INFLUENCE OF PHENOBARBITAL TREATMENT ON THE TURNOVER OF RAT LIVER MICROSOMAL LIPIDS

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1. Introduction

It is well established that short term phenobarbital (PB) treatment results in a significant increase in both liver microsomal membranes and the enzymes participating in hydroxylation reactions [1]. The underlying mechanism for this elevation in membrane constituents has been studied in detail by measuring the turnover of various microsomal proteins. It has been concluded from these studies that both increased synthesis and decreased breakdown occur [2, 3]. This latter event is of great significance in the induction phenomenon since the breakdown of several electron transport enzymes, like NADPH-cytochrome *c* reductase and cytochrome b_5, is completely abolished in the first eight days of the treatment [4]. Although reutilization of the labeled compound used in the turnover studies cannot be excluded completely [5], it does not appear sufficient to explain the experimental results indicating decreased catabolism [6].

Investigations regarding the PB-induced turnover of the lipid part of the membrane have been restricted to the use of [^{32}P]orthophosphate [7, 8], which has the disadvantage of being influenced by exchange reactions. In this paper the incorporation rate of [^{3}H]glycerol into microsomal phospholipids (PLP) and [^{3}H]mevalonate into cholesterol were studied, and the half-lives of these components were determined. The results show that lipid composition of the microsomal membranes remains unchanged after drug treatment. The mechanism by which PLP and cholesterol are maintained in the membrane is complex as shown by the present studies.

2. Materials and methods

Starved rats weighing 180–200 g were used. PB was injected intraperitoneally once a day (8 mg/100 g body weight). [2-^{3}H]Glycerol (50 mCi/mmole) and [2-^{3}H]mevalonate (60 mCi/mmole), both from the Radiochemical Centre, Amersham, were diluted in sterile Ringers solution and injected intraperitoneally, 20 μCi in the case of rate incorporation (10 min) and 40 μCi in the case of half-life studies. In these latter experiments the livers from a series of rats were removed on the days that are indicated in the figures.

Microsomes and mitochondria were prepared as described previously [9]. PLP was extracted and separated from neutral lipids on a silicic acid column [10]. Radioactivity in the methanol and chloroform phase were measured with a toluene scintillator [11]. The radioactivity values of the phenobarbital treated rats was corrected for weight increase and recovery as described by Kuriyama et al. [4].

Protein, PLP and cholesterol were determined as earlier [12].

All values represent the mean of four to nine experiments.

3. Results and discussion

The total microsomal fraction isolated after four days of PB treatment contains about twice as much protein, PLP and cholesterol as the microsomal fraction from livers of control rats (table 1). Because of

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Table 1
Chemical composition of total microsomal fraction after phenobarbital treatment.

PB treatment	Protein (mg/g liver)	PLP	Chol.	Protein / PLP	Chol. / PLP
None	20.7	6.1	0.52	3.4	0.085
4-days	36.4	10.4	0.81	3.5	0.078

Chol. represents cholesterol.

the parallel increase of all three components, the protein per PLP and the cholesterol per PLP ratios are unchanged. This distribution of the main membrane components is also found in all the other periods of incubation investigated.

The initial linear phase of incorporation of [^{3}H]glycerol into the total PLP fraction and of the [^{3}H]mevalonate into cholesterol exhibits two different patterns during PB induction (table 2). Incorporation of [^{3}H]glycerol 10 min after the injection of label increases only 10% in the first 24 hr period and after the first day there is a continuous decrease in incorporation rate, being only one third of the control value after four days treatment. On the other hand [^{3}H]mevalonate incorporation into cholesterol increases steadily and after four days it is doubled.

