STIMULATION OF LIVER CATALASE SYNTHESIS IN RATS BY ETHYL-α-p-CHLOROPHENOXYISOBUTYRATE

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SUMMARY

In male rats fed 0.25% CPIB in diet 1 to 2 weeks, the concentration of catalase protein in liver extract was 2.1 times that of controls. With immunochemical methods, it was shown that CPIB enhances the rate of synthesis of hepatic catalase by more than 80% in three days and maintains this enhanced synthesis throughout the duration of its administration.

INTRODUCTION

The male rat fed ethyl-α-p-chlorophenoxyisobutyrate (CPIB), a hypolipidemic agent, shows an elevated catalase activity together with a significant increase in the number of microbodies (peroxisomes) in the hepatic parenchymal cells (1,2). However, it has not been clear whether the elevation of catalase activity is due principally to increased synthesis of catalase or, instead, to decreased degradation of catalase protein. Accordingly, the present study was undertaken using anticatalase antibody and labeled catalase precursor. The results thus far obtained indicate that CPIB treatment increases the catalase protein concentration and the rate of catalase synthesis in rat liver.

MATERIALS AND METHODS

Animals: Inbred male F-344 rats (A. R. Schmidt Co., Madison, Wisconsin) weighing between 175 and 200 grams were used in these experiments. CPIB was administered in chow diet ad lib in a concentration of 0.25%.

Catalase activity assay: Livers were perfused with cold 0.9% NaCl solution. Deoxycholate extract of liver homogenate was prepared according to the method of Ganschow and Schimke (3). Catalase activity was assayed spectrophotometrically at 25°C and expressed as units per gram of liver tissue by using the method described by Lück (4).

Anticatalase antibody: Rat liver catalase was purified according to the method of Price, et al (5). Antiserum was prepared by injection of 12 mg of rat liver catalase (O.D. 407 mμ/O.D. 276 mμ = 1.04) emulsified in an equal volume of complete Freund's adjuvant (Difco) into foot pads of rabbits. A booster injection of 3 mg of catalase was given 4 weeks later. Three to five days after booster injection, the rabbits were bled by cardiac puncture or by severing the left carotid artery. Immune serum was stored at -20°C.

In Ouchterlony double diffusion tests, the antiserum gave a single precipitation line with purified catalase. However, with 5% liver homogenate with sodium deoxycholate, an additional minor precipitation line was noted. Since the minor line was very small compared with the major line, no attempt was made to absorb the serum to remove the substance causing the minor line.

Quantitation of catalase protein: Quantitation of catalase protein in livers of normal and CPIB-treated rats was done by the immunoprecipitation method described by Ganschow and Schimke (3).

Isotope Incorporation: H^3-δ-aminolevulinic acid (specific activity 300 mc/mmole) was obtained from Amersham/Searle, Arlington Heights, Illinois. Two to four animals in each group were injected with the isotope in a dose of 30 μc/100 gm body weight and killed 2 hours later. Deoxycholate extract of liver homogenate and immunoprecipitate of catalase with antiserum were prepared according to the method of Ganschow and Schimke (3) and Higashi and Peters (6). The immunoprecipitate was washed with 0.9% NaCl 3 times (6), solubilized with NCS solubilizer (Amersham/Searle) and counted in a liquid scintillation counter, using a mixture of 2.5-bis (5'-tertiary butylbenzoxazoyl [2'])-thiophene (BBOT from Packard Instrument Company Inc., LaGrange, Illinois) and toluene (7).

