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THE INFLUENCE OF PGE_1 AND ETHANOL AS PGE_1 SOLVENT ON THE ACTIVITY OF GALACTOSYLTRANSFERASE OF RAT LIVER GOLGI FRACTIONS – *IN VITRO* EXPERIMENTS

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Addition *in vitro* of ethanol solution of PGE_1 to isolated Golgi-rich membrane fraction caused great alterations in galactosyltransferase activity, marker enzyme of these membranes. Ethanol as a solvent of PGE_1 has an influence on the activity of the enzyme as well as the membrane permeability, different drugs penetration and availability of substrates. Then, additional control with ethanol in identical concentration as in the investigated sample was performed. In a dose 1μg PGE_1 per 1 mg of protein (and lower concentration of ethanol c. 0.09%) the stimulation of this enzyme activity (excluding 2 and 6 hour s after Golgi membrane isolation) was above 30% in comparison with the control.

Key words: *PGE_1, galactosyltransferase, Golgi memebrane fraction, in vitro activity.*

INTRODUCTION

The activity of many membrane enzymes including glycosyltransferases e.g. galactosyltransferase (1) depends on environmental phospholipids and their interaction with these enzymes (1, 2). It may depend on the influence of some organic solvents or drugs on phospholipids or enzyme alone too. On the other hand, the PGs especially from E series, in a broad spectrum of their biological activities (3) show also cytoprotective effects on gastrointestinal tract or liver damage (4, 5) and may act on lipid bilayers (6). Our previous investigations on cytoprotective function *in vivo* (16, 16' dimethylprostaglandin E_2 protects the rat liver Golgi apparatus against morphological and biochemical alterations induced by streptozotocin) show very good prevention by this derivatives (7–9). For these reasons we decided to study *in vitro* the influence of stable derivatives of PGs i.e. prostaglandin E_1 (PGE_1) on the Golgi marker enzyme (UDP-Gal → GlcNAc transferase). These investigations were carried out 0–8 hours after isolation of these Golgi-rich fractions from the rat liver.

MATERIAL AND METHODS

Animals

The experiments were carried out on female Wistar rats, about 6 months old, 170–230 g of weight and fed *ad libitum* with commercial pelleted food and tap water. The animals were not starved in order to eliminate the action of factors other than PGE_1 or ethanol. All rats were anaesthetised with ether and exsanguinated. Their livers were resected and immediately used for Golgi-rich membrane isolation.

Methods

Golgi-rich membrane isolation: These fractions were isolated according to one-step gradient procedure by Fleischer (10). After collection of the membranes (one experiment 3–4 rats) they were diluted with 0.9% NaCl to final concentration 100–200 μg of protein in 0.1 ml.

The fraction was divided into 3 groups:

1. Control group – without any treatment
2. Group with ethanol addition to final concentration 0.09% as the second control.
3. The study group with PGE_1 (in ethanol solution in concentration identical to that in group 2) in dose 1 μg PGE_1 per 1 mg of protein.

In two experiments ethanol concentration was greater than 1% but the results obtained were scattered and were not taken into account in this paper.

Assay of galactosyltransferase activity

The activity of UDP-Gal → GlcNAc transferase was estimated according to Fleischer (10). During 8 h after the membrane fraction isolation (the source of enzyme) the samples in vol. 0.05 ml were taken for 2 hours. The radioactivity of effluents from Dovex 2 × 8 (200–400 mesh) columns was determined with liquid LKB Wallac Scintillation (as ^{14}C labelled in moiety of Gal in N-acetyllactosamine).

