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INCREASED HEPATIC PHOSPHOPROTEIN PHOSPHATASE ACTIVITY INDUCED BY PHENOBARBITAL AND ITS SUPRESSION BY CYCLOHEXIMIDE AND SKF 525-A

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Abstract—Phenobarbital influences the activity of liver phosphoprotein phosphatase in rats. Daily doses of 100 mg/kg body weight induced a significant increase in phosphoprotein phosphatase activity after the fourth injection, with a plateau after the fifth dose. The rate of induction and the magnitude of response were found to be dependent on the daily doses of phenobarbital. The marked sensitivity of the enzyme induction to cycloheximide suggests that the formation of new enzyme protein is involved in the process. SKF 525-A administered 40 min before phenobarbital, completely suppressed the induced increase of phosphoprotein phosphatase. An additional series of phenobarbital injection to pretreated rats, after a pause of 5 days, when regression of drug-metabolizing enzymes and of phosphoprotein phosphatase have been reached, provoked an even more pronounced increase in the enzyme activity than the original treatment. The possible relationship between the activity of phosphoprotein phosphatase and the activity of drug-metabolizing enzymes has been investigated.

NUMEROUS investigations have shown that phenobarbital induces a marked increase in activity of drug-metabolizing enzymes in the liver.[1] The increased activity of drug-metabolizing enzymes, resulting from phenobarbital pretreatment, has been shown to be located in the microsomal fraction of the liver.[1] Besides microsomal drug-metabolizing enzymes, phenobarbital treatment of rats increases the activity of certain hepatic mitochondrial enzymes such as δ-aminolevulinic acid synthetase,[2] enzymes involved in mitochondrial oxidation utilizing succinate, pyruvate and malate,[3] etc. There is, however, a certain difference in the time interval between the administration of phenobarbital and the appearance of increased enzyme activities in microsomes and in mitochondria. The microsomal enzyme activity increases within 12–24 hr after phenobarbital treatment, whereas the mitochondrial enzymes have a characteristic 2-day lag period before this effect becomes manifest.[2,3]

In this paper we present data which show that phenobarbital increases the activity of rat liver phosphoprotein phosphatase, which is also an extramicrosomal enzyme.[4,8]

MATERIAL AND METHODS

Experiments were carried out on male albino rats weighing between 120 and 150 g. The animals were divided into four experimental groups. In the first group the effect of duration of phenobarbital treatment on the enzyme activity was examined. These animals were divided into seven subgroups and sacrificed 24 hr after the first, third, fourth, fifth, tenth, twentieth and thirtieth dose of phenobarbital. The animals of the second group were divided into three subgroups in which the dose–response was

TABLE 1. THE INFLUENCE OF PHENOBARBITAL ON RAT LIVER PHOSPHOPROTEIN PHOSPHATASE ACTIVITY

	Controls	Pb_1	Pb_3	Pb_4	Pb_5	Pb_{10}	Pb_{20}	Pb_{30}
No. of exp.	7	7	7	7	7	7	7	7
M ± S.D.	135 ± 17·30*	154 ± 22·78	146 ± 3·94	176 ± 36·05	212 ± 21·10	209 ± 43·95	199 ± 25·99	196 ± 17·66
% of controls	—	+14	+8·1	+30·0	+57·0	+55·0	+47·5	+45·0
Significance	—	P > 0·05	P > 0·05	P < 0·05	P < 0·001	P < 0·01	P < 0·01	P < 0·01

* μg P/g liver wet weight/15 min; $Pb_{1,3,4,5,10,20}$ = No. of phenobarbital injections.

examined. The animals of the third group were divided into four subgroups: the first was treated for 5 days with SKF 525-A; the second with SKF 525-A and phenobarbital simultaneously for 5 days; the third, 2 days with cycloheximide, and the fourth, 5 days with phenobarbital plus cycloheximide with fourth and fifth phenobarbital injections. In the fourth group the effect of reinduction on the activity of phosphoprotein phosphatase was examined.

Phenobarbital was injected intraperitoneally in daily doses of 100 mg/kg body weight as a 10% (w/v) solution. SKF 525-A was administered *per os* in daily doses of 25 mg/kg body weight 40 min before phenobarbital injections. Cycloheximide was given intraperitoneally, simultaneously with the fourth and the fifth phenobarbital injection in daily doses of 1 mg/kg body weight. Control animals received an equivalent volume of physiological saline.