RESULTS

Liver catalase activity: Liver catalase levels were determined in male rats treated with CPIB for 2 to 14 days. These data are presented in Fig. 1, and indicate that the activity of liver catalase rises rapidly reaching a plateau

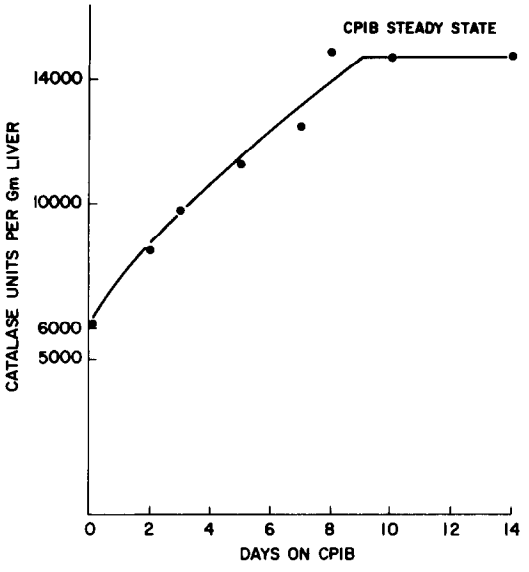

Figure 1: Liver catalase activity in CPIB-treated male rats. CPIB (0.25%) was administered in ground Purina Chow for 2 to 14 days. Three to six rats were killed at each interval and the liver catalase activity assayed spectrophotometrically at 25°C, by the method of Lück (Ref. 4), and expressed as average units of catalase per gram of liver tissue.

in 8 to 10 days. This new steady state is approximately 2.4 times greater than the normal steady state.

Quantity of catalase protein: It was found that 0.206 ml of anticatalase serum precipitated completely the catalase activity from 1 ml of 5% liver extract of rats treated with CPIB for two weeks, whereas only 0.098 ml of antiserum was required to precipitate completely the catalase activity from 1 ml of 5% liver extract from normal rats (Fig. 2). Thus, the concentration of catalase protein in the liver extract of rat in the CPIB-steady state was 2.1 times that of the extract of normal liver.

Synthetic rate of catalase: This was estimated based on the assumption that the average quantity of labeled precursor incorporated per molecule of newly-synthesized liver catalase is the same in normal and CPIB-treated rats. In this event, the comparison of the radioactivity of immunoprecipitate per gram liver would give the relative quantity of newly-synthesized catalase per gm

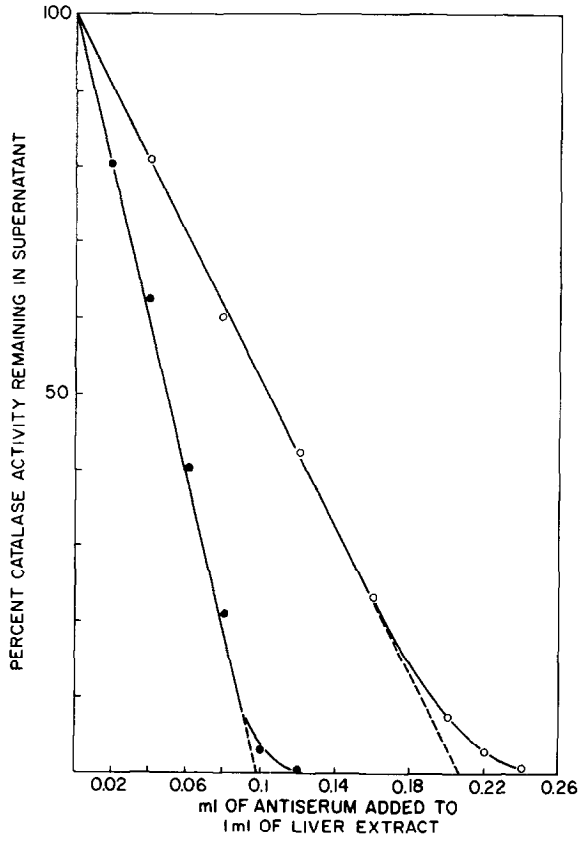

Figure 2: Immunoprecipitation of liver catalase of rats in normal and CPIB-steady states. Increasing quantities of anti-catalase antiserum were added to constant amounts of deoxycholate-treated liver extract from normal (●-●) and CPIB-treated (0-0) rats, according to the method described by Ganschow and Schimke (Ref. 3). Dashed lines are extrapolations of the linear portions of the curves to zero catalase activity.

tissue. The results are shown in Table I. It is apparent that CPIB enhances the synthetic rate of catalase by more than 80% in three days after the beginning of its administration and maintains the synthetic rate at this enhanced level through the duration of its administration.

