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QUARTERLY

Prostaglandin E ($dmPGE_2$) action *in vitro* on the activity of rat liver Golgi apparatus galactosyltransferase

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***In vitro* addition of 16,16'-dimethyl prostaglandin E_2 to Golgi-rich membrane fraction in final concentration of 0.1 μg/1 mg of protein increased generally the activity of galactosyltransferase in comparison with control. The percentage of phospholipids in the whole fraction was similar in both investigated groups, only the sum of phosphatidylethanolamine + phosphatidic acid was significantly lower after addition of $dmPGE_2$ than in the control ($0.001 < P < 0.01$).**

Within the last decade many biological functions of prostaglandins (PGs)[1] were established. The protective role of these compounds, especially I, E, and F [1 - 3], referred to as cytoprotection was demonstrated not only in gastrointestinal tract [3], but also in whole liver [2, 4, 5] as well as in liver cell culture and adipocytes [6, 7]. These compounds can protect the intestine and stomach against damage induced by several toxic compounds such as ethanol, hot water, HCl, NaOH, CCl_4 [3, 7].

In the liver Stachura *et al.* [2] found cytoprotection by 16,16'-dimethyl prostaglandin E_2 (16,16'-$dmPGE_2$) against rat hepatocyte damage caused by galactosamine application. Additionally, cytoprotection by PGs *in vivo* of biochemical activity and morphology of rat liver Golgi apparatus against streptozotocin (SZ) induced damage 6 and 11 days after SZ administration was reported [4, 5]. However, it was not clear whether PGE could influence *in vitro* the activity of galactosyltransferase (the Golgi apparatus marker enzyme) at room temperature during the first hours after isolation of the rat liver Golgi apparatus.

MATERIALS AND METHODS

Animals. The experiments were carried out on female Wistar rats (6 months old, 170 - 220 g) fed *ad libitum* with commercial pelleted food and tap water. The animals were not starved before being killed to eliminate the action of factors other than PGE_2. All rats were exsanguinated under light ether anaesthesia, their livers were resected and immediately used for isolation of Golgi-rich membrane fraction.

Methods of Golgi-rich membrane isolation and assay of galactosyltransferase activity. The Golgi-rich membrane fractions were prepared from rat liver by one-step gradient method described by Fleischer & Fleischer [8]. Then the membranes, as source of the marker enzyme, were used for activity determination [8]. The isolated membranes were suspended in 0.9% NaCl to final concentration of about 200 μg of protein in 0.1 ml. The membranes were divided into two samples:

–1. control, untreated membranes;

[1] Abbreviations: PGs, prostaglandins; PGE_2, prostaglandin E_2; PL, phospholipids; SZ, streptozotocin other abbreviations see Fig. 2.

–2. Golgi membranes to which 16,16'-dimethyl prostaglandin E_2 (final concentration 0.1 µg $dmPGE_2$/1 mg of membrane protein was added.

The activity of galactosyl transferase was assayed from 0 h to 20 h after Golgi membrane isolation at 20 - 23°C. The radioactivity of effluents from Dowex 2 × 8 (200 - 400 mesh) columns containing ^{14}C-labelled Gal moiety of *N*-acetyllactosamine was determined with Liquid LKB Wallac Scintillation.

Extraction and identification of lipids. The Golgi-rich membranes were frozen at –20°C (20 h after their isolation) and used for phospholipids (PL) estimation. PL were extracted according to Kates [9] with chloroform:methanol (1:2, v/v). Chloroform always contained 0.05% 4-hydroxybenzoic acid butyl ester. Identification and quantitative determination of PL were carried out by one dimensional micro-thin-layer chromatography on the silica gel Sigma plastic plates (9 - 12 cm). The standard PL, 10 - 20 µg, and investigated PL (100 µg) were applied and then the chromatogram was developed in the solvent composed of chloroform:methanol:acetic acid:water (50:30:8:4, by vol). PLs were detected with iodine vapour and each spot was scrapped into a test-tube, and after mineralization, inorganic phosphate was determined with the reagent of Vaskovsky *et al.* [10].

Protein determination. Protein was determined by the method of Lowry *et al.* [11] with bovine serum albumin as a standard.

Reagents. Tris, serum bovine albumin, sodium cacodylate were purchased from Koch-Light Labs.; UDP[^{14}C]Gal, sp. act. 270 mCi/mmole, was from Radiochemical Centre Amersham; UDP-Gal, Triton X-100, PC, PE, PS, S, PA, TLC silica gel plates, 250 µm layer thickness, were from Sigma Chem. Co. Dowex 2 × 8 (200 - 400 mesh) was from Fluka & Buchs, 4-benzoic acid butyl ester was obtained from Serva, 16,16'-$dmPGE_2$ was from Calbiochem Corp.; all other reagents of analytical grade were purchased from POCh- em Gliwice, Poland.

