LIVER LEVELS OF α-GLYCEROPHOSPHATE, GLYCEROPHOSPHATE DEHYDROGENASE AND ACETYL-CoA CARBOXYLASE DURING PHENOBARBITAL INDUCTION

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1. Introduction

It is well established that treatment of rats with phenobarbital causes an increase in the endoplasmic reticulum membranes of the liver [1,2]. However, because of the difficulties of measuring phospholipid synthesis and breakdown in vivo, it is not yet known whether phospholipid synthesis, breakdown, or both are involved in this process [3–5].

Another approach to this question is determination of the activity of enzymes involved in phospholipid metabolism during phenobarbital induction. Thus, it has been shown that phenobarbital increases levels of acyltransferase activity 3-fold [6], suggesting an increased synthesis of phospholipid. However, it is not yet known which steps in phospholipid metabolism are rate-limiting. Since α-glycerophosphate and fatty acids play a central role in this metabolism, we were interested in possible changes in liver levels of α-glycerophosphate, glycerophosphate dehydrogenase, and acetyl-CoA carboxylase during phenobarbital induction.

2. Materials and methods

180–200 g male Sprague-Dawley rats received 80 mg/kg phenobarbital in 0.9% NaCl IP once every 24 hr during the experiment while control animals received an equivalent vol of 0.9% NaCl. The rats were sacrificed at various times after the initial injection and a portion of the liver was used to make microsomes according to Ernster et al. [7]. Microsomal phospholipid was extracted with chloroform–methanol [8] and lipid phosphorus was determined according to Bartlett [9]. Control values of microsomal phospholipid were 7–8 μmol/g liver.

Another portion of the liver was used to determine α-glycerophosphate according to Hohorst [10] and gave control values of 0.3 μmol/g liver. A cytoplasmic fraction was prepared from a third portion of the liver by homogenizing 10 times in a Potter-Elvehjem homogenizer and carrying out standard differential centrifugation to obtain the post-microsomal supernatant. Glycerophosphate dehydrogenase was measured in this fraction according to Fondy et al. [11] and gave control values of 2.3 nmol/mg cytoplasmic protein/min (there was no significant difference in the mg cytoplasmic protein/g liver in control and phenobarbital-treated rats). Acetyl-CoA carboxylase was assayed in the cytoplasmic fraction after citrate activation according to Chang et al. [12] and gave control values of 1.43 nmol malonyl-CoA/mg cytoplasmic protein/min.

3. Results and discussion

As shown in fig.1A, there was no significant change in liver levels of α-glycerophosphate and glycerophosphate dehydrogenase during phenobarbital induction. On the other hand, fig.1B demonstrates that acetyl-CoA carboxylase activity increases 55% within 3 hr after the initial phenobarbital injection, increases slowly thereafter to 180% of control values, and returns between 48 and 96 hr after the initial injection to control levels of activity. Fig.1B also shows the approximate doubling of microsomal phospholipid/g liver during phenobarbital induction, and it can be seen that membranogenesis occurs after the

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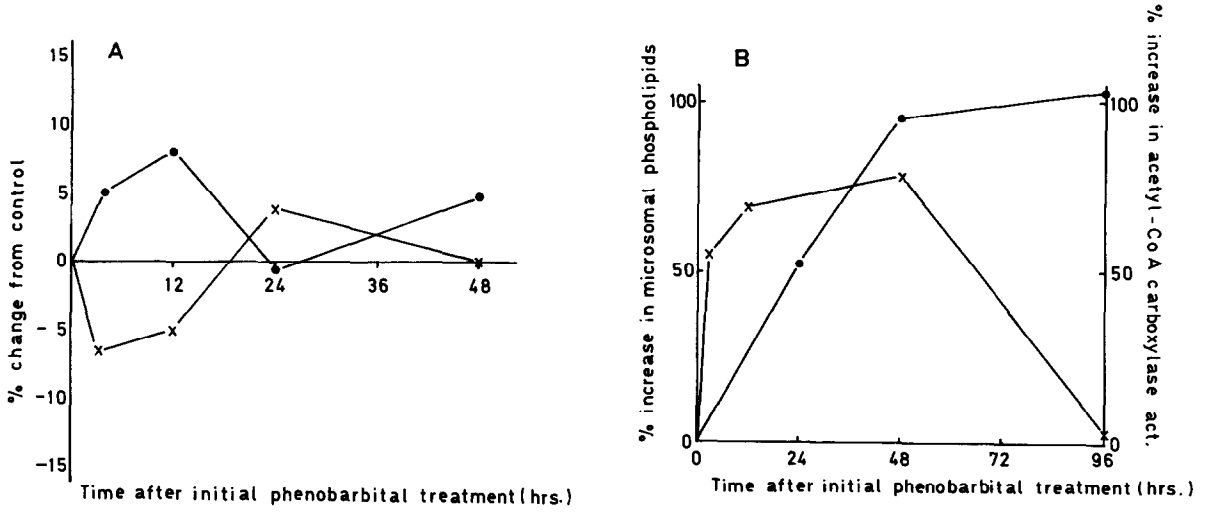

Fig.1 A) Levels of α-glycerophosphate and glycerophosphate dehydrogenase during phenobarbital induction. α-glycerophosphate and glycerophosphate dehydrogenase were measured as described in the text. (o) α-glycerophosphate. (x) glycerophosphate dehydrogenase. B) Membranogenesis and acetyl-CoA carboxylase activity during phenobarbital induction. Microsomes and a cytoplasmic fraction were prepared and microsomal phospholipids and cytoplasmic acetyl-CoA carboxylase were determined as described in the text. (o) phospholipids. (x) acetyl-CoA carboxylase.

increase in acetyl-CoA carboxylase activity and that the return of the acetyl-CoA carboxylase activity to control levels takes place after membranogenesis is complete.

Since it has been established that acetyl-CoA carboxylase, fatty acid synthetase, and fatty acid synthesis are co-ordinately controlled in rat liver [13], the findings presented here would suggest that increased fatty acid synthesis is involved in the membranogenesis induced by phenobarbital. This conclusion is in agreement with the recent report that phenobarbital stimulates the incorporation of 1[^{14}C]acetate, [^{3}H]$_2$O, 2[^{3}H]lactate, and 2,3[^{3}H]succinate into fatty acids in the liver in vivo [14]. On the other hand, liver levels of α-glycerophosphate and glycerophosphate dehydrogenase do not seem to play a role in the membranogenesis induced by phenobarbital.

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