Extraction of lipids

Phospholipids were extracted according to Kates (11). The Golgi-rich fractions containing 0.5–0.5 mg of protein in suspensions were extracted with chloroform: methanol (1:2 v/v) and chloroform : methanol : water (1:2: 0.8 v/v/v). Chloroform always included 4-hydroxybenzoic acid butylester in concentration 0.05%. Identification and quantitative determination of PL were carried out by one dimensional micro-thin-layer chromatography on the Sigma plastic plates (9 × 12 cm covered with silica gel). The 10–20 μg of standard phospholipids were applied and the the chromatogram was developed in then solvent composed of chlorophorm : methanol : acetic acid : water 50 : 30 : 8 : 4 v/v/v/v). Phospholipids were detected with iodine vapour. Each spot was scraped off into a test-tube and after mineralization the inorganic phosphate was determined with the reagent of Vaskovsky et al (12).

Protein determination

Protein content was determined by the method of Lowry et al. (13) with the crystalline serum albumin as a standard.

Reagents

Tris, serum bovine albumin, sodium cacodylate were purchased from Koch-Light Labs., UDP-Gal, Triton X-100, phosphatidylcholine from bovine liver, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, phosphatidic acid (from brain). TCL plastic plates covered with silica gel 250 μm layer thickness came from Sigma Chem. Co., UDP-Galactose labels with (U−^{14}C) sp. act. 270 mCi/mmole came from Radiochemical Centre Amersham, Dovex 2 × 8 with granulation 200−400 mesh came from Fluka and Buchs, GlcNAc was prepared in the Department of Organic Chemistry of Bialystok Medical School (Poland), sucrose came from Reachim (SU), 4-benzoic acid butylester came from Serva, the prostaglandin E_1 came from Merck and was a kindly gift from Prof. Jerzy Stachura. All other reagents of analytical grade were purchased from POChem Gliwice (Poland)

RESULTS

The activity of UDP-Gal → GlcNAc transferase *in vitro* in three groups is presented in *Fig. 1*. The tendency of action of PGE_1 (in ethanol solution) or ethanol alone (in identical concentration) on galactosyltransferase activity is

