STIMULATION OF GALACTOSYLTRANSFERASE IN LIVER MICROSOMES BY LYSOLECITHIN[1]

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SUMMARY.

Lysolecithin markedly stimulated membrane-bound UDP-galactose:glycoprotein galactosyltransferase. The parent molecule lecithin, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidic acid, lysophosphatidic acid or glycerophosphorylcholine did not activate the enzyme suggesting that both fatty acyl- and phosphorylcholine groups are required for the enzyme activation. The dose-effect of lysolecithin showed sigmoidal kinetics and the Vmax of the enzyme was increased several-fold by lysolecithin. Saturating amounts of Triton masked the effect of lysolecithin. Pre-incubation with phospholipase A also activated the enzyme. A possible role of membrane lysolecithin is indicated in regulating the enzymes of glycoprotein synthesis.

In the course of our studies on the stimulatory effect of choline derivatives on glycosyltransferases (1,2,3) we were unable to obtain any significant effect of exogenously added phospholipids on these membrane-bound enzymes (1). These membrane-bound enzymes are usually assayed in presence of Triton or other detergents. Triton is believed to cause unfolding of the protein chains in the membranes and thereby promote the access of the substrate to the active enzyme sites. However, it is possible that detergents extensively alter the organization of the membrane lipid-protein matrix and thereby obscure the attempts to study the physiological regulation of these enzymes. It is not known how the delicate cell-membrane organization allows different glycosyltransferases to operate *in vivo* in an effective and purposeful manner. Towards this goal, we decided

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to investigate the effect of phospholipids and other lipid factors on rat liver galactosyltransferase activity in absence of any detergent. The enzyme activity was barely detectable when assayed in absence of detergents, but addition of lysolecithin stimulated the enzyme very markedly.

METHODS.

A standard assay for UDP-galactose:glycoprotein galactosyltransferase, unless otherwise specified, contained rat liver microsomes (1), 25 µl (0.3 mg protein); UDP-galactose-^{14}C, 50 nanomoles (15,000 cpm); 2-(N-morpholino)ethane sulfonic acid buffer, pH 6.0, 12.5 µmoles; $MnCl_2$, 0.625 µmoles; and fetuin, depleted of sialic acid and galactose (4), 125 µg in a total volume of 50 µl. Assays were incubated at 30°C for 40 min when the reaction was terminated and the galactose-^{14}C bound protein radioactivity was counted as described before (3). All lipids were dissolved in chloroform:methanol (2:1,v/v) or in hexane solution and dried under nitrogen prior to the addition of incubation ingredients. All lipids were checked for purity by thin layer chromatography. All lysolecithin samples stained as a single band on the chromatogram. Only lysophosphatidic acid and phosphatidylglycerol gave a second minor band on the chromatogram, of which the minor band of lysophosphatidic acid showed a migration rate similar to lysolecithin. Lysolecithin was its source for commercial preparation.

Lysolecithin (egg yolk), lecithin, sphingomyelin, glycerophosphorylcholine, ganglioside and cardiolipin were obtained from Sigma (St. Louis); monoglyceride was a gift from Dr. A. Kuksis and pig liver lysolecithin, synthetic lysolecithin(oleoyl) and all other lipids were from Serdary Research Laboratories (London, Ontario). Labelled nucleotide-sugar was purchased from New Eng-

Table I. Effect of lysolecithin and other lipid factors on galactosyltransferase activity. Enzyme was assayed as described in the text.