DISCUSSION

The male rats fed CPIB showed an increase in catalase activity per gram liver and the activity reached a steady-state in 8 to 10 days after the beginning of CPIB administration. In this state, the activity per gram liver

TABLE I

Radioactivity of catalase fraction per gm. of liver*

	dpm	ratio
Normal	12,020	1
CPIB 3 days	22,340	1.86
CPIB 7 days	21,340	1.78
CPIB 14 days	21,960	1.83

*δ-aminolevulinic acid - H^3, 50 μC per 100 gm. of body weight, was given 2 hours before sacrifice. The fraction of the homogenate precipitable with anticatalase serum was considered as catalase fraction and the radioactivity of this fraction per gm. of liver tissue was calculated.

is 2.4 times that of normal liver. The concentration of antigen reacting to anticatalase antiserum is also increased to 2.1 times normal. Thus CPIB administration caused an increase in catalase protein concentration in liver. The estimate of the increase based on the catalase activity measurement was similar to that based on the use of anticatalase antiserum.

The elevation of protein concentration could be due to the increased synthesis and/or decreased degradation. Since CPIB feeding caused an increase in the rate of catalase synthesis by more than 80%, the elevation in catalase protein level is at least partly due to the stimulation of catalase synthesis by CPIB. The morphological studies on the livers of male rats treated with CPIB reported earlier (1,2) demonstrated a significant increase in the number of microbodies (peroxisomes) in liver cells, paralleling the biochemical findings.

A quantitative consideration of the kinetics of the change in the catalase level gives further suggestions. Using the formulation of Berlin and Schimke (8), the simplest model for a change in tissue content of catalase may be expressed as follows:

$$\frac{dC}{dt} = s - kC \quad \text{(Equation 1)}$$

where C is catalase concentration, s rate constant for synthesis and k the first order rate constant for degradation. In a steady state dC/dt=0, therefore, s=kC. Substituting the experimentally observed relative values for C and s, relative first order rate constants for degradation are obtainable. This constant for CPIB-treated animal is 0.75 when the relative constant for the normal animal is 1. This may imply that CPIB also reduces the first order rate constant for degradation of liver catalase, which can contribute additionally to the elevation of catalase concentration.

By solving the equation 1 and suitably substituting, it was shown that the time taken for an enzyme level to increase to one-half of the final increase at the steady-state is equal to the half-life of the enzyme (8). If this relationship is applied to the results of the present study, namely CPIB steady-state reached by 8 days and one-half of the increase reached by 4 days after the beginning of feeding (Fig. 1), the half-life of liver catalase in CPIB-treated rat is 4 days. Since half-life is inversely proportional to the first order rate constant for degradation, the half-life of liver catalase in normal rat is 4x0.75=3 days. This half-life is somewhat longer than those reported previously, 1.07 to 2.48 days (5,8). The half-life of catalase determined by H^3-leucine incorporation was 3.74 days but this longer half-life is the result of reutilization of labeled leucine (9).

While these uncertainties raised in the consideration of the kinetics of the change in catalase level may be partly clarified by further experiments, the equation 1 may also be oversimplified to express the change of catalase level *in vivo*. It is also uncertain when the CPIB effect reaches a maximum after the beginning of feeding.

These uncertainties notwithstanding, the present study clearly shows that CPIB increases liver catalase concentration largely through enhancing the rate of synthesis. The plateau of the enhanced catalase synthetic rate is reached within 3 days after the beginning of feeding and the rate is maintained at the same high level throughout the duration of CPIB administration. It

appears as if catalase synthesis system has two switch positions, normal and high, and CPIB throws the switch into high. The mechanism by which a relatively simple compound, CPIB, can increase the catalase synthesis by a finite factor remains an intriguing question.

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