Structural specificity for prostaglandin effects on hepatocyte glycogenolysis

Eric P. BRASS*† and Maureen J. GARRITY†

*Departments of Medicine and Pharmacology, Case Western Reserve University, Cleveland, OH 44106, U.S.A., and †Departments of Medicine and Physiology, University of Colorado Health Sciences Center, Denver, CO 80262, U.S.A.

Prostaglandins (PGs) are known to have effects on hepatic glucose metabolism. Some actions of PGs in intact liver systems may not involve PG effects directly at the level of the hepatocyte. To define the ability of structurally distinct prostaglandins to affect hepatocyte metabolism directly, the regulation of glycogenolysis was studied in hepatocytes isolated from male Sprague–Dawley rats. PGF_{2α} and PGB₂ inhibited glucagon-stimulated glycogenolysis in the hepatocyte system. Pinane thromboxane A₂ (PTA₂) and PGD₂ had no effect on glucagon-stimulated glycogenolysis. Consistent with their inhibition of glucagon-stimulated glycogenolysis, PGB₂ and PGF_{2α} inhibited glucagon-stimulated hepatocyte cyclic AMP accumulation. These actions of PGB₂ and PGF_{2α} are identical with those previously reported for PGE₂. Additionally, PGE₂, PGF_{2α} and PGB₂ inhibited glucagon-stimulated adenylate cyclase activity in purified hepatic plasma membranes. In contrast, PGF_{2α}, PGD₂ and PTA₂ were all without effect on basal rates of hepatocyte glycogenolysis or hepatocyte cyclic AMP content. PGE₂ also inhibited glycogenolysis stimulated by the α-adrenergic agonist phenylephrine. Exogenous arachidonic acid was not able to reproduce the effects of PGE₂ or PGF_{2α} on hepatocyte glycogenolysis, consistent with an extra-hepatocyte source of the prostaglandins in the intact liver. Thus PGE₂ and PGF_{2α} act specifically to inhibit glucagon-stimulated adenylate cyclase activity. No prostaglandin tested was found to stimulate glycogenolysis. PGE₂ and PGF_{2α} may represent intra-hepatic modulators of hepatocyte glucose metabolism.

INTRODUCTION

Several laboratories have reported effects of prostaglandins (PGs) on liver glycogenolysis and glucose production [1–9]. However, conflicting conclusions have been reported as to whether a specific PG causes stimulation, inhibition, or has no effect on hepatic glucose release. For example, PGE has been reported to stimulate glucose production in the perfused liver [5–7], whereas other laboratories have reported inhibition of hormone-stimulated glycogenolysis in isolated hepatocytes [4,9]. These observations may not be contradictory, as the perfused liver contains multiple cell types, and indirect effects of an intervention on hepatocyte metabolism are possible. The vasculature remains functional in the perfused liver, and changes in vascular tone result in altered perfusion pressures and flow, which in turn alter hepatocyte metabolism [6,10]. For PGs, which are vasoactive, the differentiation is important, as PGs are not circulating hormones, but act locally. Therefore introduction of high concentrations of PGs into the perfusate of a perfused liver system is non-physiological.

Studies using the perfused liver system have suggested that a variety of hormones, drugs and other substances can alter PG production by the liver [11–13]. This PG production by the liver has been suggested to mediate the effects of platelet-activating factor [12], phorbol esters [11,13] and other substances [6,14] on hepatocyte function. To clarify these potential diverse interactions, it is necessary to define the direct effects of PGs on hepatocyte function. The present studies were undertaken to characterize the effects of several major structurally distinct PGs on basal and agonist-stimulated glycogenolysis in isolated rat hepatocytes. Effects on glycogenolysis were compared with the effects of the PGs on hepatocyte cyclic AMP accumulation and plasma-membrane adenylate cyclase activity.

