Effect of E-Series Prostaglandins on Cyclic AMP-dependent and -independent Hormone-stimulated Glycogenolysis in Hepatocytes

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SUMMARY

The effect of E-series prostaglandins (PGE) on hormone-stimulated glycogenolysis was studied in isolated rat hepatocytes. As previously reported, the physiologically active analogue 16,16-dimethyl-PGE_2 inhibited glucagon-stimulated glycogenolysis. This effect could be reproduced by repetitive addition of PGE_2 to compensate for PGE_2 catabolism. In contrast, glycogenolysis stimulated by N^6,$O^{2'}$-dibutyryladenosine-3',5'-cyclic monophosphate (dibutyryl-cAMP) was unaffected by either PGE_2 or 16,16-dimethyl-PGE_2 (rate of glycogenolysis with 0.34 μM dibutyryl-cAMP plus 1.7 μM 16,16-dimethyl-PGE_2 = 99 ± 6% of rate with 0.34 μM dibutyryl-cAMP alone; mean ± SEM, N = 5). Similarly, glycogenolysis stimulated by 8-bromoadenosine-3',5'-cyclic monophosphate was not inhibited by PGE_2 or 16,16-dimethyl-PGE_2.

Epinephrine-stimulated glycogenolysis was inhibited by 16,16-dimethyl-PGE_2 in a dose-dependent manner. PGE inhibited the cAMP-independent stimulation of glycogenolysis resulting from phenylephrine or angiotensin II exposure (rate of glycogenolysis with 8 μM phenylephrine + 1.7 μM 16,16-dimethyl-PGE_2 = 65 ± 10% of rate with 8 μM phenylephrine alone, N = 4, $P < 0.05$; 4.9 μM angiotensin II + 1.7 μM 16,16-dimethyl-PGE_2 = 75 ± 7% of rate with 4.9 μM angiotensin II alone, N = 4, $P < 0.05$). Glycogenolysis stimulated by the calcium ionophore A23187 was also inhibited by PGE (rate of glycogenolysis with 0.55 μg/ml A23187 + 1.7 μM 16,16-dimethyl-PGE_2 = 83 ± 5% of rate with 0.55 μg/ml A23187 alone, N = 7, $P < 0.05$).

Thus, PGE can inhibit both cAMP-dependent and cAMP-independent hormone-stimulated glycogenolysis. The results suggest two sites of inhibition, one consistent with inhibition of adenylate cyclase stimulation and a second inhibiting calcium-mediated stimulation at an undefined site. DIABETES 1985; 34:291–94.

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Studies of the effects of E-series prostaglandins (PGE) on hepatic glucose metabolism have not yielded consistent results.[1-5] We have recently reported that PGE inhibits glucagon-stimulated glycogenolysis in isolated rat hepatocytes.[6] Because liver rapidly degrades PGE_1 and PGE_2,[7,8] the demonstration of the PGE effect was dependent on using synthetic, biologically active PGE analogues, or repetitive PGE_1 or PGE_2 additions to compensate for catabolism. PGE inhibition of glucagon-stimulated glycogenolysis is consistent with its inhibition of glucagon-stimulated hepatic cAMP accumulation[9,10] and the ability of PGE to desensitize hepatic adenylate cyclase to glucagon stimulation.[11]

The present study was designed to further define the effect of PGE on hormone-stimulated glycogenolysis. To determine if adenylate cyclase represented a unique site of action of PGE, the effect of PGE on glycogenolysis stimulated by hormones mediated by cAMP-dependent and cAMP-independent mechanisms was studied. The data demonstrate that PGE inhibits cAMP-dependent glycogenolysis at a level consistent with adenylate cyclase and inhibits cAMP-independent glycogenolysis at a yet undefined site.

MATERIALS AND METHODS

Hepatocyte isolation and incubations. Hepatocytes were isolated using a modification of the collagenase perfusion technique of Berry and Friend[12] as previously described.[6] Adult, male, fed Sprague-Dawley rats (300 ± 7 g, mean ± SEM, N = 35) were used in all studies. Hepatocyte preparations used in this study were 92 ± 1% viable on the basis of trypan blue exclusion (mean ± SEM, N = 35). The hepatocytes averaged 13.2 ± 1.6 mg wet wt/10^6 cells (mean ± SEM) based on assessment of seven consecutive preparations used in these studies.

Incubations were conducted at 37°C in a shaking incubator bath under an atmosphere of 95% O_2/5% CO_2. Hepatocytes (2.5–5.0 × 10^6 cells/ml) were suspended in incubation buffer (128.5 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 1.12 mM $CaCl_2$, 3.0 mM Na_2HPO_4, 5.0 mM glucose, and 10 mM Tris (hydroxymethyl) aminoethane, pH 7.4) and preincubated for 30 min at 37°C. Hormone, drug, or prostaglandins were added at time zero and as indicated. At times indicated, incubation aliquots were placed into chilled microcentrifuge tubes and the reaction terminated by rapid centrifugation. Samples were stored at −20°C until assayed for glucose.

