Prostaglandins E₂ and F_{2α} affect glycogen synthase and phosphorylase in isolated hepatocytes

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Prostaglandin E₂ (PGE₂) and prostaglandin F_{2α} (PGF_{2α}) inactivated glycogen synthase and activated glycogen phosphorylase in rat hepatocytes in a dose- and time-dependent manner. These effects were dependent on the presence of Ca²⁺ in the incubation medium. When glycogen synthase was immunoprecipitated from cells incubated with [³²P]P_i and then treated with PGE₂ or PGF_{2α}, there was increased phosphorylation of the 88 kDa subunit of the enzyme. This phosphorylation affected two CNBr fragments of the glycogen synthase, CB-1 and CB-2, the same fragments that are phosphorylated by different glycogenolytic hormones. No phosphorylation of glycogen synthase by prostaglandins was observed in the absence of Ca²⁺. Thus the effect of PGE₂ and PGF_{2α} on these glycogen-metabolizing enzymes supports a role for regulation by prostaglandins of glucose metabolism in parenchymal liver cells.

INTRODUCTION

Glycogen synthase and glycogen phosphorylase are the key enzymes in the regulation of glycogen metabolism. The activities of both enzymes are controlled through phosphorylation-dephosphorylation reactions. Phosphorylation activates phosphorylase and inactivates glycogen synthase. Glycogen synthase is phosphorylated by the action of glycogenolytic hormones (acting through cyclic AMP- or Ca²⁺-mediated mechanisms) at multiple sites contained in two CNBr fragments. In the rat liver these phosphopeptides show molecular masses of 14 kDa (CB-1) and 28 kDa (CB-2) [1,2].

It has been shown recently that prostaglandins are able to stimulate glycogenolysis in perfused rat liver [3–5] or in parenchymal liver cells [5–7]. Moreover, it has been proposed that prostaglandins may be the mediators of the effects of several agents, such as 4β-phorbol 12β-myristate 13α-acetate and platelet-activating factor [5,8–10] on hepatic glucose metabolism through an intercellular communication mechanism. These two agents are able to stimulate the secretion of prostaglandins from non-parenchymal cells [5,11,12], and in this way they may modulate parenchymal-cell metabolism.

The aim of the present study was to examine the ability of prostaglandins to regulate the glycogen-metabolizing enzymes in isolated hepatocytes. We provide evidence that prostaglandins promote the inactivation and phosphorylation of glycogen synthase and the activation of phosphorylase in a Ca²⁺-dependent manner. Thus our results support the idea that prostaglandins may exert a control on liver glycogen metabolism.

MATERIALS AND METHODS

Isolation and incubation of the cells

Suspensions of isolated parenchymal liver cells were prepared from starved (24 h) male Wistar rats as described previously [13]. Cells were resuspended in a

Krebs bicarbonate buffer (pH 7.4) free of glucose (containing 118.5 mM-NaCl, 4.8 mM-KCl, 2.5 mM-CaCl₂, 25 mM-NaHCO₃, 1.2 mM-KH₂PO₄ and 1.2 mM-MgSO₄), which was equilibrated with O₂/CO₂ (19:1). When stated, Ca²⁺ was omitted from this medium and 1 mM-EGTA was added. Samples (4 × 10⁶–5 × 10⁶ cells/ml) were pre-incubated for 20 min, and then prostaglandins were added at the indicated concentrations. Stock solutions of prostaglandins were made in dimethyl sulphoxide. At the end of the incubation, cells were centrifuged and cell pellets homogenized as described previously [14].

Isolation of ³²P-labelled glycogen synthase

Glycogen synthase was isotopically labelled by pre-incubation of hepatocytes for 45 min with [³²P]P_i (0.1 mCi/ml) in a low-phosphate (0.1 mM) version of the above Krebs bicarbonate buffer (pH 7.4). Cytosolic fractions were prepared from the 10000 g (15 min) supernatants by ultracentrifugation at 100000 g for 60 min at 4 °C. ³²P-labelled glycogen synthase was isolated by immunoprecipitation [15] by using specific antibodies. For CNBr cleavage, immunoprecipitates were treated as described in ref. [14].