MATERIALS AND METHODS

Animals and hepatocyte isolations

Male fed Sprague–Dawley rats were used in all studies. Hepatocytes were isolated from rats (310 ± 10 g; *n* = 36) by the collagenase perfusion technique of Berry & Friend [15] as previously detailed [16]. Hepatocytes prepared by this method maintain hormonal responsiveness [4,16] and PG-binding properties [17]. Hepatocytes used in the current studies contained 2.81 ± 0.16 mg of protein/10⁶ cells (*n* = 32), had a wet weight of 14.0 ± 0.8 mg/10⁶ cells (*n* = 26) and were 94 ± 1 % (*n* = 36) viable, based on Trypan Blue exclusion.

Liver plasma membranes were isolated from rats (244 ± 8 g; *n* = 20) by the sucrose-gradient technique of Smigel & Fleischer [18] as previously described [17]. Membranes were frozen at –70 °C until used in the adenylate cyclase assay.

Hepatocyte incubations

Incubations were conducted in 25 ml Erlenmeyer flasks at 37 °C under O₂/CO₂ (19:1) in a shaking water bath. Incubations contained hepatocytes (2.5 × 10⁶ cells/ml for studies of glycogenolysis and 1.0 × 10⁶ cells/ml for studies of cyclic AMP accumulation), 129 mM-NaCl, 5.0 mM-KCl, 2.0 mM-Na₂HPO₄, 1.0 mM-MgSO₄, 4.0 mM-glucose, 10 mM-tris(hydroxymethyl)-aminoethane and 1.1 mM-CaCl₂ at pH 7.4. Total incubation volume was 4.0 ml for studies of glycogenolysis and 2.5 ml for studies of cyclic AMP accumulation. Flasks were preincubated for 10 min, after which additions were made at zero time as indicated in individual experiments.

Rates of glycogenolysis were determined as the change in glucose concentration in the incubation from zero time to 10 min. Samples (300 μl) were removed in duplicate at these time points and added to an equal volume of cold 6 % (v/v) HClO₄. Cyclic

Abbreviations used: PG, prostaglandin; PTA₂, pinane thromboxane A₂.

† To whom correspondence and reprint requests should be addressed (Department of Medicine).

AMP accumulation was determined as the change in cyclic AMP content from zero time to 5 min. Samples (300 μ l) for cyclic AMP measurement were removed at these times and added to 100 μ l of cold 20% (v/v) trichloroacetic acid. These methods have been previously validated [4,19].

Assays

Glucose concentrations were determined by the glucose oxidase method adapted to kit form by Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cyclic AMP concentrations were quantified by the method of Steiner *et al.* [20] adapted to kit form by New England Nuclear Corp. (Boston, MA). Plasma-membrane adenylate cyclase activity was measured by the method of Salomon *et al.* [21], as previously described [17].

Reagents

Glucagon was a gift from Eli Lilly Corp. (Indianapolis, IN, U.S.A.). PGE₂, PGF_{2 α} , PGB₂, PGD₂ and pinane thromboxane A₂ (PTA₂) were obtained from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). Phenylephrine was purchased from Sigma, and was prepared in 10 mM-HCl fresh daily. Arachidonic acid was purchased from Nu-Check Chemicals (Elysian, MN, U.S.A.). Arachidonic acid was dissolved in hexane and stored under argon. On the day of use, the hexane was evaporated and the arachidonic acid dissolved in Na₂CO₃ and neutralized to pH 8.0 with HCl. [³²P]ATP was purchased from New England Nuclear Corp. All other chemicals and reagents were of reagent grade.

Statistics

Values are presented as means $\pm$ S.E.M. for *n* experiments, each conducted with a distinct hepatocyte or plasma-membrane preparation. Statistical significance was determined by Student's paired *t* test, comparing experiments performed simultaneously. When multiple comparisons were made, an ANOVA was performed. *P* < 0.05 was considered statistically significant.