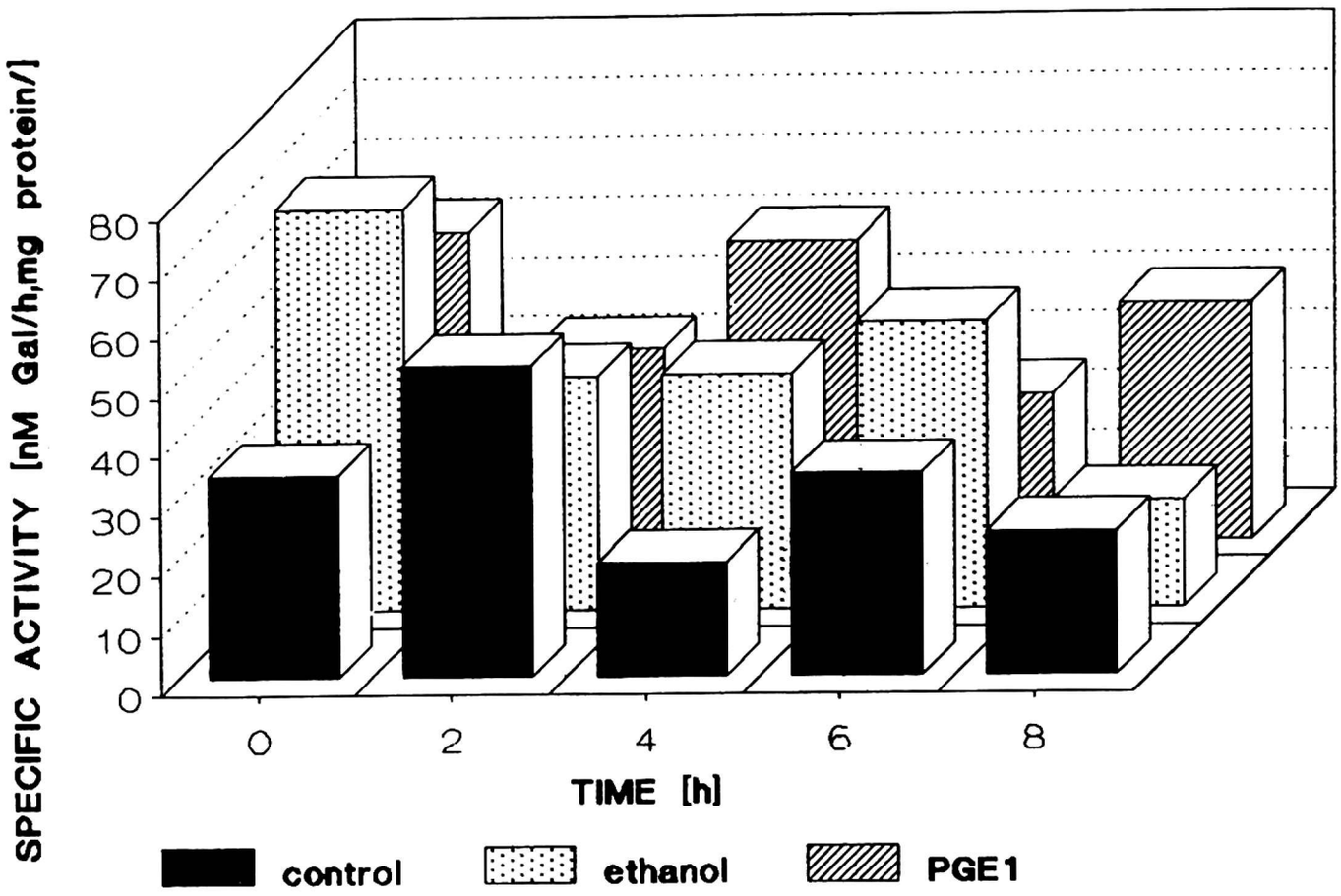

Fig. 1. The activity of UDP-Gal → GlaNAc transferase from rat liver Golgi apparatus in three groups (investigation *in vitro*): 1. control, 2. with ethanol in final concentration c. 0.09% 3. with PGE_1 (dissolved in ethanol in final concentration c. 0.09%) in a dose 1μg per 1 mg of protein in this fraction.

summarised in *Table 1*. The activity of Golgi membranes galactosyltransferase in the control group shows fluctuactions above the value 40 nMoles of Gal transferred per 1 mg of protein and per 1 h (from 20–40 nMoles transferred). There are three peaks of this activity: at 0 h, 4 h and 8 h after membrane

Table 1. PGE_1 or ethanol action *in vitro* on the activity of UDP-Gal → GlcNAc transferase from Golgi-rich membrane fraction.

+/−	activity different from control by 0–5%
+	increase of activity 5–50%
++	increase of activity from 50–200%
+++	increase of activity higher than 200%
−	decrease of actiaity from 5–33%
−−	decrease of activity from 33–67%
−−−	decrease of activity lower than 67%

Time in hours	Enzyme activity as influenced by:		
	1	2	3
	PGE_1 in comparison with control	Ethanol in comparison with control	PGE_1 in comparison with ethanol alone
0	++	++	−
2	−−	−−−	−
4	++	+++	+
6	−	++	−−
8	++	−−−	+++

Enzyme activity calculated from 3 or 4 separate experiments (each with 3–4 rats). Values of t-test calculated with control and ethanol group at 4 h and 8 h were $t < 0.100$, for control and PGE_1 groups at 2 h, 4 h and 8h $t < 0.100$.

isolation. On exclusion of two points of measurement i.e. 4 h and 8 h ethanol alone in the final concentration 0.09% can stimulate this activity. PGE_1 (in ethanol of the same concentration) stimulates the enzyme activity at the value about 50 nMoles Gal transferred per 1 h and 1 mg protein. The stimulation by PGE_1 shows a similar tendency as ethanol but is highest at 4 h and 8 h after Golgi membrane fraction isolation. At 2 h and 6 h after this preparation, the enzyme activity was lowest in comparison not only with ethanol alone but also with the control. Ethanol was used in our experiment not as a drug but as an additional control.

