Inhibition of glucagon-stimulated hepatic glycogenolysis by E-series prostaglandins

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The effect of E-series prostaglandins (PGE) on hepatic glucose metabolism is controversial. This study uses isolated rat hepatocytes and exogenously added PGE analogs or frequent native PGE additions (to compensate for hepatic PGE degradation) to define PGE's effect on hepatic glycogenolysis. 16,16-Dimethyl PGE_2, 15(*S*),15-methyl PGE_2, PGE_1 and PGE_2 all inhibit glucagon-stimulated glycogenolysis. It is concluded that E-series prostaglandins can act directly on the liver to inhibit glycogenolysis.

PGE *Prostaglandin* *Glucagon* *Glycogenolysis* *Hepatocyte* *PGE analog*

1. INTRODUCTION

Prostaglandins are recognized to play a regulatory role in a variety of tissues, but their action on hepatic glucose metabolism remains controversial. In vivo studies using prostaglandin infusions or prostaglandin synthesis inhibitors have generally supported a hyperglycemic effect of E-series prostaglandins (PGE) [1–4], resulting from increased hepatic glucose output [3]. In contrast, in vitro studies demonstrate no effect [5–7] or inhibition [8] by PGE of hepatic glucose production. In part, these discrepancies can be understood by recognition that in vivo PGE can alter circulating hormone levels [9–11], and in vitro is rapidly metabolized by liver [12–14].

Recently, authors in [15,16] reported that PGE can inhibit glucagon-stimulated hepatocyte cAMP production. Also, those in [17] demonstrated PGE-induced desensitization of hepatic adenylate cyclase to glucagon stimulation. Based on these observations, the present study was designed to define the effect of PGE on glucagon-stimulated glycogenolysis. Studies were performed in suspended, isolated hepatocytes to avoid possible secondary influences of PGE seen in vivo. Additionally, to compensate for the rapid metabolism of PGE by hepatocytes, synthetic PGE analogs as well as multiple additions of natural PGEs were used. The study demonstrates that PGE inhibits glucagon-stimulated glycogenolysis, consistent with its reported effects on cAMP accumulation.

2. MATERIALS AND METHODS

2.1. Hepatocyte isolation

Hepatocytes were isolated using a modification of the collagenase perfusion technique in [18]. All experiments were initiated at 7:30 a.m. Male, fed, Sprague-Dawley rats (270–320 g) were anesthetized with pentobarbital (50–100 mg/kg intraperitoneally). The liver was perfused through the portal vein with bicarbonate buffer [116 mM NaCl, 6 mM KCl, 0.6 mM $MgSO_4$, 0.74 mM KH_2PO_4, 12 mM $NaHCO_3$, 15 mM glucose (pH 7.1–7.4), 37°C, equilibrated with 95% O_2/5% CO_2] for 10 min while the liver was isolated. The liver was then placed in a recirculating perfusion chamber, and collagenase (40 mg/100 ml) and $CaCl_2$ (1 mM final concentration) were added to the perfusion medium. This perfusion was continued for 15 min, after which the liver cells were carefully dispersed in fresh perfusion buffer. Cells were filtered and washed 3 times at 65 × *g* in a wash buffer [128.5 mM

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NaCl, 5.2 mM KCl, 0.9 mM Mg SO_4, 0.12 mM $CaCl_2$, 3 mM Na_2HPO_4, 5.0 mM glucose (pH 7.35)]. Cells were finally resuspended in incubation buffer [wash buffer plus 10 mM Tris, 1 mM $CaCl_2$ (pH 7.4)]. Hepatocyte quality was determined by trypan-blue exclusion and glucagon-stimulated glucose output. All preparations were at least 85% viable (91 ± 3%, mean ± SD, $n = 13$) on the basis of trypan-blue exclusion, and demonstrated increased glucose output over basal of at least 40% in the presence of 5×10^{-7} M glucagon (see section 3).

2.2. Incubations

All incubations were conducted at 37°C in a shaking incubator bath continuously gassed with 95% O_2/5% CO_2. Cells ($2.5–5.0 \times 10^6$ ml) were suspended in incubation buffer and pre-incubated at 37°C for 30 min. Glucagon or prostaglandins were added at time zero or as indicated. Incubation aliquots were placed on ice and reactions terminated by rapid centrifugation. Dilvent used to dissolve prostaglandins contained trace amounts of ethanol. Addition of diluent alone to hepatocyte incubations caused no change in any parameters measured.