RESULTS

The effects of distinct PGs on glucagon-stimulated glycogenolysis and cyclic AMP accumulation in the hepatocyte system were initially studied (Table 1). Glucagon (0.5 μ M) increased glucose production and cyclic AMP accumulation, and PGF_{2 α} and PGB₂ (each at 1 μ M) inhibited glucagon-stimulated glyco-

Table 1. Effect of PGs on glucagon-stimulated hepatocyte glycogenolysis and cyclic AMP accumulation

Rates of glycogenolysis and cyclic AMP accumulation in the presence of glucagon (0.5 μ M) and PGs as indicated were determined as described in the text. Rates of glycogenolysis are in nmol of glucose/10 min per 2.5×10^6 cells and are corrected for basal rates of glycogenolysis (basal rates of glycogenolysis were 620 ± 50 , *n* = 16). Accumulation of cyclic AMP is presented as change in cyclic AMP content (pmol/10⁶ cells) from 0 to 5 min after glucagon. **P* < 0.05 versus absence of prostaglandin.

PG	Concn.	<i>n</i>	Glycogenolysis	<i>n</i>	Cyclic AMP
None	—	14	330 ± 50	11	5.54 ± 0.62
PGB ₂	1 μ M	4	$24 \pm 110^*$	—	—
PGD ₂	1 μ M	5	285 ± 50	3	5.03 ± 0.96
PGF ₂	1 μ M	6	$120 \pm 10^*$	5	$3.96 \pm 0.58^*$
	0.3 μ M	5	$200 \pm 40^*$	3	4.93 ± 0.51
	0.03 μ M	3	240 ± 20	—	—
PTA ₂	1 μ M	5	300 ± 110	3	7.95 ± 2.58

Table 2. Effect of PGs on basal hepatocyte glycogenolysis and cyclic AMP accumulation

Rates of glycogenolysis and accumulation of cyclic AMP were determined in the presence of the PGs indicated. Rates of glycogenolysis are in nmol of glucose/10 min per 2.5×10^6 cells, and cyclic AMP responses are the change in cyclic AMP content (pmol/10⁶ cells) from 0 to 5 min.

PG	Concn.	<i>n</i>	Glycogenolysis	<i>n</i>	Cyclic AMP
None	—	16	620 ± 50	13	-0.45 ± 0.26
PGB ₂	1 μ M	3	640 ± 30	3	-0.73 ± 1.10
PGD ₂	3 μ M	4	660 ± 90	—	—
	1 μ M	9	610 ± 70	4	-0.12 ± 0.03
PGF ₂	1 μ M	8	710 ± 70	6	0.00 ± 0.20
PTA ₂	1 μ M	5	520 ± 70	5	0.00 ± 0.60

genolysis. Inhibition of glucagon-stimulated glycogenolysis by PGF_{2 α} was concentration-dependent, with 64% inhibition observed at 1 μ M-PGF_{2 α} , 39% inhibition at 0.3 μ M-PGF_{2 α} and 27% (non-statistically significant) inhibition at 0.03 μ M-PGF_{2 α} . PGF_{2 α} also inhibited glucagon-stimulated cyclic AMP accumulation. In contrast, PGD₂ and PTA₂ (a physiologically active stable analogue of thromboxane A₂) had no effect on glucagon-stimulated glycogenolysis or cyclic AMP accumulation.

Similar studies were conducted to define the potential effects of the PGs on basal rates of hepatocyte glycogenolysis and cyclic AMP accumulation (Table 2). PGF_{2 α} , PGB₂, PGD₂ and PTA₂ (1 μ M) all had no effect on basal rates of glycogenolysis, nor did they alter cyclic AMP content over the 0–5 min period after PG addition.