RESULTS AND DISCUSSION

The activity of galactosyltransferase in Golgi membranes in the investigated ($dmPGE_2$ containing) and control groups is summarized in Fig. 1. Table 1 shows the existence of a general

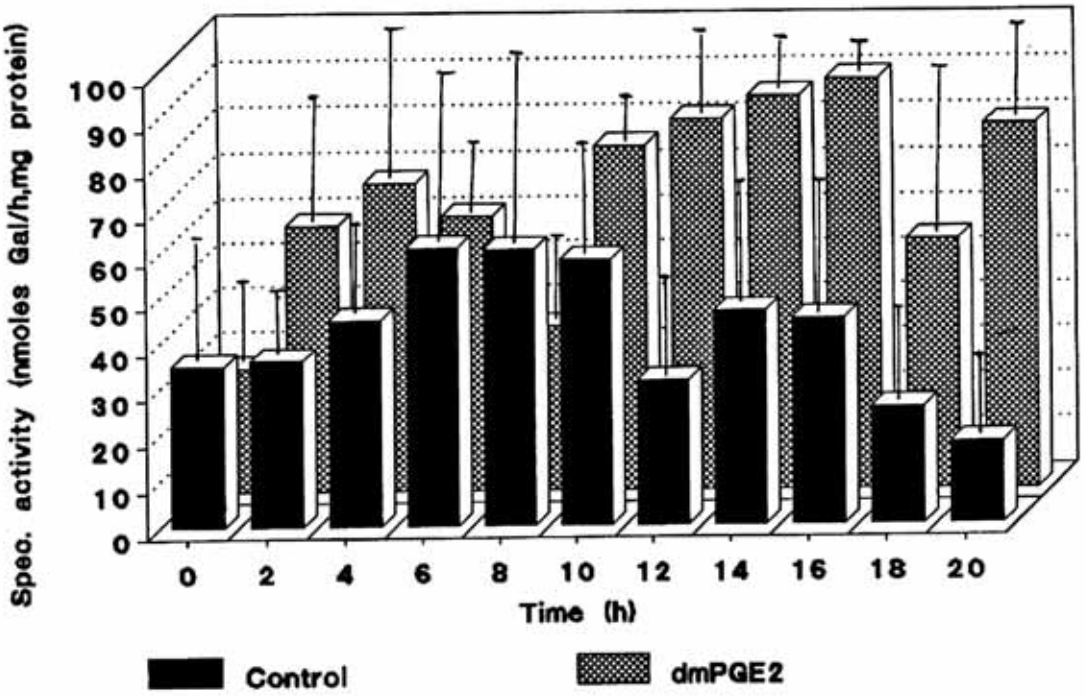

Fig. 1. The effect of 16,16'-$dmPGE_2$ (0.1 µg/mg protein) on the activity of UDP-Gal→GlcNAc transferase. The activity is expressed as nmole of Gal transferred from UDP-[^{14}C]Gal to N-acetylglucosamine per 1 h and per 1 mg protein. Each bar represents the mean result from 3 or 4 individual experiments, each done on 3 - 4 animals. For statistical analysis see Table 1.

Table 1

Changes in galactosyltransferase activity in Golgi-rich membrane preparations treated with 16,16'-dmPGE$_2$ (0.1 μg/mg protein) measured at 0 - 20 h after isolation of these membranes with respect to control

Designations: +, ++, or +++ increase of the enzyme activity by 5 - 50%, 50 - 200% or more than 200%, respectively; +/–, the enzyme activity differing by 0 - 5% from control values ; –, – –, and – – –, decrease of the enzyme activity by 5 - 33%, 33 - 67% or more than 67%, respectively. The results were calculated on the basis of 3 or 4 separated experiments and evaluated by Student's *t* test

Time (h)	General trends in enzyme activity	*t*	*P*
0	–		
2	+		
4	+		
6	+/–		
8	– –		
10	+		
12	+	3.0341	$0.02 < P < 0.5$
14	+	3.4022	$0.02 < P < 0.05$
16	+	2.6441	$0.05 < P < 0.10$
18	+		
20	+++	2.7036	$0.05 < P < 0.10$

trend toward alterations of the enzyme activity. The appropriate signs designate an increase or decrease of this activity with respect to control. The increase in the enzyme activity consistently observed (except at 6 h and 8 h) was on the border of statistical significance only in the samples tested 10 - 20 h after isolation of Golgi membranes. The dispersion of the results was probably due to great differences in individual response to the drug of the Golgi apparatus. The activity of galactosyltransferase was increased in the presence of 16,16'-dmPGE$_2$ in comparison with controls (except at two points of measurements: 6 h and 8 h after Golgi membrane isolation). This increase may point to the protective role of 16,16'-dmPGE$_2$ not only on intact liver [4, 5] but also on the membrane organelle outside of the living cell.

