INDUCTION OF HEPATIC MITOCHONDRIAL FERROCHELATASE BY PHENOBARBITAL

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Summary

Ferrochelatase, the catalyst for the last step in heme synthesis, is induced by chronic phenobarbital administration and this induction is prevented by the simultaneous administration of cycloheximide. This induction is revealed when the pyridine hemochrome was measured under anaerobic conditions but cobalt chelatase activity in hepatic mitochondria was not increased by phenobarbital. Furthermore, certain metals inhibited ferrochelatase activity but had no effect on cobalt chelatase. Although both delta aminolevulinic acid synthetase and ferrochelatase are located in a similar region in the mitochondria, the time course of induction for ferrochelatase is different than that observed for delta aminolevulinic acid synthetase.

One of the earliest measurable responses to inducers of microsomal mixed function oxidase reactions is the induction of delta aminolevulinic acid synthetase (ALAS), the catalyst for the first step in heme synthesis (1,2,3). Within 2 hours after the administration of phenobarbital or 3,4-benzpyrene significant increases in ALAS are observed (3) which coincide with increased incorporation of glycine-2-^{14}C into microsomal heme and which precede elevations in microsomal protoheme, P-450 and certain microsomal oxidations. Since ALAS and ferrochelatase (protoheme ferrolyase) are both associated with the inner membrane of the mitochondrion (4,5) and since ferrochelatase mediates the critical last step in heme synthesis, it was of interest to examine whether it is induced by phenobarbital and, if so, whether it would be increased at a rate which is similar to the rate of increase of ALAS. This report shows that phenobarbital induces ferrochelatase activity, but at a time course different than that observed for ALAS, and that this induction is not observed when cobalt chelatase activity was measured.

Methods and Materials

Sprague-Dawley, male rats weighing 50 grams were used in all studies. Water was allowed ad libitum but animals were fasted for 24 hours prior to sacrifice. Sodium phenobarbital was given at a daily dose of 60 mg/kg and cycloheximide was employed at a dose of 1 mg/kg and 2 mg/kg, 48 and 24 hours before sacrifice.

Ferrochelatase activity was studied using rat liver mitochondria prepared as described by Jones and Jones (4) except that glycine buffer was substituted for Tris buffer used by those investigators. Mitochondrial pellets were suspended finally in 0.25 M sucrose-0.1 M glycine buffer, pH 8.0. Tris buffer was not employed because a spectrum was produced non-enzymatically with cobalt at wavelengths which were used to measure the activity of the enzyme.

Two assays were used to study the activity of the enzyme. The aerobic formation of cobalt-protoporphyrin IX complex was measured at 430 mμ in a Shimadzu split beam spectrophotometer. Reaction mixtures were composed of protoporphyrin IX, 50 μM; cobalt, 50 μM; Tween 80, 0.1%; mitochondria, 1-4 mg protein per milliliter of reaction mixture and glycine buffer, 0.1 M, pH 7.8. At 37°, the increase in absorbance at 430 mμ was linear with protein concentration up to 4 mg/ml of reaction mixture and 75 minutes of incubation (Figure 1). Boiled enzyme and the omission of either cobalt or protoporphyrin IX yielded no absorbance bands. Control reactions were usually carried out without cobalt until measurements were desired, at which time cobalt chloride was added and spectral measurements were taken.

In the second assay system the formation of the pyridine hemochrome was measured from ferrous ion and protoporphyrin IX under a nitrogen atmosphere in the presence of rat liver mitochondria. This is the method described by Porra and Jones (6) except that reduced glutathione was omitted from reaction mixtures. Its omission appeared not to affect results reported here. Assays were conducted while product formation was linear with time and protein concentrations.

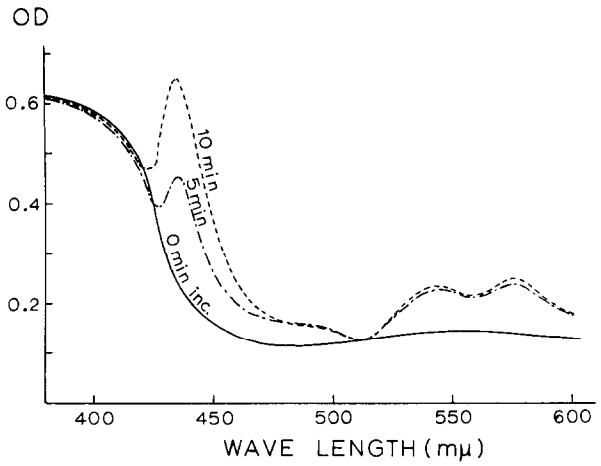

Figure 1. The formation of cobalt-protoporphyrin IX complex in rat liver mitochondria. Reactions were carried out aerobically at 37° and they contained 50 μM protoporphyrin IX, 50 μM cobalt chloride, 0.1% Tween 80, mitochondria, 2 mg/ml of reaction mixture and glycine buffer, 0.1 M, pH 7.8. The spectra were measured on a Shimadzu split beam recording spectrophotometer with the blank reaction having cobalt added just prior to recording.