PGD₂ has been previously reported to increase hepatocyte glycogenolysis [13,22], and has been implicated as a mediator of enhanced glycogenolysis in the perfused liver system [7,13]. Therefore, additional studies were undertaken to characterize any potential effect of PGD₂ in the hepatocyte model. Increasing the PGD₂ concentration to 3 μ M did not result in any effect on basal rates of hepatocyte glycogenolysis (Table 2). PGs are rapidly catabolized in the hepatocyte system, limiting expression of their biological activity after single additions [23]. β -Oxidation is responsible for the rapid hepatic catabolism of PGs [24], and, for PGE₂, the addition of non-eicosanoid fatty acids such as octanoate inhibits the breakdown of PGE₂ and potentiates its actions [25]. Based on these observations, the effect of PGD₂ on hepatocyte glycogenolysis was also studied in the presence of 0.8 mM-octanoate (Table 3). Again, concentrations of PGD₂ as high as 3 μ M had no effect on rates of glycogenolysis.

The inhibition of glucagon-stimulated glycogenolysis by PGE₂

Table 3. Effect of PGD₂ on basal glycogenolysis in the presence of octanoate

Rates of glycogenolysis were determined in the presence of 0.8 mM-octanoate and PGD₂ at the concentrations indicated. Rates of glycogenolysis are in nmol of glucose/10 min per 2.5×10^6 cells (*n* = 3).

[PGD ₂] (μ M)	Glycogenolysis
0	770 ± 160
3	610 ± 210
1	660 ± 190
0.3	640 ± 200

Table 4. Effects of PGs on hepatic plasma-membrane adenylate

Adenylate cyclase activity determined in liver plasma membranes. PGs were added in the absence of other hormones/drugs ('basal activity') or in the presence of 0.1 mM-forskolin. Studies with guanosine 5'-[β -imido]triphosphate (p[NH]ppG) were done in the absence of the 100 μ M-GTP usually included in the adenylate cyclase assay. Values are expressed as the percentage of adenylate cyclase activity in the absence of PG. Basal adenylate cyclase activity (absence of PGs and forskolin) was 6.4 ± 0.8 pmol/min per mg of protein ($n = 20$). Forskolin-stimulated adenylate cyclase activity was 106 ± 23 pmol/min per mg of protein ($n = 7$). * $P < 0.05$ versus absence of prostaglandin.

Activity	PG	Concn. ...	1 nM	0.1 μ M	
Basal	PGD ₂		113 ± 9 (3)	123 ± 23 (6)	
	PGF _{2α}		107 ± 8 (3)	97 ± 13 (6)	
	PGE ₂		100 ± 8 (3)	105 ± 1 (3)	
	PGB ₂		72 ± 6 (3)*	81 ± 14 (6)*	
	PTA ₂		142 ± 12 (5)*	143 ± 25 (5)	
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		Concn. ...	1 pM	0.1 nM	10 nM
Forskolin-stimulated	PGD ₂		106 ± 12 (3)	97 ± 1 (4)	100 ± 6 (3)
	PGF _{2α}		76 ± 5 (4)*	100 ± 8 (5)	93 ± 4 (5)
	PGE ₂		77 ± 2 (4)*	85 ± 1 (4)*	96 ± 9 (3)
	PGB ₂		90 ± 6 (4)	83 ± 4 (4)*	78 ± 5 (4)*
	p[NH]ppG		—	72 ± 4 (7)*	—

Table 5. Effects of PGs on phenylephrine-stimulated hepatocyte glycogenolysis

Rates of glycogenolysis in the presence of phenylephrine (12.5 μ M) and PGs (1 mM), as indicated, were determined. Rates of glycogenolysis are in nmol of glucose/10 min per 2.5×10^6 cells and are corrected for basal rates of glycogenolysis (510 ± 70 , $n = 8$). * $P < 0.05$ versus absence of PGs.