Phosphatidylcholine and phosphatidylethanolamine (and their lysoforms) are known to stimulate the activity of galactosyltransferase [12]. Therefore we estimated additionally the content of these phospholipids after 3 intraperitoneal injections of 16,16'-dmPGE$_2$ *in vivo* to rats. This caused a statistically significant lowering of the total phospholipid content in rat liver Golgi-rich membrane fraction [13] due to lowering of the percentage of the sum of phosphatidylethanolamine and phosphatidic acid. The contents of phospholipids reported in this paper are similar to those previously described [13]. The differences on distribution of individual phospholipids between the experimental and control groups, calculated by Student's *t* test, were not statistically significant. We did not expect that addition of 16,16'-dmPGE$_2$ to isolated rat liver membrane fractions would cause any change in the percentages of individual phospholipids, and these estimations were done only to verify this supposition. Figure 2 shows the results of these estimations.

The prostaglandins are compounds with wide spectrum of biological activity. *Inter alia* they can protect some organs [2, 4, 5], tissues [6, 14 - 17] and cells [18 - 24] against damage caused by various drugs.

The prostaglandins from the E, F and I series are especially active. The problem of cytoprotective action of PGs so far has not been extensively investigated yet, so we try to find some data concerning this subject. As demonstrated previously, PGE$_1$ could protect *in vitro* the activity of galactosyltransferase up to eight hours

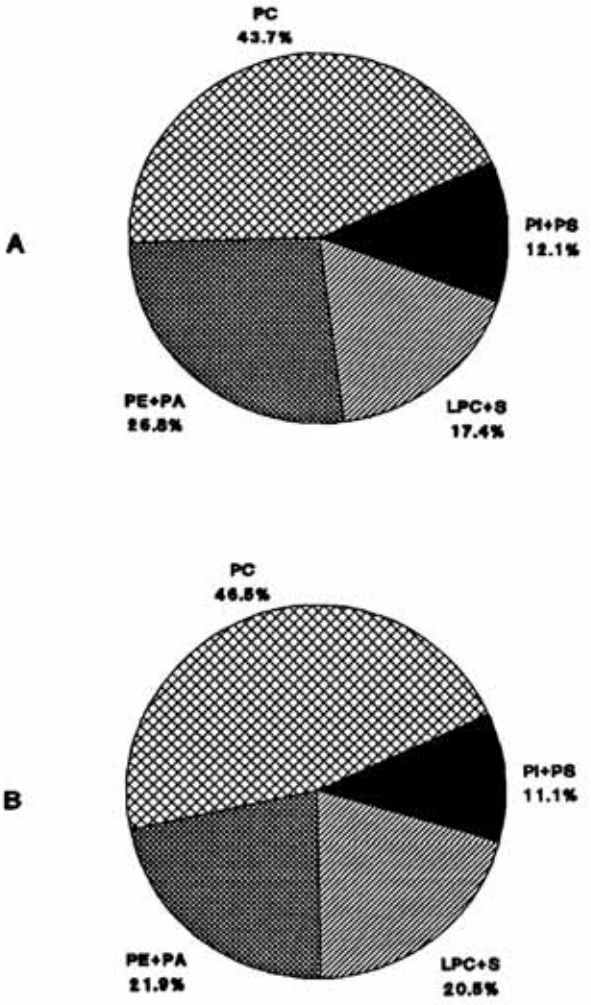

Fig. 2. Diagrams of phospholipids distribution (A) without and (B) after addition of 16,16'-dmPGE$_2$ (0.1 μg/mg protein) to the Golgi membrane preparations. Abbreviations used: PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidlinositol; PS, phosphatidylserine; LPC, lisophospatidylcholine; S, sphingomyelin

after rat liver Golgi membrane isolation (Kordowiak *et al.*, unpublished). The present results show that 16,16'-dmPGE$_2$ can cause a strong increase in the activity of galactosyltransferase *in vitro* for a much longer time. Further investigations on the mechanisms of cytoprotective role of prostaglandins [25], especially *in vitro*, are necessary to gain more understanding of this problem.

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