Experiment	Additions (µg/50µl)	Picomoles ^{14}-C galactose/mg protein/40 min.	Activation (times over control).
control	none	208	----
Lysolecithin, egg	83	1489	7.2
	250	3363	16.2
Lysolecithin, pig liver	83	1249	6.0
	250	3145	15.1
Lysolecithin, synthetic	100	2003	9.6
	300	2856	13.8
Lecithin, egg	100	212	1.0
	600	232	1.1
Phosphatidylethanolamine, pig liver	100	304	1.5
	300	338	1.6
Lysophosphatidylethanolamine, pig liver	83	234	1.1
	250	267	1.3
Phosphatidic acid, egg	25	181	0.87
	150	307	1.5
	375	406	1.9
Lysophosphatidic acid, egg	25	323	1.6
	150	423	2.0
	375	590	2.8
Sphingomyelin, bovine brain	100	138	0.66
	300	179	0.86
Glycerophosphorylcholine, Cd^{++}	51	140	0.67
	102	170	0.82
Phosphatidylglycerol, egg	25	241	1.2
	150	247	1.2
Ganglioside, bovine brain	83	257	1.2
	250	198	0.95
Cardiolipin, bovine heart	75	478	2.3
	225	325	1.5
Diglyceride (1,2-diolein)	250	221	1.1
	750	200	0.96
Monoglyceride (1-monoolein)	270	254	1.2
	810	358	1.7
Oleic acid	100	260	1.3
	500	262	1.3
Palmitic acid	83	272	1.3
	250	378	1.8

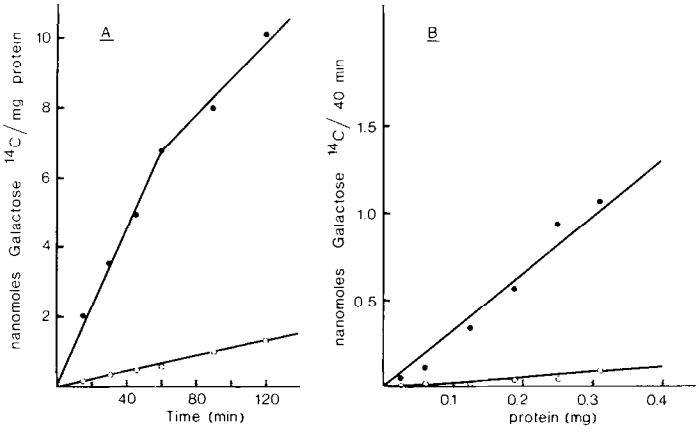

Fig. 1. The dependence of galactosyltransferase activity on time (panel A) and on microsome protein concentration (panel B) in presence (250 µg) ——●—— and in absence ——o—— of lysolecithin (egg). The method of enzyme assay is described in the text.

land Nuclear (Dorval, P.Q.) and phospholipase A (Crotalus artox) was from Ross Allen Reptile Inst. (Silver Springs, Florida).

RESULTS AND DISCUSSION.

The stimulatory effect (13 to 16-fold) on the galactose enzyme was observed by three different types of lysolecithin, i.e., egg yolk, pig liver and synthetic (Table I). The effect was markedly specific for lysolecithin since various other phospho- and lyso-phospholipids did not stimulate the enzyme. A 2.8-fold stimulation of the enzyme by lysophosphatidic acid was probably due to the presence of lysolecithin as a contaminant.

In the presence of lysolecithin the enzyme activity was almost linear up to 60 min and to the microsomal protein of about 0.3 mg (Fig. 1, A and B). The enzyme activity was increased in a linear fashion with the increase of Triton dose up to 0.3% or 4.5 mM (Fig. 2B), whereas the dose effect of lysolecithin showed sigmoidal kinetics (Fig. 2A). The lysolecithin effect was evident at a concentration of 0.56 to 1.12 mM and was saturated at a