PG	n	Rate of glycogenolysis
None	8	160 $\pm$ 10
PGE ₂	3	80 $\pm$ 30*
PGD ₂	3	180 $\pm$ 90
PGF _{2α}	4	110 $\pm$ 60
PTA ₂	4	130 $\pm$ 10

Table 6. Effect of arachidonic acid on hepatocyte glucagon-stimulated glycogenolysis

Rates of glycogenolysis were determined in the presence of glucagon (0.5 μ M) and other additions as indicated. When added, arachidonic acid was added at 8.8 μ M and ibuprofen at 8 μ M. Rates of glycogenolysis are in nmol of glucose/10 min per 2.5×10^6 cells after subtracting the basal rate of glycogenolysis (140 ± 50).

Condition	n	Glycogenolysis
Glucagon	7	360 $\pm$ 100
Glucagon + arachidonic acid	7	250 $\pm$ 90
Glucagon + arachidonic acid + ibuprofen	3	310 $\pm$ 110

is associated with inhibition of forskolin- or glucagon-stimulated adenylate cyclase activity, which can be demonstrated by using purified liver plasma membranes [26]. PGD₂, PGF_{2 α} and PGB₂ were all without effect on basal adenylate cyclase activity in liver plasma membranes (Table 4). PTA₂ (1 nM) stimulated adenylate cyclase activity by 42 % (in contrast with the 16-fold stimulation by forskolin). Consistent with their effects on intact hepatocytes, PGE₂, PGF_{2 α} and PGB₂ inhibited forskolin-stimulated adenylate cyclase activity, whereas PGD₂ had no effect (Table 4).

Hormone stimulation of hepatocyte glycogenolysis can occur independently of changes in cyclic AMP content through an increase in the cytosolic Ca²⁺ concentration [27]. Phenylephrine, an α -adrenergic agonist, stimulates glycogenolysis through this signal-transduction system [27]. PGE₂ inhibited phenylephrine-stimulated glycogenolysis in the hepatocyte system by 50 %, whereas PGF_{2 α} , PGD₂ and PTA₂ had no effect (Table 5).

To clarify further the role of the hepatocyte with respect to PG-related responses in the perfused liver, the potential for arachidonic acid to mimic the action of PGs was studied in the hepatocyte system. Arachidonic acid (8.8 μ M) had no effect on glucagon-stimulated hepatocyte glycogenolysis (Table 6). Incubations done in the presence of arachidonic acid (8.8 μ M) and ibuprofen (8 μ M) demonstrated rates of glucagon-stimulated glycogenolysis that were not different from incubations with either no additions (control) or arachidonic acid alone.

DISCUSSION

PGs modulate glucose and lipid metabolism in a variety of liver systems *in vitro* [1–9,13,14,19,22]. Apparently contradictory conclusions have been made as to PG effects on glucose metabolism, based on observations using isolated hepatocytes and perfused liver systems. This may be in part due to the presence of non-hepatocyte cell types in the perfused liver which can respond to PGs in the perfusate, and thus cause secondary metabolic effects. Using the isolated rat hepatocyte model, the current studies demonstrate that PGE₂, PGF_{2 α} and PGB₂ inhibit hepatocyte glucagon-stimulated glycogenolysis. No PG evaluated stimulated basal rates of hepatocyte glycogenolysis.

Inhibition of glucagon-stimulated glycogenolysis by PGE₂ and PGF_{2 α} is consistent with observations by several laboratories [2–4,9]. PGE₂ [22] has been reported to stimulate basal rates of hepatocyte glycogenolysis, but the magnitude of the stimulation was small compared with other glycogenolytic hormones. Further, both PGE₂ and PGF_{2 α} also inhibit glucagon-stimulated hepatocyte cyclic AMP accumulation (Table 2; refs. [2,19,28]), providing support for an action to antagonize glucagon's effects. The action of PGE₂ and PGF_{2 α} on hepatocyte glucose metabolism and cyclic AMP accumulation is abolished by pre-treatment with pertussis toxin, consistent with coupling of the PG receptors to inhibition of cyclic AMP formation [28,29]. PGB₂, although not a naturally occurring PG, also significantly inhibited glucagon-stimulated glycogenolysis. Although the relevance of this observation is at present unclear, PGB₂ has actions similar to PGE₂ in other physiological systems [30]. PGD₂ and PTA₂ had no effect on glucagon-stimulated glycogenolysis. Thus the effects of PGE₂, PGF_{2 α} and PGB₂ were not non-specific actions of PG-like lipids. Additionally, only PGE₂ inhibited hepatocyte glycogenolysis stimulated by phenylephrine, an agent that stimulates Ca²⁺ mobilization (Table 5; ref. [4]). This structural specificity for prostaglandin inhibition of α -adrenergic stimulated glycogenolysis is the same as that observed by Okumura and colleagues [29]. The activities of PGE₂ and PGF_{2 α} thus are as modulators of hormone-stimulated glycogenolysis.

