Pentobarbital-Induced Increase of Rat Liver Phosphoprotein Phosphatase

Earlier papers from this laboratory demonstrated that phenobarbital[1,2] and certain steroids which possess a resistence-increasing or 'catatoxic' effect (e.g. pregnenolone-16α-carbonitrile (PCN), SC-11927, and ethylestrenol (EE), etc.), increase the hepatic level of extramicrosomal phosphoprotein phosphatase (PPP-ase) by selectively increasing the rate of enzyme synthesis[3,4]. Between the tested steroids only dexamethasone was ineffective in elevating the level of hepatic PPP-ase[3]. Besides, it was found that the induction of PPP-ase was inhibited by SKF 525-A, if given 40 min before the inducers[2-4]. The latter results raised the question of the possible relationship of PPP-ase induction with enhanced activity of microsomal drug-metabolizing enzymes.

PPP-ase is located in the soluble fraction of liver cells[5]. It acts on intracellular phosphoproteins by splitting the high energy phosphate links[6]. The liberated energy and phosphate could be used for synthetizing ATP[6,7]. It is also noteworthy to mention that PPP-ase may act on those intracellular proteins which are capable of acting as phosphate acceptors for protein phosphokinases[7].

Since phenobarbital stimulates the formation of microsomal drug-metabolizing enzymes in the rat liver, as does phenobarbital[8] and the 'catatoxic' steroids[9], it was of interest to test pentobarbital as a potential inducer of extramicrosomal PPP-ase. The results presented here show that pentobarbital induced an increase in PPP-ase level already 20 min after a single injection.

Material and methods. Male rats of Wistar strain (Vinca, Belgrade, Yugoslavia) averaging 100 g, maintained on corn diet (equal proportions of maize and oat) and tap water ad libitum, were used in the experiments.

The animals were divided into 4 experimental groups. In the 1st group the effect of a single dose of pentobarbital was examined. In the 2nd group, the effect of duration of pentobarbital treatment on the enzyme activity was tested. In the 3rd group, the effect of different daily doses of pentobarbital was examined. In the 4th group of animals, the effect of simultaneous administration of pentobarbital with SKF 525-A or cycloheximide on basal and induced enzyme level was examined.

Pentobarbital was injected i.p. in daily doses of 2 mg or 4 mg/100 g body wt. SKF 525-A was administered per os, 40 min before pentobarbital, in daily doses of 2.5 mg/100 g body wt. Cycloheximide was given i.p. in a dose of 100 μg/100 g body wt. Control animals received an equivalent volume of physiological saline.

All the animals were killed by decapitation between 08.00 and 09.00 h. Samples of whole liver homogenate were taken for determination of PPP-ase activity. The enzyme activity was determined according to the method of FEINSTEIN and VOLK[10] and expressed in μg of phosphorus liberated from casein per g of wet liver tissue.

Results and discussion. Figure 1 shows that a single dose of pentobarbital (4 mg/100 g body wt.) already after 20

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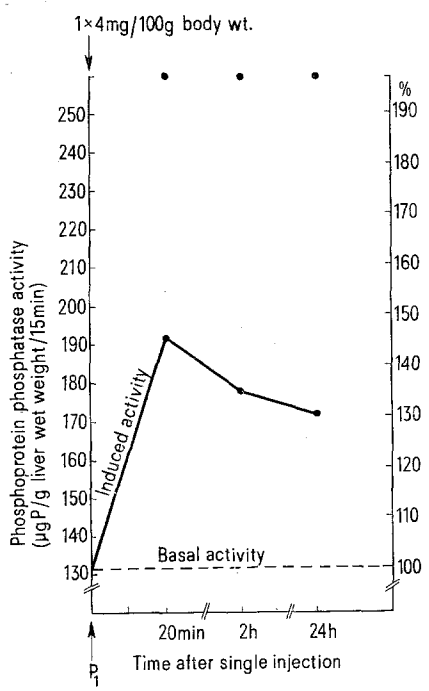

Fig. 1. Course of phosphoprotein phosphatase induction after a single pentobarbital injection. Arrows indicate pentobarbital injections. Each point represents the mean of 7 rats.

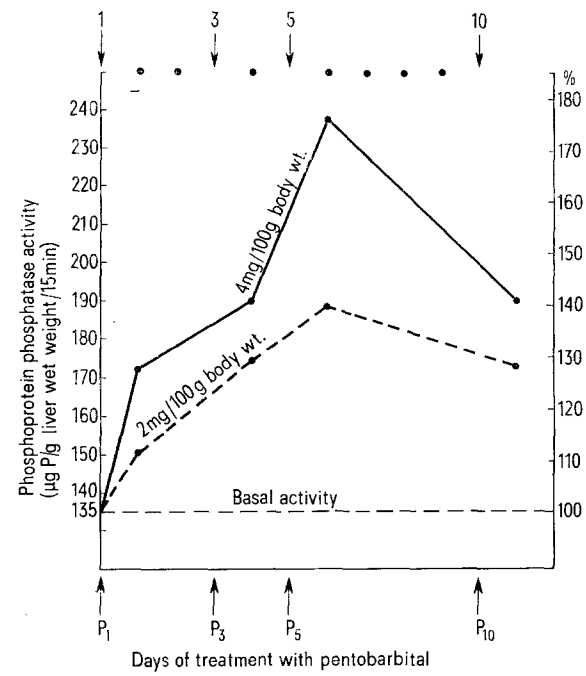

Fig. 2. Enhanced rate of phosphoprotein phosphatase activity after different daily doses of pentobarbital. Arrows indicate pentobarbital injections, and points the mean of 7 rats.