Glycogenolysis. Glycogenolysis was measured as the rate of glucose production in the incubation in the absence of substrates for gluconeogenesis.[13] Glucose was measured using a glucose-oxidase method.[14] Glucose concentrations were determined from incubation aliquots obtained after 0, 10, 20, and 30 min of incubation and expressed as μg glucose/10^6 cells. The rate of glycogenolysis was calculated as the slope of a linear least-squares regression through these time points. Rates of glycogenolysis were proportional to cell concentration over the range 1–5 × 10^6 cells/ml.

Reagents. All chemicals used were of reagent grade. Collagenase (type II) was obtained from Worthington Diagnostic Systems, Inc., Freehold, New Jersey. (−)-Epinephrine, phenylephrine, angiotensin II, N^6,$O^{2'}$-dibutyryladenosine-3',5'-cyclic monophosphate (dibutyryl-cAMP), 8-bromo-adenosine-3',5'-cyclic monophosphate (8-bromo-cAMP) and A23187 were obtained from Sigma Chemicals, St. Louis, Missouri. Solutions were prepared daily using 10 mM HCl (for epinephrine and phenylephrine) or incubation buffer (for angiotensin II, 8-bromo-cAMP, and dibutyryl-cAMP) as solvents. A23187 was dissolved in ethanol and stored at −20°C. Glucagon was a gift of Eli Lilly and Company and stored at −20°C in 10 mM NaOH. Prostaglandins were the gift of Dr. J. Pike, Upjohn Pharmaceuticals, Kalamazoo, Michigan, and were dissolved in ethanol. Addition of drugs in HCl resulted in an HCl concentration of 24 μM in the incubation. Incubation ethanol concentrations after addition of prostaglandins was 0.006%.

Statistics. As an additional verification of hepatocyte viability, only incubation sets in which the agonist increased glucose output by at least 20% over basal glucose output were used for subsequent analysis. For each study, sets of incubations were conducted for basal, hormone-stimulated, and hormone-plus-prostaglandin rates of glycogenolysis. Paired comparisons were then made within each incubation set. "N" refers to the number of incubation sets each run on separate hepatocyte preparations. Data were analyzed on a paired basis with statistical significance determined using Student's *t*-test, with a single-tailed $P < 0.05$ considered significant.

RESULTS

As previously reported,[6] glucagon-stimulated glycogenolysis was inhibited by the physiologically active PGE_2 analogue 16,16-dimethyl-PGE_2, as well as by additions of PGE_2 after 0, 10, and 20 min of incubation designed to compensate for prostaglandin catabolism (Table 1). 16,16-Dimethyl-PGE_2 has no affect on basal rates of glycogenolysis.[6] To localize the level of PGE inhibition, the effect of 16,16-dimethyl-PGE_2 on dibutyryl-cAMP-stimulated glycogenolysis was examined. Both 6.8 μM and 0.34 μM dibutyryl-cAMP resulted in significant stimulation of glycogenolysis, but this stimulation was unaffected by 16,16-dimethyl-PGE_2 (Table 1). Similarly, PGE_2 added at 10^{-6} M at 0, 10, and 20 min had no effect on dibutyryl-cAMP-stimulated glycogenolysis (Table 1). Addition of butyric acid did not result in significant nonspecific stimulation of glycogenolysis (basal = 1.84 ± 0.33 μg glucose/10^6 cells/min, 360 μM butyric acid = 2.13 ± 0.23 μg glucose/10^6 cells/min; N = 4, mean ± SEM, $P > 0.05$). The use of 8-bromo-cAMP, a cAMP analogue with action similar to that of dibutyryl-cAMP, also stimulated glycogenolysis and was not inhibited by 16,16-dimethyl-PGE_2 or repetitive additions of PGE_2 (Table 1).

16,16-Dimethyl-PGE_2 inhibited epinephrine-stimulated glycogenolysis in a dose-dependent manner (Figure 1) similar to that previously reported for glucagon-stimulated glycogenolysis.[16] Addition of the ethanol-diluent used for the PGE had no effect on epinephrine-stimulated glycogenolysis (10^{-5} M epinephrine = 3.40 ± 0.4 μg glucose/10^6 cells/min, 10^{-5} M epinephrine plus 0.006% ethanol final concentration = 3.25 ± 0.2 μg glucose/10^6 cells/min, basal =