2.3. Glycogenolysis

Glycogenolysis was estimated by the rate of glucose appearance in the incubation as no gluconeogenic substrates were added [19]. Glucose was measured using a glucose oxidase method [20]. Incubation glucose content was determined at 0, 10, 20 and 30 min of incubation, and the data expressed as μg glucose per 10^6 cells. The rate of glycogenolysis was concluded as the slope of a linear regression through these points and was proportional to cell concentrations over the range $1–5 \times 10^6$ cells/ml.

2.4. Reagents

All chemicals used were of reagent grade. Collagenase (type II) was obtained from Worthington Diagnostic Systems, Freehold, NJ. Glucagon was obtained from Eli Lilly and stored at −20°C in 10 mM NaOH. Prostaglandins were the gift of Dr J. Pike, Upjohn Pharmaceuticals, Kalamazoo, MI.

2.5. Statistics

Data were analyzed on a paired basis. Glucagon-stimulated glycogenolysis was compared to simultaneously incubated cells under basal conditions. Similarly, incubations including prostaglandins were compared to incubations, run simultaneously, excluding prostaglandins. 'N' refers to the number of separate incubations each run on separate hepatocyte preparations. Statistical significance was determined using Student's t-test (paired form) with a single-tailed $p < 0.05$ considered significant.

3. RESULTS AND DISCUSSION

Basal and glucagon stimulated glycogenolysis were linear for the 30 min observation period (fig.1). Glucagon (5×10^{-7} M) resulted in an average 175 ± 76% stimulation over basal glucose output (basal 2.19 ± 0.41 μg/10^6 cells per min, 5×10^{-7} M glucagon 4.48 ± 0.50 μg/10^6 cells per min, mean ± SE, $n = 9$). Addition of the synthetic PGE analog, 16,16-dimethyl PGE_2 inhibited glucagon-stimulated glycogenolysis (fig.1) in a dose-dependent fashion (fig.2). As fig.2 indicates, 16,16-

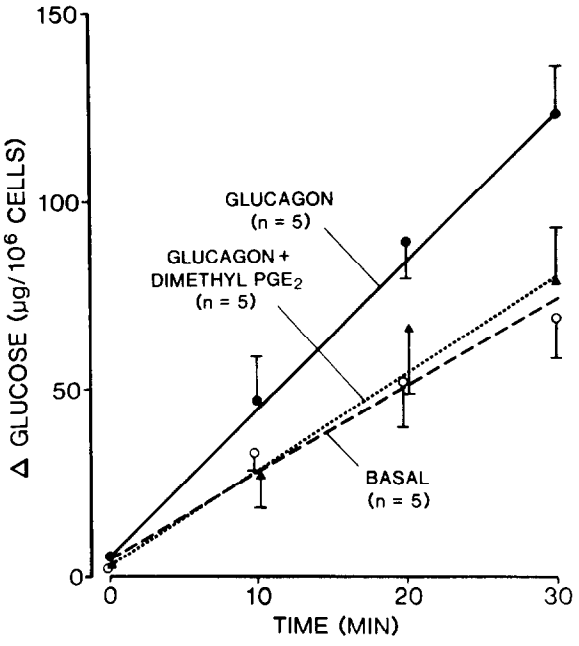

Fig.1. Hepatic glycogenolysis. Incubations as per text. Data are the mean ± SE of 5 incubations; 2.5×10^6 hepatocytes/ml. Lines plotted represent least-square regressions. (○) Cells alone, (●) 5×10^{-7} M glucagon, (▲) 5×10^{-7} M glucagon plus 8×10^{-7} M 16,16-dimethyl PGE_2.