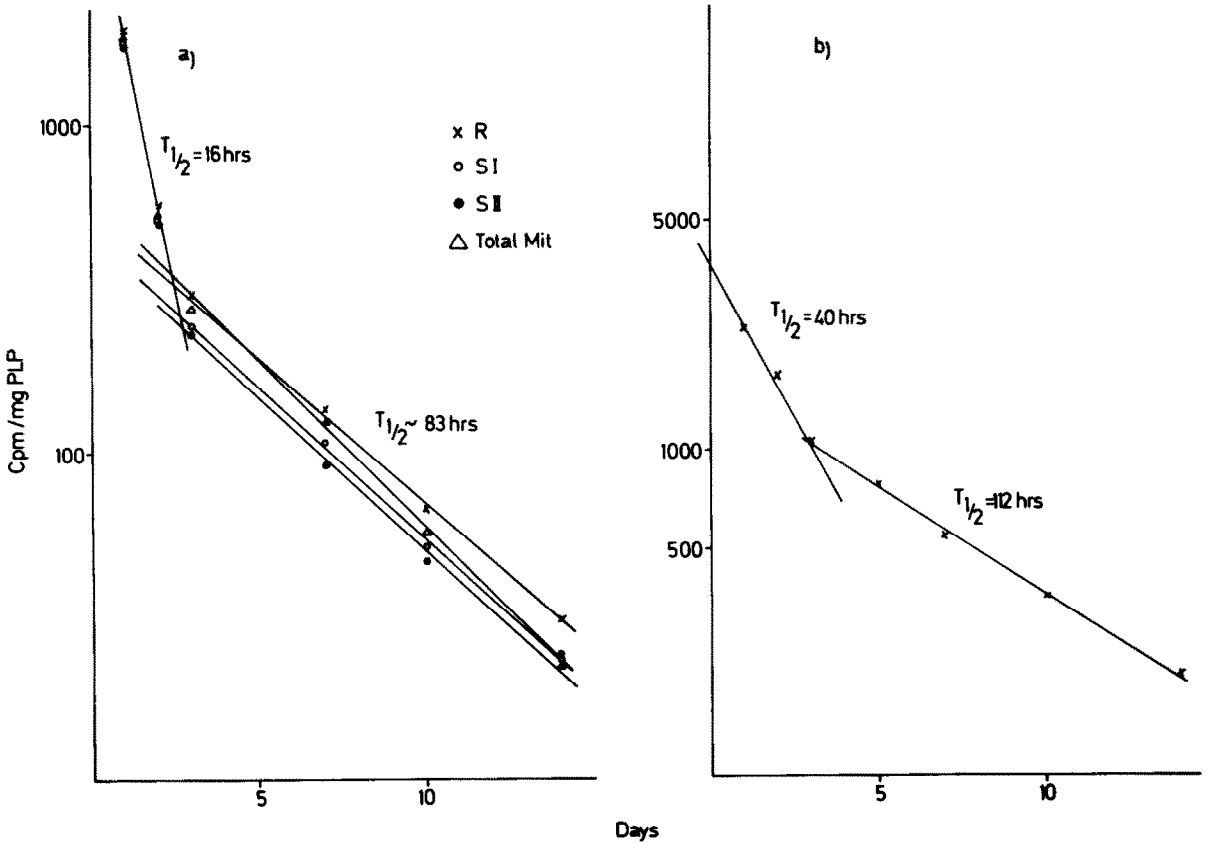

Fig. 1. Decay of specific radioactivity of [^{3}H]glycerol in the phospholipid fractions. [^{3}H]Glycerol incorporation was followed in the methanol fraction after silicic acid chromatograohy. a) Decay in control mitochondria (Mit), rough (R), smooth I (S I) and smooth II (S II) microsomes. b) Decay in total microsomal fraction during phenobarbital treatment. Phenobarbital was injected once a day by giving the first injection at day one.

Table 2
Incorporation of [^{3}H]glycerol and [^{3}H]mevalonate into microsomal lipids after phenobarbital treatment.

PB treatment	Incorporation rate	
	[^{3}H]glycerol (cpm/mg PLP)	[^{3}H]mevalonate (cpm/mg cholesterol)
None	405	120
1 day	443	148
4 days	131	202

Injection was given as described in Materials and methods. Glycerol incorporation (10 min) was measured in the methanol phase after silicic acid chromatography, and mevalonate incorporation (10 min) was determined by following the radioactivity in the chloroform phase of the silicic acid chromatography.

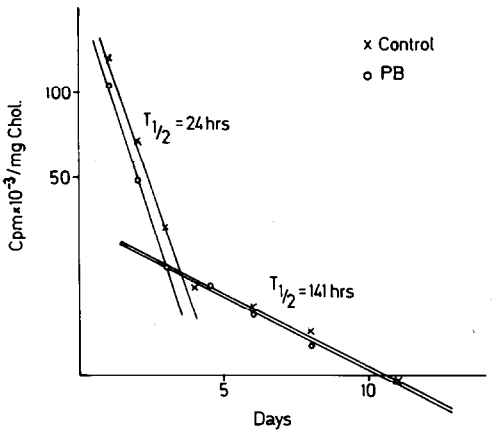

Fig. 2. Decay of specific radioactivity of [^{3}H]mevalonate in the neutral lipid fraction. The lipid extract from total microsomes was subjected to silicic acid chromatography and radioactivity was measured in the chloroform phase. In the phenobarbital (PB) experiments, PB was injected once a day by giving the first injection at day one. The values indicated in the figure concern control rat. Chol. represents cholesterol.

The decrease in specific activity of incorporated [^{3}H]glycerol during the period of day 1 to day 14 is shown in fig. 1a. The decay pattern is identical for mitochondria and the three microsomal subfractions: rough, smooth I and smooth II microsomes. The experiment indicates that the total microsomal fraction can be used in such an investigation and isolation of various subfractions does not give additional information. The identical decay curve of the mitochondrial fraction suggests that the protein carrier mediated lipid exchange process, which is demonstrated to occur under *in vitro* conditions [13], also takes place *in vivo*. There are two slopes, one with a graphically determined half-life of 16 hr and one with a much longer half-life, about 83 hr. The decay of radioactivity of the PLP is much slower when the rat is treated with PB during the whole period of investigation (fig. 1b). The half-lives calculated from the two slopes are 40 and 112 hr, respectively. The reason for the two slopes is not clear and cannot be explained exclusively by appearance of some label in the fatty acid moiety. After deacylation, the hydrophilic fractions still exhibit the two main features described above, i.e., two components with different turnover rates. Furthermore, both components decay more slowly in PB-treated rats.

Similarly to PLP, cholesterol also displays two separate compartments as regards half-lives, one of 24 hr and one of 141 hr (fig. 2). On the other hand, PB does not influence the decay slopes to any appreciable extent since the two values are 22 and 141 hr, respectively.

It appears that the unchanged lipid composition of the liver microsomes isolated from PB-treated rats is maintained by a complex mechanism. Both PLP and cholesterol increase to a great extent, and in a parallel fashion, but the elevations are caused in different ways. The main reason for the increase of PLP appears to be a decreased breakdown paralleled by an unchanged or decreased rate of synthesis, while the elevation of membrane cholesterol occurs because of the increased rate of synthesis with no change in degradation.

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