The analysis of glycogen synthase subunit or CNBr phosphopeptides was carried out by electrophoresis in polyacrylamide gels in the presence of SDS, with 7%-acrylamide or 6–20%-gradient-acrylamide slab gels as described in ref. [16]. Samples for SDS/polyacrylamide-gel electrophoresis were evaporated to dryness and resuspended in 30 μl of sample buffer, containing 40 mM-Tris (pH 6.5), 5% (w/v) SDS, 0.6 M-2-mercaptoethanol, 20% (w/v) sucrose and 0.0125% (w/v) Bromophenol Blue as a marker. Autoradiograms were obtained, and the amount of radioactivity was quantified by scanning the developed films at 546 nm.

Enzyme assays

Glycogen synthase activity ratio was measured by using the low-glucose 6-phosphate/high-glucose 6-

Abbreviations used: PGE₂, PGF_{2α}, prostaglandin E₂ and F_{2α}.

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phosphate method as described previously [17]. Glycogen phosphorylase activity was measured as in ref. [18]. Protein was determined by the biuret method [19] as in ref. [20].

Materials

[^{32}P] P_i was obtained from Amersham International. Prostaglandins were from Sigma. All other reagents were analytical grade.

RESULTS

Effects of PGE_2 and $\text{PGF}_{2\alpha}$ on the activation state of glycogen synthase and glycogen phosphorylase activity

The incubation of isolated hepatocytes with $25\text{ }\mu\text{M}$ - PGE_2 or $20\text{ }\mu\text{M}$ - $\text{PGF}_{2\alpha}$ for different times caused an activation of glycogen phosphorylase and an inactivation

of glycogen synthase in a time-dependent manner (Fig. 1). The maximal activation of glycogen phosphorylase was achieved for both prostaglandins after 1–2 min of incubation, and the activity was increased more than 2-fold over the control values. The maximal decrease in the glycogen synthase activity ratio was observed in both cases at 5 min (about 25%).

In order to investigate the possible role of Ca^{2+} in the mechanism of action of prostaglandins, the cells were resuspended in a Ca^{2+} -free medium which included 1 mM -EGTA. In the absence of Ca^{2+} neither PGE_2 nor $\text{PGF}_{2\alpha}$ modified the activity of glycogen phosphorylase or altered the activity ratio of glycogen synthase (Fig. 1).

When hepatocytes were treated with different concentrations of PGE_2 and $\text{PGF}_{2\alpha}$, a dose-dependent activation of glycogen phosphorylase was observed (Fig. 2a). The half-maximal effect was obtained with $1\text{ }\mu\text{M}$ - PGE_2 or $0.5\text{ }\mu\text{M}$ - $\text{PGF}_{2\alpha}$. The effect on glycogen synthase was also dependent on the concentration of prostaglandin used; $0.5\text{ }\mu\text{M}$ - PGE_2 or $0.5\text{ }\mu\text{M}$ - $\text{PGF}_{2\alpha}$ was already effective (Fig. 2b).

Fig. 1. Time-dependent effects of PGE_2 and $\text{PGF}_{2\alpha}$ on glycogen synthase and glycogen phosphorylase activities

Hepatocytes incubated in normal ($\blacktriangle$, $\blacksquare$) or Ca^{2+} -depleted medium ($\triangle$, $\square$) were treated with $25\text{ }\mu\text{M}$ - PGE_2 ($\blacksquare$, $\square$) or $20\text{ }\mu\text{M}$ - $\text{PGF}_{2\alpha}$ ($\blacktriangle$, $\triangle$) for different times. Extracts were then prepared, and glycogen phosphorylase activity (a) and glycogen synthase activity ratio (b) were measured as indicated. Results are expressed as means $\pm$ S.E.M. of at least four independent experiments.

Fig. 2. Concentration-dependent effects of PGE_2 and $\text{PGF}_{2\alpha}$ on glycogen synthase and glycogen phosphorylase activities

Cells were incubated with different concentrations of PGE_2 ($\blacksquare$) and $\text{PGF}_{2\alpha}$ ($\blacktriangle$) for 5 min. Then glycogen phosphorylase activity (a) and glycogen synthase activity ratio (b) were measured. Results are expressed as means $\pm$ S.E.M. of at least four independent experiments.