TABLE 1

Effect of certain metals on ferrochelatase activity and cobalt chelatase activity

Metal Ion	Concentration (M)	Per Cent Inhibition	
		Iron Method	Cobalt Method
Cu^{++}	10^{-4}	52	6
Ni^{++}	10^{-4}	67	0

Reactions were carried out as described in Methods and Materials. Chloride salts were employed where indicated and chloride ion itself was shown not to have any effects.

Results

Several series of studies such as lability on heating, pH optima and stimulation of activity by ether (7) gave responses in a similar direction

whether the enzyme was measured by the cobalt or iron assay. Previously, the distribution of cobalt and ferrochelatase activity had been shown to be similar (4). However, it was surprising to find that a differential sensitivity existed to certain metals such as copper and nickel. Table 1 shows that when cupric ion or nickel was used in reaction mixtures there was a marked inhibition of the formation of the pyridine hemochrome while no effect was observed using the cobalt assay.

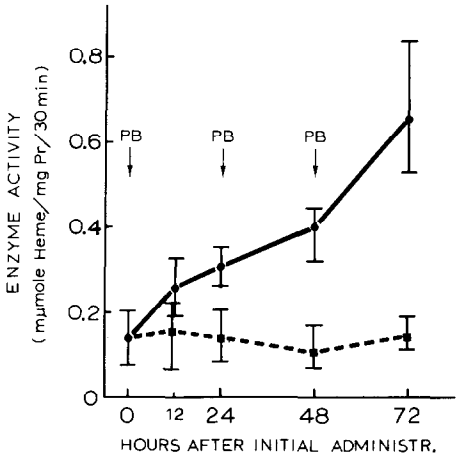

Figure 2. The effect of phenobarbital administration on rat liver mitochondrial ferrochelatase. Male, Sprague-Dawley rats (50 grams) were treated with phenobarbital sodium (60 mg/kg) at the arrows (solid line) or saline (dashed line). Activity was measured by the pyridine hemochrome assay (7) except that reduced glutathione was omitted from the reaction. Each point represents the mean value ± S.E.M. of 4 animals.

Figure 2 shows the effect of chronic phenobarbital treatment on rat hepatic mitochondrial ferrochelatase activity. No increase in activity was seen when the cobalt assay was employed but when the activity was measured by the pyridine hemochrome formation a marked enhancement of activity was observed. At 72 hours after phenobarbital treatment a 4-fold increase in specific activity was observed. This stimulation of ferrochelatase activity by phenobarbital is inhibited by the simultaneous administration of cycloheximide (Table 2) but the addition of phenobarbital to incubation mixtures did not alter enzymatic activity. This indicates that the effect of phenobarbital is probably mediated through an increase in protein synthesis.

TABLE 2

The effect of cycloheximide on the induction of ferrochelatase activity produced by phenobarbital treatment.

Treatment	Ferrochelatase Activity (mμmoles heme/mg protein/30 minutes)
Control	0.13
Phenobarbital	0.28
Cycloheximide	0.14
Phenobarbital + Cycloheximide	0.16

Pyridine hemochrome formation was used to study ferrochelatase activity as described in Methods and Materials. Phenobarbital sodium (60 mg/kg) and cycloheximide (1 mg/kg and 2 mg/kg) were injected into rats 48 and 24 hours prior to sacrifice. Control rats received injections of saline.

Discussion

These studies document an induction of rat hepatic mitochondrial ferrochelatase by the chronic treatment of rats with phenobarbital. However, this enhancement of activity was observed only when pyridine hemochrome formation was measured and not when the formation of the cobalt protoporphyrin IX complex was examined. Previously, we had observed the insensitivity of cobalt chelatase activity to copper and nickel, metals which inhibited pyridine hemochrome formation. Since other investigators (5) have used cobalt as a substrate for this reaction, attention is drawn to the dissociation of these activities, and since ferrous ion is considered to be the physiologic substrate it is suggested that the pyridine hemochrome assay is more truly representative of ferrochelatase activity. One likely explanation for this dissociation is that a different enzyme mediates cobalt complexation. However, one cannot exclude the possibility that a metal carrier system for iron is being affected with phenobarbital treatment

and with certain metals but that no carrier or a different carrier is required for cobalt transport to the enzyme.

It is interesting to note that the temporal response of ferrochelatase activity after phenobarbital administration is quite different than that observed with ALAS activity. ALAS activity increases markedly and rapidly in rat liver after phenobarbital administration reaching a maximum at 12 hours (1800% of control) followed by a return toward the normal level after 2-4 days of treatment (3). One may suggest that the ALAS activity and ferrochelatase are reciprocally related and that increases in ferrochelatase activity are promoted through increased flux of porphyrin substrate brought about by increased ALAS activity. In turn, the increased heme formed by these events may be responsible for the decreases in ALAS activity upon prolonged phenobarbital administration. It has been suggested by Granick and his colleagues (8,9,10) that heme participates in the regulation of the synthesis of ALAS on the basis of a feedback repression. Also, Scholnick et al. (11) have described the direct inhibition of soluble ALAS by heme which could account for another manner of control of heme synthesis in the mammal. It is also possible that the synthesis of ALAS and ferrochelatase are independent of each other and that the turnover of ferrochelatase is much slower than that of ALAS.

Acknowledgements

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