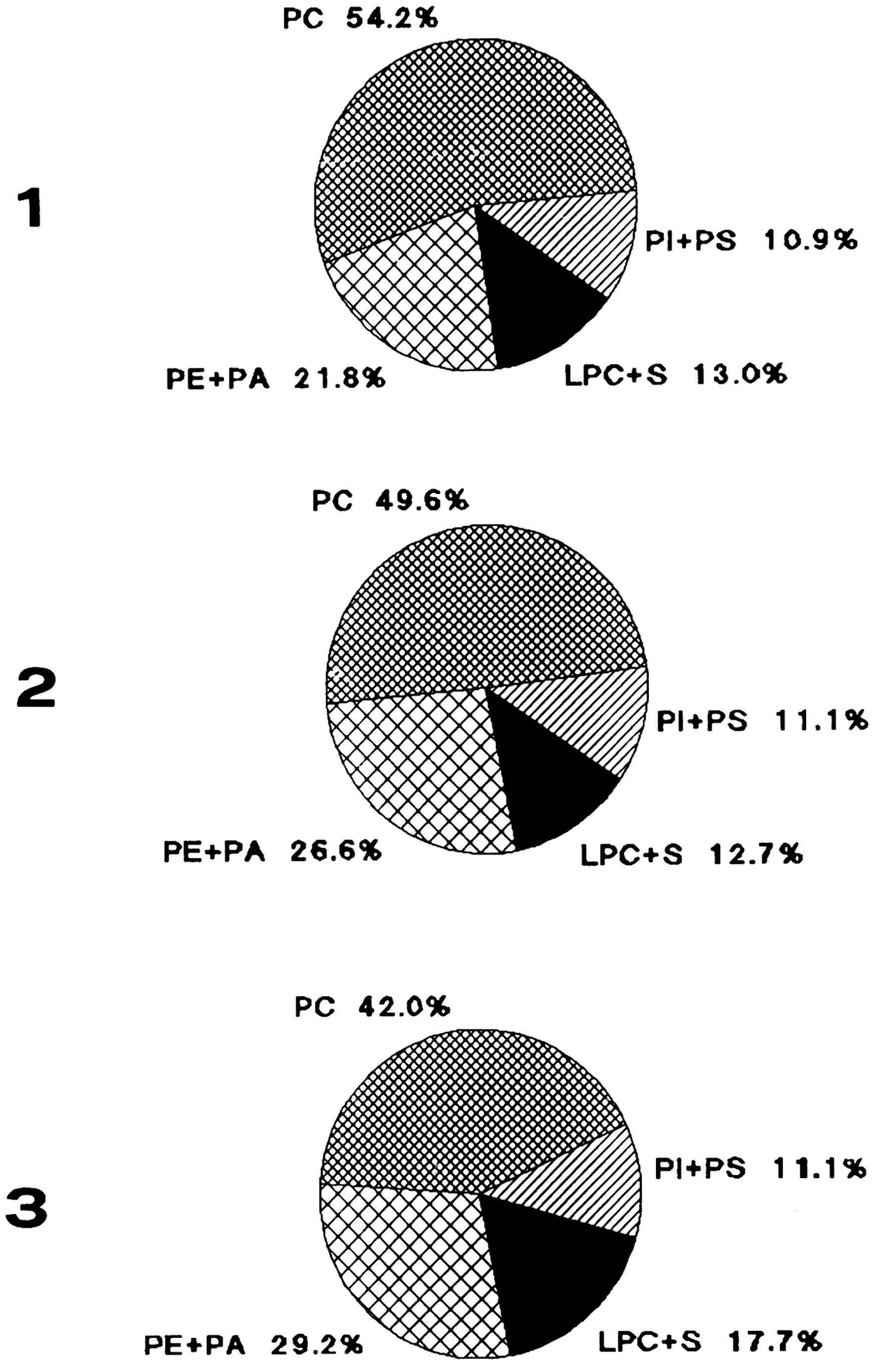

Fig 2. The percentage of phospholipids in the groups: 1 – control, 2 – with ethanol concentration c. 0.09% 3 – with PGE_1 addition in a dose 1μg per 1 mg of protein dissolved in ethanol in concentration identical to that in group 2.