The influence of pentobarbital on rat liver phosphoprotein phosphatase activity

	Controls	No. of Pentobarbital injections 1	3	5	10	20	30
No of experimental animals in groups	7	7	7	7	7	7	7
M ± SD	135 ± 17.30[a]	172 ± 35.61	190 ± 5.95	233 ± 32.96	189 ± 31.06	222 ± 28.91	203 ± 28.87
of Controls	—	+ 27.3	+ 40.8	+ 73	+ 40	+ 64.5	+ 50.4
p-value	—	= 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[a] Enzym activity as μg P/g liver wet weight/15 min.

min produced measutable changes in the activity of rat liver PPP-ase. This effect lasted for the next 24 h. In the case of repeated administration of pentobarbital (Table), the maximal response was obtained after 5 days of treatment with the drug.

These results and the results obtained earlier with phenobarbital[2] show that the pattern of PPP-ase induction is very similar to the induction process of microsomal drug-metabolizing enzymes[11]. A plateau of activity in both induction processes was reached after 5 days of treatment with the drugs. It seems that at that time a repressor (or repressors) appears, which inhibits a further increase in extramicrosomal PPP-ase activity, as well as in the activity of microsomal drug-metabolizing enzymes system[11].

The rate of induction and the magnitude of response were found to be dependent on daily doses of pentobarbital (Figure 2). With maximal doses of pentobarbital (4 mg/100 g body wt. per day), a plateau of activity was reached after 5 daily injections. Lower doses (2 mg/100 g body wt.) provoked also a plateau of activity after 5 daily injections but of lower intensity (Figure 2).

Cycloheximide, at a concentration inhibiting protein synthesis in mammalian cells[12], abolished induction by the pentobarbital (Figure 3). These results lend support to the conclusion that enhanced de novo synthesis of PPP-ase molecules underlies the action of the drug.

When the metabolism of the drug was blocked by simultaneous administration of SKF 525-A, no detectable changes were registered in the liver PPP-ase activity. The enzyme activity remained at the control level (Figure 3). According to these results, it is reasonable to suppose that the induction of PPP-ase might be stimulated by pentobarbital or by some of its metabolites, or by both.

While these data suggest that pentobarbital, or some of its metabolites, may stimulate the induction of PPP-ase, they also raise other questions. First, what is the mechanism through which the drug effects the induction of PPP-ase? And second, what is the significance of an induction of extramicrosomal PPP-ase by pentobarbital and other enzyme inducers? As to the first question, the drug may induce the increase in PPP-ase level by acting directly at the level of liver cells. The experiments with SKF 525-A partially support this possibility. However, it is quite possible that the increase in the PPP-ase level may be secondary to some endocrine changes provoked by pentobarbital treatment. The answer to the second question at the moment may be of a speculative nature only. Starting from the known facts that PPP-ase acts on intracellular proteins, especially those capable of acting as phosphate acceptors for protein phosphokinase[6,7] and that liberated energy and phosphate could be used for synthetizing ATP[6,7], it is possible to suppose a regulatory role in the synthesis of some coenzymes and in the activation of some apoenzymes that participate in the metabolic transformations of the drugs and steroids at the level of liver cells.

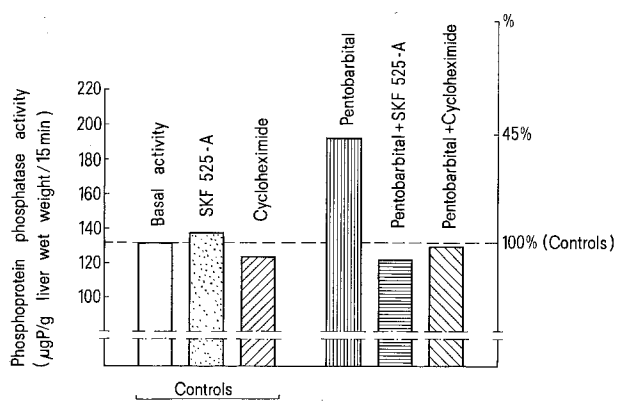

Fig. 3. Inhibition of the pentobarbital-induced phopshoprotein phosphatase activity by simultaneous administration of SKF 525-A and cycloheximide. Cycloheximide (100 μg/100 g body wt.) was given simultaneous with pentobartital (4 mg/100 g body wt.) and SKF 525-A (2.5 mg/100 g body wt.) 40 min before pentobarbital. The animals were sacrificed 20 min after the pentobarbital injection. Each column represents the mean of 5 rats.

Résumé. Le pentobarbital provoque un accroissement important de l'activité de la phosphoprotein-phosphatase extramicrosomiale du foie du rat. L'accroissement de l'activité de l'enzyme se produit dejà 20 min après l'administration du médicament (+40%) et dure autant que le traitement (+50–70%). Le niveau de l'induction dépend de la dose du pentobarbital. L'induction est bloquée par l'administration simultanée du cycloheximide ou du SKF 525-A.

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