SHORT COMMUNICATION

Estimation of the Turnover Rate of Barbiturate Side-Chain Oxidation Enzyme in Rat Liver

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SUMMARY

Hepatic side-chain oxidation of hexobarbital was estimated in rats before, during, and after administration of sodium phenobarbital in increasing dosage for 1 to 84 days. The half-life of the enzyme was virtually the same during and after drug administration. These results indicate that phenobarbital increases synthesis of the enzyme, and they illustrate the estimation of enzyme degradation by analysis of the change in enzyme level from one steady state to another.

Knowledge of the rates of turnover of the hepatic microsomal, oxidative, NADPH-requiring enzymes involved in drug metabolism is essential in the study of their regulation. None of these enzymes has been isolated and, therefore, turnover studies after incorporation of a suitable isotopic label have not been performed. In animal tissues, increased enzyme levels related to drug administration can theoretically result from increased enzyme synthesis and/or decreased enzyme degradation. The rate of change in enzyme level from a basal steady state to a new, drug-induced steady state is determined by the rate of degradation of the enzyme. The half-time of the change to the new level is equal to the half-life of the enzyme in the presence of the drug (1–3). After cessation of drug administration, the half-time for the return of enzyme level to the original steady state is the half-life of the enzyme in the absence of the drug. If the drug increases the level of the enzyme by enhancing its synthesis, the time courses of change to a new steady state during and after drug administration should be identical. If the drug primarily retards enzyme degradation, the return to the basal enzyme level after cessation of drug administration should be faster than the rise in enzyme level in the presence of the drug.

The effect of phenobarbital administration on barbiturate oxidation by rat liver has been repeatedly demonstrated (4–7). However, the full time course of the response during and after prolonged and maximal drug administration has not been presented, and the mechanisms whereby the drug affects enzyme levels are unsettled (8–12). In the present study, the half-life of the hepatic enzyme catalyzing the side-chain oxidation of barbiturate was estimated from the full time course of change in the steady-state enzyme level during and after the administration of phenobarbital to rats.

Male homozygous glucuronyl transferase-deficient (Gunn) rats weighing 200–300 g were given water and mouse breeder

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chow ad libitum and injected subcutaneously with sodium phenobarbital for 1 to 84 days (13). Mutant Gunn rats were selected for study in order to determine whether prolonged phenobarbital administration affects unconjugated hyperbilirubinemia in these animals; the results of this investigation will be reported separately. Sodium phenobarbital was administered in a dose of 60 mg/kg per day for 1 week, and the dose was progressively increased at a rate of approximately 20 mg/kg per day each week to a maximum of 200 mg/kg per day. At the end of the twelfth week, drug administration was stopped. A control group of Gunn rats of similar age and weight received daily subcutaneous injections of isotonic saline. All animals were weighed every other day throughout the study. Rats were selected at random from treated and control groups and killed by decapitation 18 hours after a dose of phenobarbital or saline. The liver was promptly removed, placed in 0.25 M sucrose (pH 7.4) at 2°, blotted and weighed; a 40% homogenate was prepared in buffered sucrose using a glass homogenizer with a motor-driven Teflon pestle. The 9000 *g* supernatant fraction was prepared and incubated according to Dixon *et al.* (14). The metabolism of hexobarbital by side-chain oxidation was determined by estimating the disappearance of hexobarbital according to Cooper and Brodie (15). Arithmetic means of basal and drug-stimulated enzyme levels were used as measures of the corresponding steady state levels. Because of the impossibility of accomplishing instantaneous onset and cessation of drug action, the first-order rate constant and half-life in the presence and absence of drug were estimated from the linear slopes of logarithmic plots of the data expressed as fractions of the total change in enzyme activity.

The schedule of drug administration and the observed changes in hepatic barbiturate side-chain oxidation are presented in Fig. 1. Maximal enzyme activity was attained after 3 days of phenobarbital administration and was not affected by serially increased drug dosage. There was no significant change in enzyme activity 24 hr after cessation of drug administration; however, enzyme levels subsequently decreased rapidly and reached the original steady-state level on the sixth posttreatment day. The half-time for reaching the new steady state during drug administration was 2.2 days and for return to the original steady state after cessation of drug administration was 2.6 days. No significant change in enzyme activity occurred in control rats during the experiment. Treated and control rats gained weight at the same rate.

Although the theoretical model used in this study is oversimplified, and all enzymes may be continuously changing in response to regulatory stimuli, the model seems well suited to analysis of enzyme responses to drug stimulation as the exogenous stimulus (i.e., the drug) dominates over endogenous influences.

Within the methodologic limitations of these experiments, the half-life of the enzyme catalyzing the side-chain oxidation of hexobarbital was about the same during and after drug administration. The half-life for return of enzyme activity to the basal steady state after cessation of drug administration was slightly greater than the half-life for attaining the new steady state during drug administration, whereas, if phenobarbital had stabilized the enzyme, the half-life of the enzyme in the absence of drug should have been less than the half-life of the enzyme in the presence of drug. These findings suggest that phenobarbital increases the rate of enzyme synthesis rather than decreasing the rate of enzyme degradation.

In the present study, enzyme cytochemical and electron microscopic studies of liver from rats treated with phenobarbital for 84 days (A. B. Novikoff, unpublished observations) revealed abundant smooth endoplasmic reticulum similar to that occurring in rats treated for only 4 days with phenobarbital (6). These observations suggest that the turnover of the smooth endoplasmic reticulum may be similar to that observed for the enzyme catalyzing the side-chain oxidation of phenobarbital as well as other smooth endoplasmic

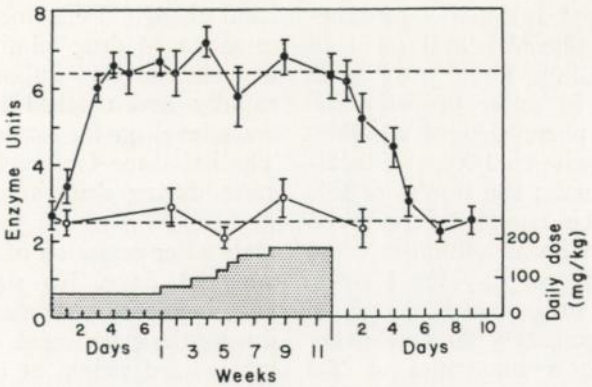

FIG. 1. *The effect of prolonged phenobarbital administration on hepatic hexobarbital oxidation by glucuronyl transferase-deficient (Gunn) rats.*

Treated rats (●——●) received daily subcutaneous injections of phenobarbital sodium in increasing dosage for 12 weeks as indicated by the stippled area. Control rats (○——○) received daily subcutaneous injections of saline. During the first and last week of the experiment, four animals from each group were used for each time period studied. In studies performed from the first through the twelfth week, three animals from each group were used for each time period studied. Data are expressed as means ± standard error. A unit of enzyme activity is equal to 1 μmole of hexobarbital oxidized per gram of liver in 30 min. Note that the scale on the abscissa is in days for the first 7 and last 9 days of the experiment, whereas the intervening period is in weeks. The upper and lower broken lines represent arithmetic means of enzyme activity at the drug-stimulated and basal steady states, respectively.

reticulum components such as cytochromes P450 and b_5 (16, 17).

Further studies of full time courses of responses of other microsomal enzymes to stimulation with different drugs should be of interest particularly where different mechanisms of changing enzyme levels have been postulated (18).

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