compounds on ALAS activity. One explanation is as follows. As discussed in the introduction, heme manifests control on ALAS in three ways: transcription, translation, and translocation. It is possible that the level of free heme required to exert optimal control on each of these processes varies. Thus, inhibition of FC by *N*-ethylPP may reduce levels of free heme to the point where one or two but not all three control mechanisms are derepressed. Destruction of the heme moiety of cytochrome P-450 may lower the remaining heme levels to the point where all three control mechanisms are derepressed, and ALAS activity is able to reach maximum attainable levels. It is of interest that AIA has been shown to decrease the microsomal and cytosolic but not the nuclear heme pools. On the other hand, inhibitors of heme biosynthesis, such as cobalt chloride and 3-amino-1,2,4-triazole, are reported to lower all three heme pools [35].

In summary we have shown that lowering of cellular heme levels by 4-alkyl DDC analogues and *N*-ethylPP will produce an induction of ALAS activity. This induction of ALAS by 4-ethyl DDC cannot be explained completely by its inhibition of FC by *N*-ethylPP. It also appears that FC inhibition is more effective in lowering the levels of the cellular free heme pool than is the lowering of cytochrome P-450 levels through cytochrome P-450-heme alkylation. The levels of induction of ALAS activity obtained here with *N*-ethylPP and 4-isobutyl DDC suggest a synergistic interaction between these two compounds in lowering the levels of free heme in chick embryo hepatocyte culture.

REFERENCES

1. Kappas A, Sassa S and Anderson KE, The Porphyrias. In: *The Metabolic Basis of Inherited Disease* (Eds. Stanbury JB, Wyngaardern JB, Frederickson DS, Goldstein JL and Brown MS), pp. 1301–1384. Academic Press, New York, 1983.
2. Whiting MJ, Synthesis of δ-aminolevulinic acid synthase by isolated liver polyribosomes. *Biochem J* **158**: 391–400, 1976.
3. Sassa S and Granick S, Induction of δ-aminolevulinic acid synthetase in chick embryo liver cells in culture. *Proc Natl Acad Sci USA* **67**: 517–522, 1970.
4. Tyrrell DJL and Marks GS, Drug-induced porphyrin biosynthesis—V. Effect of protohemin on the transcriptional and post-transcriptional phases of δ-aminolevulinic acid synthetase induction. *Biochem Pharmacol* **21**: 2077–2093, 1972.
5. Hayashi N, Kurashima Y and Kikuchi G, Mechanism of allylisopropylacetamide-induced increase of δ-aminolevulinate synthetase in liver mitochondria. V. Mechanism of regulation by hemin of the level of δ-aminolevulinate synthetase in liver mitochondria. *Arch Biochem Biophys* **148**: 10–21, 1972.
6. Nakakuki M, Yamamuchi K, Hayashi N and Kikuchi G, Purification and some properties of δ-aminolevulinate synthetase from the rat liver cytosol fraction and immunochemical identity of the cytosolic enzyme and the mitochondrial enzyme. *J Biol Chem* **255**: 1738–1745, 1980.
7. Marks GS, McCluskey SA, Mackie JE, Riddick DS and James CA, Disruption of hepatic heme biosynthesis after interaction of xenobiotics with cytochrome P-450. *FASEB J* **2**: 2774–2783, 1988.
8. May BK, Borthwick IA, Srivestava G, Pirola BA and Elliott WH, Control of 5-aminolevulinate synthase in animals. *Curr Top Cell Regul* **28**: 233–262, 1986.
9. Onisawa J and Labbe RF, Effects of diethyl-1,4-dihydro-2,4-trimethylpyridine-3,5-dicarboxylate on the metabolism of porphyrins and iron. *J Biol Chem* **238**: 724–727, 1963.
10. Augusto O, Beilan HS and Ortiz de Montellano PR, The catalytic mechanism of cytochrome P-450. Spin-trapping evidence for one-electron substrate oxidation. *J Biol Chem* **257**: 11288–11295, 1982.
11. Tephly TR, Gibbs AH and DeMatteis F, Studies on the mechanism of experimental porphyria produced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. Role of a porphyrin-like inhibitor of protohaem ferrolyase. *Biochem J* **180**: 241–244, 1979.
12. Cole SPC and Marks GS, Structural requirements in dihydropyridines for ferrochelatase inhibition and δ-aminolevulinic acid synthetase induction. *Int J Biochem* **12**: 989–992, 1984.
13. Cole SPC, Whitney RA and Marks GS, Ferrochelatase-inhibitory and porphyrin-inducing properties of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and its analogues in chick embryo liver cell culture. *Mol Pharmacol* **20**: 395–403, 1981.
14. Marks GS, Allen DT, Johnston CT, Sutherland EP, Nakatsu K and Whitney RA, Suicidal destruction of

cytochrome P-450 and reduction of ferrochelatase activity by 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and its analogues in chick embryo liver cells. *Mol Pharmacol* **27**: 459–465, 1985.