All the animals were killed by decapitation between 8 and 9 a.m., 24 hr after the last injection of phenobarbital. Samples of whole liver homogenate were taken for determination of phosphoprotein phosphatase activity. The enzyme activity was determined according to the method of Feinstein and Volk[5] and expressed in micrograms of phosphorus liberated from casein per gram of wet liver tissue.

RESULTS

The results of our experiments presented in Table 1 show that daily injection of phenobarbital (100 mg/kg body weight) induced a marked increase in the activity of phosphoprotein phosphatase in the liver of treated rats. The first significant rise of phosphoprotein phosphatase activity was observed after the fourth injection of phenobarbital (30·0 per cent over control), while the maximum increase of its activity was noted after five daily injections (57 per cent over controls). Further treatment did not significantly elevate the enzyme activity above this value. Cessation of phenobarbital treatment after 5 days resulted in a decrease in the level of phosphoprotein phosphatase activity (Fig. 1). After another 5–6 days the values were about the same as those for the control rats.

Smaller doses, given daily for the same time, can also induce a detectable increase in phosphoprotein phosphatase activity. With the lower doses tested, 33 mg of phenobarbital per kg body weight, a significant rise in phosphoprotein phosphatase activity was achieved only after ten daily injections (Fig. 2). However, with daily doses of 66 mg/kg body weight, a significant rise in enzyme activity was reached also after five daily injections of phenobarbital.

Inhibition of phenobarbital-induced increase in phosphoprotein phosphatase activity by simultaneous administration of cycloheximide and SKF 525-A

Cycloheximide blocks enzyme synthesis at the level of translation of mRNA information by suppressing the transfer of amino acids from soluble RNA to polypeptide chains.[6] It also produces nucleolar ultrastructural lesions in parenchymal liver cells and has a secondary effect on RNA synthesis, transport and stability.[6] In the dose given simultaneously with the fourth and fifth phenobarbital injections, cycloheximide completely abolished the phenobarbital-stimulated increase in phosphoprotein phosphatase activity (Fig. 3).

Several compounds are known as inhibitors of drug-metabolizing enzymes–iproniazid, CFT 1201, Lilly 18 947, SKF 525-A, etc.[7] One of the most potent among

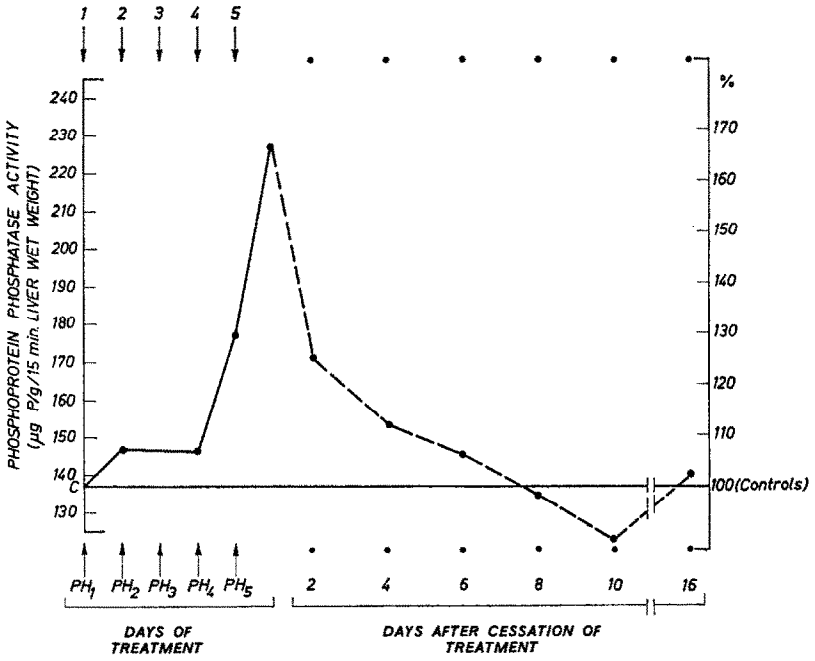

FIG. 1. Effect of phenobarbital treatment on liver phosphoprotein phosphatase activity (solid line). Dashed line: enzyme activity after cessation of treatment. Arrows indicate phenobarbital injections.

