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LOCALIZATION OF GAMMA-GLUTAMYLTRANSFERASE IN SUBCELLULAR FRACTIONS OF RAT AND RABBIT LIVER: EFFECT OF PHENOBARBITAL

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Abstract—We compared the gamma-glutamyltransferase (GGT) activities in the various subcellular fractions and in plasma membrane preparations, and found that phenobarbital caused an increase in the activity of almost solely this enzyme in the plasma membranes of rat and rabbit liver. We conclude that the enzyme is highly concentrated in plasma membranes. An inductive effect of phenobarbital-like compounds was thus demonstrated even in the rat, where the phenomenon has been difficult to demonstrate before.

Our results permit the use, once again, of the rat as a model for the study of enzymatic induction of GGT, provided the enzyme's activity is measured in liver plasma membranes and not only in microsomes.

The use of the enzyme gamma-glutamyltransferase (GGT, EC 2.3.2.2.) has recently been introduced in clinical chemistry for the study of hepatic function and biliary disorders. High serum or plasma GGT activity is usually found in cases of liver disease, especially when cholestasis occurs [1, 2]; in cases of excessive alcohol intake [3, 4]; or after the administration of drugs, particularly those of the phenobarbital (PB) group [5, 6]. In order to explain these increases, it is necessary to know the physiological variations of the enzyme [7], its cellular and subcellular origins, and the mechanisms of its release into the plasma [8], especially after treatment with drugs.

The localization of GGT in the subcellular fractions of the liver is not yet exactly known. Many authors have suggested that it is localized in the endoplasmic reticulum fraction [9–13] and that this localization might explain the effect of inducing drugs. But recent papers emphasize the possibility of its preferential localization in plasma membranes [14] or in brush border membranes [15].

The localization of GGT in the different liver cells is also controverted. Thus hepatocytes and hepatic nonparenchymal cells were isolated from control and PB-treated animals [16], and the GGT activity was measured in the various cells.

In this paper, we report on a study of the distribution of GGT in the various hepatic subcellular fractions. Because the enzymatic activities in rat plasma and liver are very low, the study was done simultaneously on rats and rabbits. With the differential ultracentrifugation used to prepare microsomes, plasma membranes are distributed amongst the nuclear and microsomal fractions. It then was necessary to prepare a specific plasma membrane fraction. The method described for the rat [17] has been adapted to the rabbit.

MATERIALS AND METHODS

We used male Fauve de Bourgogne rabbits weighing 1.5–2.0 kg and male Sprague–Dawley rats weighing 180–250 g.

Plasma membranes were prepared from rat liver cells as described by Neville [17]. The same method was used for rabbits, with a slight modification: the homogenization step required fifteen up and down strokes of the Dounce homogenizer piston because of the presence of large amounts of connective tissue, and the stirring time was increased (5 min instead of the 3 min recommended). The protein level in our preparations was 1.3–1.7 mg per gram of liver.

Subcellular fractions were obtained by ultracentrifugation as described by Amar-Costesec *et al.* [18]. The purity of the preparations was assessed by electron microscopy observations and by the assay of marker enzymes.

GGT was measured with Szasz's technique [19] using carboxylated L-γ-glutamyl 4-nitroanilide* as a substrate. In some cases, deoxycholate (DOC) was added to the incubation medium to a final concentration of 1% (w/v). This detergent did not change at all the measured activity of GGT, but its clarifying effect on the medium permitted us to obtain a linear slope. Readings were made on an LKB 8600 at a wavelength of 405 nm. Proteins were determined by the method of Lowry *et al.* [20] with bovine serum albumin† used as a standard.

For the PB treatment, the rats and the rabbits were injected with PB‡ at doses of 100 mg/kg and 50 mg/kg, respectively, daily for 4 days. The injections were given intraperitoneally in the rats and subcutaneously in the rabbits. The animals were killed on day 5, after 15 hr fasting.

* Boehringer, Mannheim, France: Ref. No. 125954.
† Fluka: Ref. No. 05480.
‡ Specia (Gardenal): Ref. No. 550427.7.

RESULTS AND DISCUSSION

Distribution of GGT in the liver cellular fractions. In the control rats, GGT activity, although found in all the subcellular fractions of the liver, was very low in the mitochondria–lysosomal fraction and in the postmicrosomal supernatant (cytosol) (Table 1). High GGT activity was found associated with two fractions: namely the fraction rich in nuclei and the one which contains plasma membranes. Although the microsomal GGT activity was considerable, that found in the plasma membranes was 9-fold more when the results were expressed as specific activity. When the results were expressed per gram of liver, the amount was 3-fold greater in microsomes, because one gram of liver yields 30 times more microsomal proteins than plasma membranes do.

In the rabbits, the distribution of GGT in the liver was the same as for the rats but there was more enzyme activity per gram of liver (microsomes × 2, plasma membranes × 2, supernatant × 3.5) (Table 2). Specific activities are always higher in rabbits than in rats subcellular fractions.

The ratios of the activity in plasma membrane to that in microsomes were slightly lower in the rabbits (5.5) than in the rats (9.0).

Effects of phenobarbital. The same study was reported after induction by phenobarbital.

In the PB-treated rats, GGT activity was significantly increased in the plasma membrane fraction (3.5 times that in the controls) but only slightly increased (by 1.5) in microsomes (Table 1). In the mitochondria-lysosomal fractions, enzyme activities also seemed to be slightly increased, to the same degree as in microsomes. In the postmicrosomal supernatant, however, the change in the enzymatic activity was very small. These results are particularly clear when exposed in specific activities.