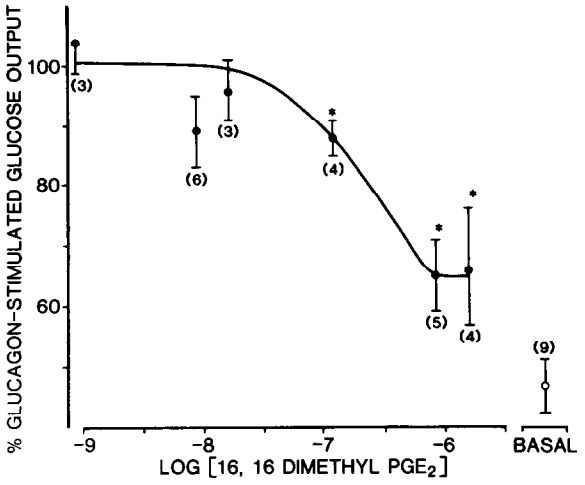

Fig.2. Inhibition of glucagon-stimulated glycogenolysis by 16,16-dimethyl PGE_2. Incubations as per text containing 2.5×10^6 hepatocytes/ml. Data are presented as mean ± SE. Values are expressed as percent of glycogenolysis in the presence of 5×10^{-7} M glucagon alone (mean 100% value = 4.48 ± 0.50, $n = 9$). Concentrations of 16,16-dimethyl PGE_2 indicated were added in addition to 5×10^{-7} M glucagon at time zero. Basal value represents glucose output in the absence of glucagon and without 16,16-dimethyl PGE_2 expressed as percentage of glucagon (5×10^{-7} M) stimulated glucose production (mean basal glucose output = 2.19 ± 0.41 μg glucose/10^6 cells per min, $n = 9$).

Table 1

Effect of PGE on glucagon-stimulated hepatocyte glycogenolysis

Conditions	Glucose production (μg/10^6 cells per min)
Set I	
Basal ($n = 5$)	2.25 ± 1.23
5×10^{-7} M glucagon	4.12 ± 1.51
5×10^{-7} M glucagon + 1.7×10^{-6} M 16,16-dimethyl PGE_2	2.74 ± 1.35[a]
Set II	
Basal ($n = 3$)	2.20 ± 1.97
10^{-7} M glucagon	3.77 ± 2.25
10^{-7} M glucagon + 10^{-6} M 15(*S*),15-methyl PGE_2	2.79 ± 1.64[a]
Set III	
Basal ($n = 5$)	2.46 ± 1.92
10^{-7} M glucagon	4.63 ± 1.40
10^{-7} M glucagon + PGE_1	3.16 ± 0.96[a]
10^{-7} M glucagon + PGE_2	2.66 ± 1.42[a]

Incubations as per text. Additions made at time zero except PGE_1 and PGE_2 which were added at 0, 10 and 20 min. [a]$p < 0.05$ as compared to addition of glucagon alone. Values, mean ± SD

dimethyl PGE_2 inhibited approx. two-thirds of the glucagon-stimulated glucose output. Addition of 1.7×10^{-6} M 16,16-dimethyl PGE_2 alone did not affect basal glucose output (basal 1.93 ± 0.79 μg glucose/10^6 cells per min; basal + 16,16-dimethyl PGE_2 1.97 ± 0.73 μg glucose/10^6 cells per min; $n = 6$; mean ± SD, $p > 0.05$). Similarly, 10^{-6} M 15(*S*),15-methyl PGE_2 inhibited glucagon-stimulated glycogenolysis to a level of 70 ± 7% of 10^{-7} M glucagon alone (table 1).

PGE_1 and PGE_2 are metabolized by hepatocytes [12–14] with half-lives of approx. 5 min at 10^{-6} M [13]. Based on these data, the effect of addition of 3×10^{-9} mol PGE_1 or PGE_2 every 10 min to a 3 ml hepatocyte incubation was studied. This protocol was adopted to maintain a concentration greater than 10^{-7} M over the entire incubation period. Added in this manner, PGE_1 and PGE_2 inhibited glucagon-stimulated glycogenolysis (table 1).

These studies demonstrate that PGE can act directly on the hepatocyte to inhibit glucagon-stimulated glycogenolysis, consistent with its known effect on glucagon-stimulated cAMP production [15,16] and adenylate cyclase activity [17]. This suggests that the hyperglycemic effect of PGE observed in vivo [1–4] may be secondary to PGE-induced hormonal changes, such as inhibition of insulin secretion [9] or stimulation of glucagon secretion [21]. Further the lack of reproducible in vitro hepatic PGE effects in the past may be explained by failure to compensate for hepatic PGE metabolism [12–14] as well as failure to examine stimulated states of glycogenolysis specifically.

In liver membrane systems, PGE stimulates adenylate cyclase [22,23], however in the intact hepatocyte, PGE appears to act as an adenylate cyclase antagonist. Similar situations have been described in the renal medulla where PGE stimulates adanylate cyclase but inhibits the ability of ADH to stimulate cyclase [24], and in corpora lutea where $PGF_{2\alpha}$ inhibits leutenizing hormone-stimulated cAMP accumulation [25].

In summary, PGE can act on hepatocytes to inhibit glucagon-stimulated glycogenolysis consistent with PGE's effect on glucagon-stimulated hepatic adenylate cyclase. This suggests that prostaglandins may play a physiologic role in modulating the action of hormones on the liver.

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