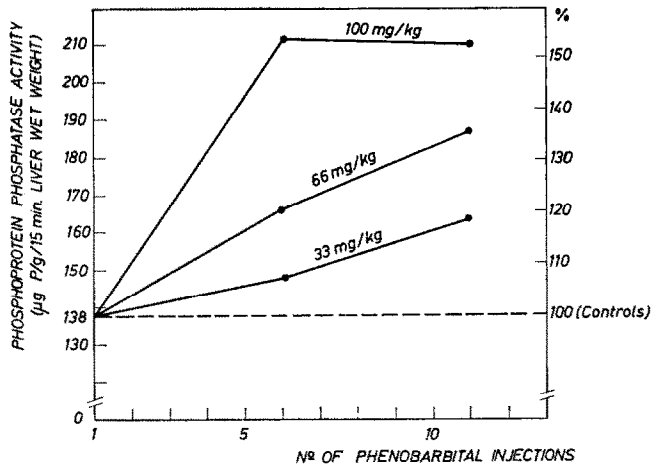

FIG. 2. Enhanced rate of phosphoprotein phosphatase activity after different daily doses of phenobarbital.

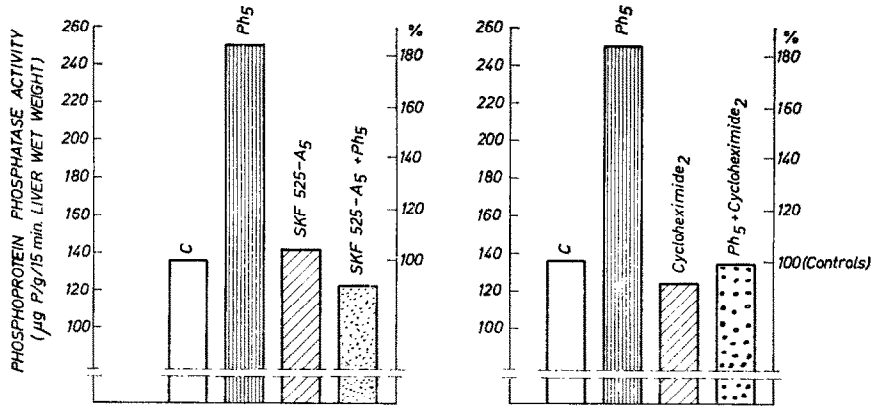

FIG. 3. Inhibition of the phenobarbital-induced enzyme activity by simultaneous administration of cycloheximide and SKF 525-A.
C, controls; Ph_5, enzyme activity induced by five phenobarbital injections; SKF 525-A_5, enzyme activity in animals injected with five doses of SKF 525-A; Ph_5 plus cycloheximide$_2$, rats received two cycloheximide injections with fourth and fifth phenobarbital injections.

these agents is SKF 525-A. The mechanism by which SKF 525-A exerts its inhibitory effect on drug-metabolizing enzymes is not completely clear. Brodie[7] has suggested two possibilities for the action of SKF 525-A: (a) inhibition of a common component of the microsomal enzyme systems; and (b) effect on the permeability of the microsomal membrane to drugs. Administered alone, in five daily doses of 25 mg/kg body weight it has no effect on the phosphoprotein phosphatase activity in the liver. But, administered in five daily doses of 25 mg/kg body weight, 40 min before phenobarbital, it completely suppresses the increase of phosphoprotein phosphatase induced by phenobarbital in the rat liver (Fig. 3).

The influence of "reinduction" on the activity of phosphoprotein phosphatase

In order to see whether any parallelism exists between the degree of induction of drug-metabolizing enzymes and that of phosphoprotein phosphatase, experiments were performed in which rats were reinjected with phenobarbital after a pause of 5 days after the first five injections of the drug. The data obtained from the "reinduction" experiments are summarized in Fig. 4. Figure 4 shows that the second period of treatment with phenobarbital caused an increase in phosphoprotein phosphatase activity after the first injection of the drug. The degree of increase of phosphoprotein phosphatase activity after the first injection of phenobarbital during the "reinduction" experiments was as high as after the fifth injection during the original induction.