PG effects on glucagon-stimulated glycogenolysis are consistent with inhibition of hormone-stimulated adenylate cyclase

activity, and this effect has been previously directly demonstrated for PGE₂ [26]. In the present studies PGF_{2α} and PGB₂ also inhibited forskolin-stimulated adenylate cyclase activity in plasma membranes, whereas PGD₂ was without effect. This structural specificity parallels the actions of these PGs in the intact hepatocyte, and further supports the conclusions that PGE₂ and PGF_{2α}, but not PGD₂, directly influence hepatocyte function. The maximal inhibition of forskolin-stimulated adenylate cyclase caused by PGE₂, PGF_{2α} or PGB₂ was similar to that induced by guanosine 5'-[βγ-imido]triphosphate (Table 4). This suggests that N_i (the guanine nucleotide-binding protein responsible for inhibition of adenylate cyclase) may limit maximal adenylate cyclase inhibition, and is consistent with the incomplete inhibition of glucagon-stimulated glycogenolysis and cyclic AMP accumulation observed in the intact hepatocytes.

No stimulation of hepatocyte basal rates of glycogenolysis or cyclic AMP content were observed with PGF_{2α}, PGB₂, PGD₂, or PTA₂. Previous studies have reported a stimulation of glycogenolysis by PGD₂ in isolated hepatocytes [13,31]. The basis for these discrepant observations is unknown. Attempts to potentiate the action of PGD₂ by inhibiting its catabolism with octanoate also failed to demonstrate any stimulation of glycogenolysis by the eicosanoid. An animal-strain difference in PG action may exist, as all reported findings of a glycogenolytic action of PGD₂ utilized Wistar rats [13,31,32], whereas the present studies were performed with Sprague-Dawley rats.

An important contribution of the work with the perfused liver model has been the demonstration that various hormones, drugs and other components induce hepatic PG synthesis [13,14,31]. Studies using purified preparations of hepatocytes, Kupffer cells and hepatic endothelial cells demonstrate that hepatocytes have a very limited capacity for prostaglandin biosynthesis [33]. Functional studies also support the conclusion that endogenous PG production by hepatocytes is quantitatively insignificant. Inhibition of any endogenous PG synthesis with non-steroidal anti-inflammatory drugs has no effect on rates of hepatocyte glycogenolysis [34]. Similarly, exogenous arachidonic acid could not reproduce the action of exogenous PGs in the hepatocyte system (Table 6). Additionally, studies with a stable-isotope-labelled prostanoid showed no change in label specific radioactivity after addition to hepatocyte incubations [35], demonstrating directly that no endogenous prostanoid (or metabolites) was present in the hepatocyte incubation.

Eicosanoids play a role in the integrated regulation of hepatic metabolism. In the intact organ, several cell types contribute to the generation of PGs and the response to PGs that ultimately results in altered hepatocyte metabolism. The present studies provide evidence that PGE₂ and PGF_{2α} act directly on the hepatocyte to antagonize glucagon action and glucagon-stimulated glycogenolysis, whereas no PG tested stimulated glycogen breakdown. Further studies are needed to elucidate the importance of these observations in the physiological regulation of liver glucose and lipid metabolism.

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