Effects of PGE₂ and PGF_{2α} on the phosphorylation state of glycogen synthase

We next studied whether the inactivation of glycogen synthase by PGE₂ or PGF_{2α} was the result of the phosphorylation of the enzyme. Isolated hepatocytes were preincubated with [³²P]P_i and, after treatment with prostaglandins, glycogen synthase was immunoprecipitated, separated by polyacrylamide-gel electrophoresis and subjected to autoradiography. Treatment of the cells with 25 μM-PGE₂ or 20 μM-PGF_{2α} for 5 min

Fig. 3. Electrophoretic analysis of ³²P-labelled glycogen synthase

³²P-labelled glycogen synthase was immunoprecipitated from [³²P]P_i-preincubated cells and subjected to polyacrylamide-gel electrophoresis before (a) or after (b) CNBr treatment. Lane 1 is an autoradiogram of immunoprecipitated protein from control cells; lane 2, immunoprecipitated protein from cells exposed to 25 μM-PGE₂ for 5 min; lane 3, immunoprecipitated protein from cells exposed to 20 μM-PGF_{2α} for 5 min. Molecular masses (in kDa) of standard proteins are shown on the left of the Figure. The bottom arrow shows the position of the front.

increased by about 2-fold the amount of ³²P bound to the 88 kDa subunit of the enzyme (Fig. 3a, Table 1), suggesting that the inactivation by prostaglandins observed was related to the phosphorylation of glycogen synthase.

In order to determine the effects of both prostaglandins on the CNBr fragments of glycogen synthase, immunoprecipitated ³²P-labelled glycogen synthase was subjected to CNBr cleavage and the resulting phosphopeptides were resolved by electrophoresis in 6–20%-gradient polyacrylamide gels. Both PGE₂ and PGF_{2α} increased the content of [³²P]P_i in two CNBr phosphopeptides, CB-1 and CB-2 (Fig. 3b). Radioactivity associated with CB-2 was increased by PGE₂ or PGF_{2α} about 1.8-fold, whereas radioactivity present in CB-1 was increased 2.2-fold (Table 1) with respect to the control. These results demonstrate that prostaglandins stimulate the phosphorylation of hepatic glycogen synthase at multiple sites.

In agreement with the results presented above, when Ca²⁺ was omitted from the incubation medium and 1 mM-EGTA was included, no effects of either PGE₂ or PGF_{2α} on the phosphorylation state of the glycogen synthase subunit or CNBr fragments were observed (Table 1).

DISCUSSION

We have shown that PGE₂ and PGF_{2α} inactivated glycogen synthase and activated glycogen phosphorylase in rat hepatocytes. The time courses of the effects of prostaglandins on both enzymes were similar to those of the glycogenolytic hormones such as glucagon, adrenaline and vasopressin, or glycogenolytic agents such as vanadate [13,21–24]. Maximal inactivation of glycogen synthase occurred at about 5 min, and activation of phosphorylase reached a peak at about 2 min, indicating a rapid response of the glycogenolytic process. The half-maximal concentration of prostaglandins affecting both enzymes was 1 μM, in agreement with the concentrations of prostaglandins reported to stimulate glucose output in perfused rat livers and hepatocytes [3–7]. This concentration seems rather high for a biological effect. However,

Table 1. Effects of PGE₂ and PGF_{2α} on the phosphorylation state of glycogen synthase

³²P-labelled glycogen synthase from control or prostaglandin-treated cells, incubated in normal or Ca²⁺-depleted medium, was immunoprecipitated. Electrophoresis was performed before or after CNBr treatment. Autoradiograms of gels were scanned, and the peaks were integrated. Results are expressed as a percentage of the values of the peaks from control cells, and are means ± S.E.M. of at least four experiments performed on different days.

Glycogen synthase ³² P-labelled subunit		³² P-CNBr fragments		
		CB-2	CB-1	CB-2/CB-1
Ca ²⁺				
Control	100	100	100	3.1 ± 0.3
PGE ₂	188 ± 12	174 ± 18	224 ± 15	1.9 ± 0.2
PGF _{2α}	190 ± 10	180 ± 15	221 ± 20	1.8 ± 0.2
No Ca ²⁺				
Control	100	100	100	
PGE ₂	105 ± 7	98 ± 10	105 ± 9	
PGF _{2α}	103 ± 5	110 ± 8	102 ± 12	

hepatocytes are able to metabolize prostaglandins rapidly [25–27]. Also, it is possible that a high local intrahepatic concentration of prostaglandins exists, since it appears that prostaglandins secreted from non-parenchymal liver cells may influence parenchymal liver-cell metabolism [7].