DISCUSSION

It is known from the data of Paigen and Griffiths[4] that phosphoprotein phosphatase is an extramicrosomal enzyme, located predominantly in lysosomes and cytosol in mice liver. They have also found that there is an endogenous inhibitor of the soluble enzyme which has little effect on the particulate enzyme in the lysosomal fraction. On the basis of this finding they have supposed the existence of two phosphoprotein phosphatases in the liver—the particulate and the soluble one. According to the data

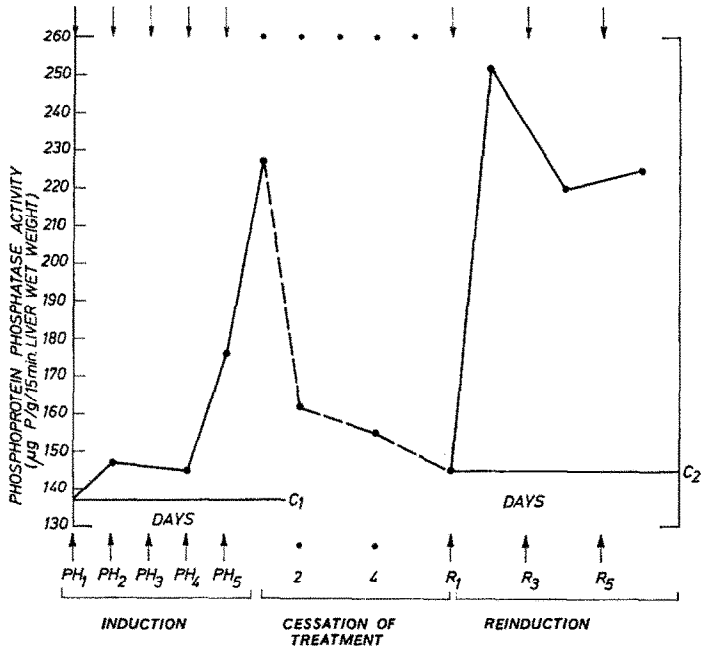

FIG. 4. Enhanced rate of phosphoprotein phosphatase activity after administration of phenobarbital to untreated and previously treated rats. The previously treated rats had received another series of phenobarbital injections after the fifth days of cessation of treatment with the first series of phenobarbital injections. Solid line: induction and reinduction of phosphoprotein phosphatase activity. Dashed line: decrease in phosphoprotein phosphatase activity after cessation of phenobarbital treatment. Arrows indicate phenobarbital injections.

of Caravaglio *et al.*[8] rat liver phosphoprotein phosphatase is located predominantly in the cytosol.

The results reported in this paper show that phenobarbital in chronically-treated animals induces a significant increase of phosphoprotein phosphatase activity in the liver (Table 1). There is however, a 4-day lag period before this effect becomes manifest. The degree of increase of enzyme activity is, however, lower than in drug-metabolizing enzymes system at the level of maximal response.[9]

The rate of induction and the magnitude of response were found to be dependent on the daily doses of phenobarbital injected (Fig. 2). With the maximal dose injected, 100 mg/kg body weight per day, a plateau of activity was reached after five daily injections. The lower doses needed prolonged treatment to reach the maximal response.

The marked sensitivity of the enzyme induction to cycloheximide (Fig. 3) strongly suggests that the formation of new enzyme protein is involved in this process.

The results of the experiments with SKF 525-A show that there is some relationship between the activity of the drug-metabolizing enzymes and the activity of phosphoprotein phosphatase (Fig. 3). When metabolism of phenobarbital was inhibited by administration of SKF 525-A, no detectable changes were registered in the phosphoprotein phosphatase activity (Fig. 3). These results suggest that the increase of phosphoprotein phosphatase activity correlates in some way with the accelerated drug metabolism. However, the rate of induction and magnitude of response were not

found to be dependent on the degree of drug-metabolizing enzyme activity. The "reinduction" experiments support this. The effect of "reinduction" on the activity of drug-metabolizing enzyme system has been studied by Orrenius and Ericsson.[9] They found that the second period of treatment with phenobarbital, after a pause of 5 days, when "drug-metabolizing" activity returns to normal level, "causes an increase in oxidative demethylation activity which was less rapid than that caused by administration of the drug to previously untreated animals. This phenomenon was most pronounced during the early phase of the reinduction".[9] Tested at the same time, under the same experimental conditions, the activity of phosphoprotein phosphatase increases after the first injection during the "reinduction" period, as after the fifth injection during the original treatment. Moreover, the enhanced enzyme activity persists all the time at the high level during the period of "reinduction" (Fig. 4) when Orrenius and Ericsson[9] have found slower induction of drug-hydroxylating enzyme system.

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