Abbreviations: PA – phosphatidic acid, PC – phosphatidylcholine, PE – phosphatidylethanolamine, PI – phosphatidylinostitol, LPC – lysophosphatidylcholine, S – sphingomyeline.

Fig. 2 shows the percentage of individual phospholipids in three investigated groups. In comparison with the control, ethanol addition caused a decrease in total phosphorus content (expressed as μg P_i per 1 mg of protein. It was on the border line of statistical significance ($t = 2.7556$, $0.01 < p < 0.02$). Other differences between percentage of individual phospholipids class were not statistically significant.

DISCUSSION

Prostaglandis (PGs) and other derivatives of polyunsaturated fatty acids are thought to have a broad biological spectrum of actions on many biological processes. Prostaglandins influence lipolysis, regulation of intracellular cholesterol (cholesterol esters balance) (4, 14), ion flux and regulation (15, 16), temperature regulation (16, 17), reproductive biology, cell aggregation and host-parastite interaction (3), not only in vertebrates but also in invertebrates (18).

Among these multiple activities, some of them especially from I, E, and F series show protective effects against tissue or cell damage induced by various agents (4, 5, 17, 19). These properties were shown not only *in vivo* (1, 5, 7, 8, 17) but also *in vitro* (4, 20–23). These facts prompted us to investigate the effect of PGE_1 addition on the activity *in vitro* of liver Golgi membrane fraction marker enzyme: UDP-Gal → GlcNAc transferase. The catalytic part of this enzyme is exposed to lumen of cisternae of Golgi apparatus, then after mild isolation (Fleischer method) we hoppe to achieve approximate to normal condition, action of enzyme, especially in a short time after isolation.

The influence of ethanol alone, which was used as solvent of powder of PGE_1 was investigated as the second control. It must be taken into consideration that ethanol alone, as lipid solvent could change membrane permeability and influence drug penetration. On the other hand it can induce alterations in the availability of substrates in this way altering enzyme activities. In comparison with the control, ethanol stimulated (excluding 2 h after Golgi membrane fraction isolation) enzyme activity at each point of the experiment.

Contrary to this action, ethanol solution of PGE_1 caused lower activity of galactosyltransferase not only after 2 h, but also after 6 h. The facts are difficult to explain. They suggest fluctuations of galactosyltransferase activity during the 4-hour period.

In our previous study the $dmPGE_2$ caused a decrease in galactosyltransferase activity at 0 h and 8 h after isolation of the fraction of Golgi membranes; at 6 h the activity of the enzyme was similar to that of the control. The decrease in the activity of galactosyltransferase 6–8 hours after Golgi

membrane isolation, induced both by PGE_1 and 16, 16' dm PGE_2 (*in vitro*) in comparison with the control is very interesting.

Phospholipid membrane content was estimated in comparison to their proportion, because some of them (1, 2) affect galactosyltransferase activity. We could not expect great changes in phospholipid proportion after ethanol or PGE_1 addition *in vitro*, the differences we found were due to rather individual alterations among rats. After ethanol addition, lower than the control level (on the border of statistical significance of inorganic phosphorus per 1 mg of protein was only found. After 16,16' dm PGE_2 administration *in vivo* (9) similarly as after *in vitro* addition the statistically significant lower than in the control group both the level of phosphorus, expressed as μg P_1 per mg of protein and PE + PA percent had been found previously in our study.

The mechanism of cytoprotection by PGs, especially E series, is not clear *in vivo* as it is *in vitro* (24, 25), only further experiments will allow us to understand the action of these coumpounds *in vitro* on some isolated cell organelles.

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