On the basis of results obtained in the rats, we limited our study in rabbits to the plasma membranes and microsomes. The results confirmed those obtained in the rats, in that induction was greater in plasma membranes than in microsomes (Table 2).

Our results, obtained after subcellular fractionation, show that the hepatic GGT is mainly in the plasma membranes. However, Neville's technique [17], although very convenient and satisfactory for obtaining plasma membranes from all the hepatic cell types, did not permit the separation of membranes from the various individual cell types (Kupffer cells, cells from the endothelial reticulum, hepatocytes, etc.)

The large amount of GGT activity that we found in the nuclear fraction is not surprising, because the plasma membranes practically all sediment with nuclei at the centrifugation speed used; we therefore consider that the GGT activity found in the nuclear fraction arose from the plasma membranes. In organs other than the liver, it has already been shown that GGT is bound to plasma membranes [15, 21].

We do not agree with the other authors who have suggested that liver GGT is bound mainly to the membranes of the endoplasmic reticulum [9–13] and can be

Table 1. Proteins content and GGT activities in rat livers

	Proteins mg/g liver		GGT mU/g liver		GGT mU/mg proteins	
	Controls	PB treated	Controls	PB treated	Controls	PB treated
Homogenate	230.6 ± 2.1	208.2 ± 1.3	880.5 ± 55.2	965.9 ± 17.4	3.8 ± 0.3	4.6 ± 0.1
Subcellular fractions						
Nuclei	33.0 ± 1.8	37.4 ± 1.9	317.0 ± 3.1	526.4 ± 17.1	9.6 ± 0.6	14.0 ± 0.3
Mitochondria–lysosomes	46.0 ± 1.0	41.0 ± 0.7	43.3 ± 3.1	72.5 ± 4.7	0.9 ± 0.04	1.7 ± 0.12
Microsomes	40.7 ± 0.8	42.7 ± 1.5	213.7 ± 39.4	341.3 ± 5.2	5.3 ± 2.4	8.0 ± 0.4
Post-microsomal supernatant	79.2 ± 1.4	81.9 ± 3.2	31.6 ± 5.0	28.5 ± 1.8	0.4 ± 0.07	0.3 ± 0.04
Plasma membranes	1.5 ± 0.1	1.5 ± 0.08	68.9 ± 3.6	237.7 ± 14.4	46.5 ± 3.9	158.4 ± 1.6

The values are the means ± S.D. from 12 experimental animals.

Table 2. Proteins content and GGT activities in rabbit livers

	Proteins mg/g liver		GGT mU/g liver		GGT mU/mg proteins	
	Controls	PB treated	Controls	PB treated	Controls	PB treated
Homogenate	210.0 ± 22.6	220.0 ± 1.9	1561.9 ± 178.8	4972.0 ± 35.5	7.4 ± 0.8	22.6 ± 0.3
Subcellular fractions						
Nuclei	40.4 ± 0.8	N.D*	498.7 ± 73.7	N.D.*	12.3 ± 1.6	N.D*
Mitochondria–lysosomes	26.7 ± 0.5	N.D*	42.3 ± 5.4	N.D*	1.6 ± 0.2	N.D*
Microsomes	28.0 ± 1.8	30.0 ± 1.4	441.6 ± 22.3	649.8 ± 39.4	15.8 ± 0.3	21.6 ± 0.9
Post-microsomal supernatant	85.7 ± 6.7	N.D*	118.7 ± 10.1	N.D*	1.4 ± 0.1	N.D*
Plasma membranes	1.6 ± 0.08	1.7 ± 0.1	140.0 ± 16.8	629.0 ± 95.2	87.8 ± 10.5	370.0 ± 56.4

* N.D: Not determined.
The values are the means ± S.D. from 6 experimental animals.

used as an indicator of enzyme induction in microsomes by drugs or alcohol [10, 12, 13]. In fact, those authors did not study the enzyme in other subcellular fractions, particularly the plasma membranes.

We also detected GGT activity in microsomal fractions, with values similar to those reported by Ivanov *et al.* [9] and Teschke *et al.* [10]. However, we consider that this activity is due to contamination of the microsomes by plasma membranes. Indeed, Wisher and Evans [22] have demonstrated that plasma membranes can be obtained from the microsomal fraction by ultracentrifuging it on a sucrose gradient. On the other hand, by analyzing enzymes present in the various organelles after subfractionation of the microsomal fractions from rat liver, Tilleray and Peters [14] found that GGT is distributed as 5'-nucleotidase is. Since the latter is definitely associated with plasma membranes, they concluded that GGT is bound to the same membranes.

Induction with phenobarbital strengthens the hypothesis that GGT is located in plasma membranes. Indeed, after induction with this drug, the ratio of GGT activity in plasma membranes to that in microsomes rose from 5.5 to 17 in rabbits and from 9 to 20 in rats; in other words, the amount of GGT increased 2 to 3 times more in plasma membranes than in microsomes.

As to the use of GGT as an indicator for microsomal enzyme induction, we think that the level of increase in microsomes compared with that in plasma membranes after PB treatment is too low and too hard to detect. The ratio of GGT in microsomes from treated animals to GGT in those from control animals was about 1.5, while the ratio for plasma membranes was 3.4 in rats and 4.2 in rabbits. In our opinion, hepatic GGT is usable as an indicator of enzyme induction by drugs only if its activity is assayed in plasma membranes.

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