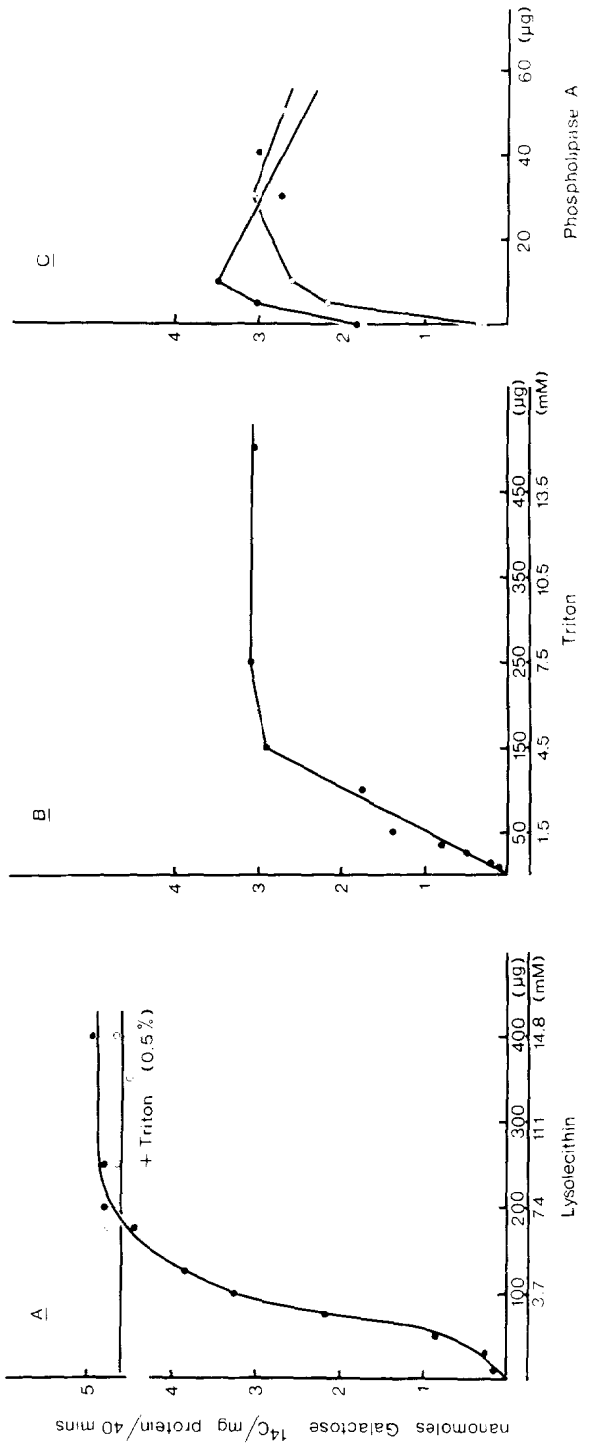

Fig. 2. The effect of different doses of lysolecithin (panel A), Triton (panel B) and phospholipase A, pre-incubated for 10-20 min, (panel C) on galactosyltransferase activity. The method of enzyme assay is described in the text. Egg lysolecithin was used in the experiments. Panel A also shows a masking effect of 0.5% Triton —o— superimposed on a dose-response study with lysolecithin —●—. Panel C also shows the effect of phospholipase A in presence (83 ug) —●— and in absence —o— of lysolecithin. The molecular weight of 536 for lysolecithin was arbitrarily calculated (as oleoyl lysolecithin) and the molecular weight of Triton X-100 as 635 was calculated (13) on the basis of a structure of polyoxyethylated octylphenol (ethylene oxide:9.7 units).

concentration of 7.5 mM. Pre-incubation with phospholipase A for 10-20 min also stimulated the enzyme in a dose-dependent manner (Fig. 2C), probably because of the hydrolytic generation of lysolipids in the membranes. The effect of phospholipase A was enhanced in the presence of lysolecithin. Furthermore, when the effect of different doses of lysolecithin was studied in the presence of a saturating concentration of Triton, the stimulatory effect of lysolecithin was completely masked (Fig. 2A). This masking effect of Triton would explain why the stimulatory action of lysolecithin on the enzyme was not observed before. The effect of varying concentrations of the nucleotide-sugar substrate on the initial velocity of the enzyme activity was studied (Fig. 3). The remarkable stimulatory effect of lysolecithin is evident at all concentrations of the substrate tested (Fig. 3A). From these results it was possible to plot the data on the basis of Michales-Menten kinetics and the apparent Km value for UDP-galactose was calculated to be 0.33 mM. There was no change in the Km value when the enzyme was assayed in presence or in absence of lysolecithin. However, lysolecithin has a profound effect on the Vmax of the enzyme, which was increased 6-fold, from a value of 0.22 to 1.33 in the presence of 250 µg of lysolecithin.