The effects of PGE_2 and $\text{PGF}_{2\alpha}$ on glycogen synthase and phosphorylase were dependent on the presence of Ca^{2+} in the incubation medium, suggesting that these effectors act in a manner similar to the Ca^{2+} -dependent hormones. Several studies in perfused rat livers indicate that the actions of prostaglandins on glycogenolysis are closely correlated with Ca^{2+} concentrations [3,4]. Although it has been reported that prostaglandins stimulate phosphoinositide breakdown in different cell systems [28–30], no information is at present available to indicate that prostaglandins exert this effect in liver parenchymal cells. However, Altin & Bygrave [4] suggested that the action of $\text{PGF}_{2\alpha}$ in liver may involve phosphatidylinositol turnover. It could be considered that our finding that in the absence of Ca^{2+} no effects of prostaglandins are seen is inconsistent with the report that in perfused rat liver $\text{PGF}_{2\alpha}$ stimulated glucose output and other responses in both $10\ \mu\text{M}$ - and $1.3\ \text{mM}$ - Ca^{2+} [4]. However, under our conditions the calculated concentration of Ca^{2+} is about $30\ \text{nM}$ [31], whereas the lower concentration used in ref. [4] is $10\ \mu\text{M}$. This difference in free Ca^{2+} concentration is decisive in order to observe Ca^{2+} -mediated effects in liver cells [31,32].

We demonstrated that the inactivation of glycogen synthase by PGE_2 and $\text{PGF}_{2\alpha}$ was correlated with an increase in the phosphorylation state of the subunit of the enzyme. The increase in $[\text{P}^{32}]\text{P}_i$ content occurred in two CNBr fragments, CB-1 and CB-2, indicating that the enzyme was phosphorylated at multiple sites. Although more total phosphate was incorporated in CB-2, on a percentage basis the increase in the $[\text{P}^{32}]\text{P}_i$ content of CB-1 was more pronounced. This is similar to the pattern of phosphorylation observed after treatment of hepatocytes with glycogenolytic agents such as vasopressin, adrenaline and vanadate [1,2,14]. These data prove the covalent nature of the modification of glycogen synthase by prostaglandins. As with glycogen synthase and phosphorylase activities, this effect of PGE_2 and $\text{PGF}_{2\alpha}$ required the presence of Ca^{2+} in the incubation medium. In the absence of Ca^{2+} , the phosphorylation state of glycogen synthase subunit or CNBr fragments was unchanged by PGE_2 or $\text{PGF}_{2\alpha}$. These results suggest that Ca^{2+} -dependent glycogen synthase kinases are involved in the mechanism of action of prostaglandins.

Although PGE_2 and $\text{PGF}_{2\alpha}$ appear to cause the breakdown of glycogen in hepatocytes via a similar mechanism to that of Ca^{2+} -dependent hormones, the physiological meaning of this effect seems to be very different. Prostaglandins are thought to mediate the effects of different glycogenolytic agents. It has been proposed that effectors such as phorbol esters, arachidonic acid, platelet-activating factor or heat-aggregated IgG [5,8–11], activate glycogenolysis in liver parenchymal cells by stimulating the synthesis of prostaglandins such as PGE_2 , $\text{PGF}_{2\alpha}$ or PGD_2 from non-parenchymal cells. The released prostaglandins would then be responsible for the activation of glycogenolysis in the parenchymal cells. Our results, indicating that PGE_2 and $\text{PGF}_{2\alpha}$ are able to modify the activation state of glycogen synthase and phosphorylase, support the above proposal.