15. McCluskey SA, Marks GS, Sutherland EP, Jacobsen N and Ortiz de Montellano PR, Ferrochelatase-inhibitory activity and *N*-alkylprotoporphyrin formation with analogues of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine. *Mol Pharmacol* **30**: 352–357, 1986.
16. De Matteis F and Marks GS, The effect of *N*-methylprotoporphyrin and succinyl-acetone on the regulation of heme biosynthesis in chick hepatocyte culture. *FEBS Lett* **159**: 127–131, 1983.
17. Ortiz de Montellano PR, Kunze KL, Cole SPC and Marks GS, Differential inhibition of hepatic ferrochelatase by isomers of *N*-ethylprotoporphyrin IX. *Biochem Biophys Res Commun* **103**: 581–586, 1981.
18. DeMatteis F, Gibbs AH and Smith AG, Inhibition of protohaem ferro-lyase by N-substituted porphyrins. Structural requirements for the inhibitory effect. *Biochem J* **189**: 645–651, 1980.
19. Morgan RO, Fischer PWF, Stephens JK and Marks GS, Thyroid enhancement of drug-induced porphyrin biosynthesis in chick embryo liver cells maintained in serum-free Waymouth medium. *Biochem Pharmacol* **25**: 2609–2612, 1976.
20. Brooker JD, Srivestava G, May BK and Elliott WH, Radiochemical assay for δ-aminolevulinate synthase. *Enzyme* **28**: 109–119, 1982.
21. Marks GS, Powles JE, Lyon ME, Zelt DT and McCluskey SA, Patterns of porphyrin accumulation in response to xenobiotics. *Ann NY Acad Sci* **514**: 113–127, 1987.
22. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
23. Cole SPC, Marks GS, Ortiz de Montellano PR and Kunze KL, Inhibition of ferrochelatase by *N*-methylprotoporphyrin IX is not accompanied by δ-aminolevulinic acid synthetase induction in chick embryo liver cell culture. *Can J Physiol Pharmacol* **60**: 212–215, 1982.
24. Ortiz de Montellano PR and Correia MA, Suicidal destruction of cytochrome P-450 during oxidative drug metabolism. *Annu Rev Pharmacol Toxicol* **23**: 481–503, 1983.
25. Farrell GC, Schmid R, Kunze KL and Ortiz de Montellano PR, Exogenous heme restores *in vivo* functional capacity of hepatic cytochrome P-450 destroyed by allylisopropylacetamide. *Biochem Biophys Res Commun* **89**: 456–463, 1979.
26. Farrell GC and Correia MA, Structural and functional reconstitution of hepatic cytochrome P-450 *in vivo*. Reversal of allylisopropylacetamide-mediated destruction of the hemoprotein by exogenous heme. *J Biol Chem* **255**: 10128–10133, 1980.
27. Correia MA, Farrell GC, Schmid R, Ortiz de Montellano PR, Yost GS and Mico BA, Incorporation of exogenous heme in hepatic cytochrome P-450. *J Biol Chem* **254**: 15–17, 1979.
28. Bhat KS, Sardana MK and Padmanaban G, Role of heme in the synthesis and assembly of cytochrome P-450. *Biochem J* **164**: 295–303, 1977.
29. Correia MA, Farrell GC, Olson S, Wong JS, Schmid R and Ortiz de Montellano PR, Cytochrome P-450 heme moiety. The specific target of drug-induced heme alkylation. *J Biol Chem* **256**: 5466–5470, 1981.
30. Ortiz de Montellano PR, Costa AK, Grab LA, Sutherland EP and Marks GS, Cytochrome P-450 destruction and ferrochelatase inhibition. In: Colloque INSERM *Porphyrins and Porphyrias* (Ed. Nordman Y), pp. 109–117. John Libbey Eurotext, London, 1985.
31. McCluskey SA, Marks GS, Whitney RA and Ortiz de Montellano PR, Differential inhibition of hepatic ferrochelatase by regioisomers of *N*-butyl, *N*-pentyl, *N*-hexyl, and *N*-isobutylprotoporphyrin IX. *Mol Pharmacol* **34**: 80–86, 1988.
32. DeMatteis F, Hepatic porphyrias. In: *Heme and Hemoproteins* (Eds. DeMatteis F and Aldridge WN), pp. 129–155. Springer, New York, 1978.
33. Krupa V, Creighton JC, Freeman M and Marks GS, Drug-induced porphyrin biosynthesis—XII. Levels of cytochrome P-450 in chick embryo liver following administration of allyisopropylacetamide and propylisopropylacetamide. *Can J Physiol Pharmacol* **52**: 891–895, 1974.
34. Hamilton JW, Bement WJ, Sinclair PR, Sinclair JF and Wetterhahn K, Expression of 5-aminolevulinate synthase and cytochrome P-450 mRNA's in chick embryo hepatocytes *in vivo* and in culture. *Biochem J* **255**: 267–275, 1988.
35. Dwarki VJ, Francis VNK, Bhat GJ and Padmanaban G, Regulation of cytochrome P-450 messenger RNA and apoprotein levels by heme. *J Biol Chem* **262**: 16958–16962, 1987.