The possible role of glycosyltransferase enzymes in the synthesis of secretory glycoproteins (1,5), in development (6), in intercellular adhesion (7,8) and in surface recognition phenomenon (8,9) is well documented. Of the various enzymes studied, galactosyltransferase activity has been found to be specially important in the development of the embryonic chicken brain (6) and in the adhesion of embryonic chicken neural retinal cells (8). However, in all these intra- and intercellular functions, glycosyltransferases are probably amenable to physiological regulation. If

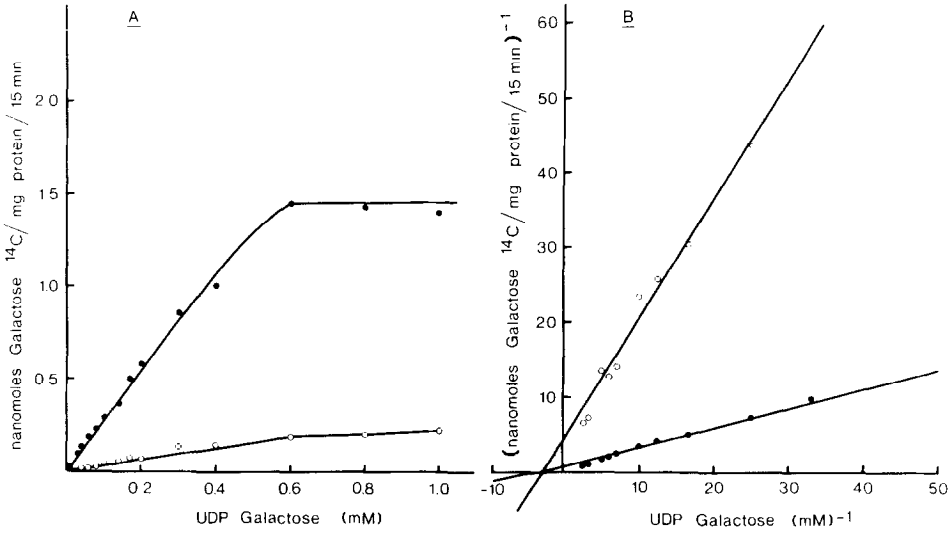

Fig. 3. The effect of varying UDP-galactose concentration on galactosyltransferase activity in presence (250 µg) —●— and in absence —o— of egg lysolecithin (panel A) and the Lineweaver-Burk plot of the data (panel B). The apparent Km for UDP-galactose was calculated to be 0.33 mM and the value remained unchanged in presence or in absence of lysolecithin. The Vmax in absence of lysolecithin was 0.22 which was increased to 1.33 in presence of lysolecithin. The enzyme assay conditions are as described in the text with the exception that the incubation time was 15 minutes.

some of the membrane lipid components play such a role, attempts to elucidate the role of lipids or other factors could be obscured due to the presence of Triton in the enzyme assay systems.

To our knowledge, the results presented in this paper are the first evidence of an activation of a membrane-bound enzyme required for serum glycoprotein synthesis by addition of a phospholipid itself. The physiological concentration of lysolecithin in rat liver is about 3 mM assuming its uniform distribution within the cell (10). However, lysolecithin is mainly localized in the membranes and consequently its concentration will be much higher in the membrane sites. It is therefore conceivable that the amount of lysolecithin present in the membrane sites is sufficient to cause an activation of the membrane-bound glycosyl-

transferases. The concentration of lysolecithin in different membrane locations may further be varied depending upon local differences in phospholipase A and acyltransferase activities. This, however, adds complexity to the suggestion of a physiological control of the enzyme by lysolecithin. Studies on the level of glycosyltransferase enzymes in serum in various disease conditions showed that the serum enzyme concentration was increased only in liver diseases (11,12). It is possible that defective lysolecithin metabolism in liver may be related to the process of hypersecretion of these enzymes into the serum.

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