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REFERENCES

1. Ariño, J., Mor, A., Bosch, F., Baanante, I. V. & Guinovart, J. J. (1984) *FEBS Lett.* **170**, 310–314
2. Ciudad, C., Camici, M., Ahmad, Z., Wang, Y., DePaoli-Roach, A. A. & Roach, P. J. (1984) *Eur. J. Biochem.* **142**, 511–520
3. Buxton, D. B., Fisher, R. A., Briseno, D. L., Hanahan, D. J. & Olson, M. S. (1987) *Biochem. J.* **243**, 493–498
4. Altin, J. G. & Bygrave, F. L. (1988) *Biochem. J.* **249**, 677–685
5. Casteleijn, E., Kuiper, J., Van Rooij, H. C. J., Kamps, J. A. A. M., Koster, J. F. & Van Berkel, T. J. C. (1988) *Biochem. J.* **250**, 77–80
6. Casteleijn, E., Kuiper, J., Van Rooij, H. C. J., Kamps, J. A. A. M., Koster, J. F. & van Berkel, T. C. J. (1988) *J. Biol. Chem.* **263**, 2699–2703
7. Casteleijn, E., Kuiper, J., Van Rooij, H. C. J., Koster, J. F. & Van Berkel, T. J. C. (1988) *Biochem. J.* **252**, 601–605
8. Altin, J. G., Dieter, P. & Bygrave, F. L. (1987) *Biochem. J.* **245**, 145–150
9. Garcia-Sainz, J. A. & Hernandez-Sotomayor, S. M.-T. (1985) *Biochem. Biophys. Res. Commun.* **132**, 204–209
10. Mendlovic, F., Corvera, S. & Garcia-Sainz, J. A. (1984) *Biochem. Biophys. Res. Commun.* **123**, 507–514
11. Birmelin, M. & Decker, K. (1984) *Eur. J. Biochem.* **142**, 219–225
12. Dieter, P., Shulze-Specking, A. & Decker, K. (1986) *Eur. J. Biochem.* **159**, 451–457
13. Massague, J. & Guinovart, J. J. (1977) *FEBS Lett.* **82**, 317–320
14. Bosch, F., Ariño, J., Gomez-Foix, A. M. & Guinovart, J. J. (1987) *J. Biol. Chem.* **262**, 218–222
15. Ariño, J. & Guinovart, J. J. (1986) *Biochem. Biophys. Res. Commun.* **134**, 113–119
16. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
17. Guinovart, J. J., Salavert, A., Massague, J., Ciudad, C. J., Salsas, E. & Itarte, E. (1979) *FEBS Lett.* **106**, 284–288
18. Van der Werve, G., Hue, L. & Hers, H.-G. (1977) *Biochem. J.* **162**, 135–142
19. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–756
20. Layne, E. (1957) *Methods Enzymol.* **3**, 450–451
21. Hutten, N. J., Brumley, F. T., Assimacopoulos, F. D., Harper, S. C. & Extton, J. H. (1976) *J. Biol. Chem.* **251**, 5200–5208
22. Hems, D. A., Rodrigues, L. M. & Whitton, P. D. (1978) *Biochem. J.* **172**, 311–317
23. Strickland, W. G., Blackmore, P. F. & Extton, J. H. (1980) *Diabetes* **29**, 617–622
24. Massague, J. & Guinovart, J. J. (1978) *Biochim. Biophys. Acta* **543**, 269–272
25. Dawson, W., Jessup, S. J., McDonald-Gibson, W., Ramsell, P. & Shaw, J. E. (1970) *Br. J. Pharmacol.* **39**, 585–598
26. Garrity, M. J., Brass, E. P. & Robertson, R. P. (1983) *Clin. Res.* **32**, 47

27. Okumura, T., Nakayama, R., Sago, T. & Saito, K. (1985) *Biochim. Biophys. Acta* **838**, 197–207
28. Macphee, C. H., Drummond, A. H., Otto, A. M. & De-Asua, L. J. (1984) *J. Cell. Physiol.* **119**, 35–40
29. Mene, P., Dubyak, G. R., Scarpa, A. & Dunn, M. J. (1987) *Biochem. Biophys. Res. Commun.* **142**, 579–586
30. Davis, J. S., Weakland, L. L., Weiland, D. A., Farese, R. V. & West, L. A. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3728–3732
31. Blackmore, P. F., Strickland, G. W., Bocckino, S. B. & Exton, J. H. (1986) *Biochem. J.* **237**, 235–242
32. Renard, D., Poggioli, J., Berthon, B. & Claret, M. (1987) *Biochem. J.* **243**, 391–398

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