TABLE 1
Effect of PGE on cAMP-dependent stimulation of glycogenolysis

Conditions	Rate of glycogenolysis (μg/10^6 cells/min)
Group 1 (N = 4)	
Basal	2.38 ± 0.63
5 × 10^{-7} M Glucagon	4.39 ± 0.82
5 × 10^{-7} M Glucagon + 1.7 × 10^{-6} M 16,16-dimethyl-PGE_2	2.96 ± 0.80*
Group 2 (N = 5)	
Basal	2.46 ± 0.86
5 × 10^{-7} M Glucagon	4.63 ± 0.63
5 × 10^{-7} M Glucagon + PGE_2	2.66 ± 0.64*
Group 3 (N = 5)	
Basal	1.63 ± 0.33
6.8 × 10^{-6} M Dibutyryl-cAMP	3.79 ± 0.34
6.8 × 10^{-6} M Dibutyryl-cAMP + 1.7 × 10^{-6} M 16,16-dimethyl-PGE_2	3.59 ± 0.41
Group 4 (N = 5)	
Basal	1.34 ± 0.44
3.4 × 10^{-7} M Dibutyryl-cAMP	2.86 ± 0.67
3.4 × 10^{-7} M Dibutyryl-cAMP + 1.7 × 10^{-6} M 16,16-dimethyl PGE_2	2.75 ± 0.61
Group 5 (N = 5)	
Basal	1.79 ± 0.42
3.4 × 10^{-7} M Dibutyryl-cAMP	3.23 ± 0.84
3.4 × 10^{-7} M Dibutyryl-cAMP + PGE_2	2.83 ± 0.41
Group 6 (N = 4)	
Basal	1.90 ± 0.33
7.8 × 10^{-6} M 8-Bromo-cAMP	3.83 ± 0.07
7.8 × 10^{-6} M 8-Bromo-cAMP + 1.7 × 10^{-6} M 16,16-dimethyl-PGE_2	3.71 ± 0.11
7.8 × 10^{-6} M 8-Bromo-cAMP + PGE_2	3.42 ± 0.42

Incubations were conducted as detailed in the text. Additions were made at time zero except for PGE_2, which was added at 10^{-6} M at 0, 10, and 20 min. Values are mean ± SEM, *$P < 0.05$, agonist versus agonist + PGE.

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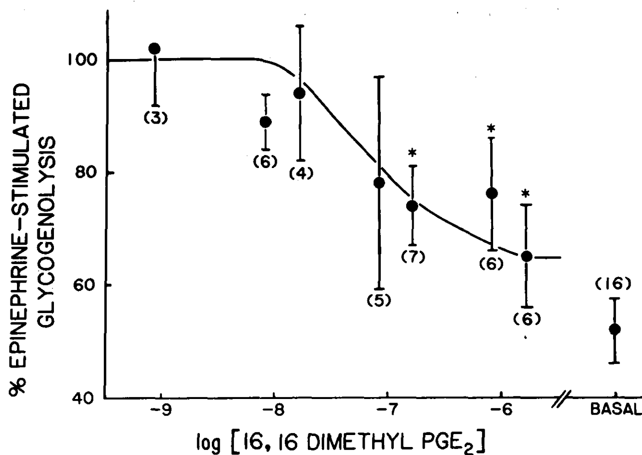

FIGURE 1. Effect of 16,16-dimethyl-PGE_2 on epinephrine-stimulated glycogenolysis. Incubations were conducted as detailed in the text. Data are normalized to stimulated glycogenolysis (epinephrine alone) equals 100% for each set of incubations. Epinephrine concentration equals 10^{-5} M. The line drawn represents an artist's approximation of the curve to fit to the data. Values are mean ± SEM. *$P < 0.05$. Mean 100% rate = 3.94 ± 0.36 μg glucose/10^6 cells/min, N = 16. Numbers in parentheses = N.

1.84 ± 0.5 μg glucose/10^6 cells/min; mean ± SEM, N = 4). In adult, male rats, epinephrine-stimulated glycogenolysis results primarily from alpha-adrenergic stimulation[15,16] and is cAMP independent.[15-17] This suggests that PGE may have an effect independent of adenylate cyclase- and cAMP-mediated processes. To clarify this issue, the effect of 16,16-dimethyl-PGE_2 on phenylephrine, an alpha-adrenergic agonist, and on angiotensin II-stimulated glycogenolysis was determined. Table 2 demonstrates that 16,16-dimethyl-PGE_2 inhibited glycogenolysis stimulated by both these agonists. This effect was confirmed by inhibition of phenylephrine-stimulated glycogenolysis by PGE_2 when added at 0, 10, and 20 min of incubation (Table 2). Phenylephrine and angiotensin II stimulate glycogenolysis by cAMP-independent mechanisms involving changes in cytoplasmic calcium concentration.[18] The calcium ionophore A23187 was used to stimulate glycogenolysis in a manner similar to phenylephrine and angiotensin II, while bypassing the specific hormone-hepatocyte interaction.[19] Addition of 16,16-dimethyl-PGE_2 or repetitive additions of PGE_2 resulted in a small, but significant, inhibition of A23187-stimulated glycogenolysis (Table 2).

DISCUSSION

These results demonstrate that PGE can act directly on hepatocytes to inhibit hormone-stimulated glycogenolysis. This demonstration of the inhibitory effect of PGE in liver required: (1) the use of isolated hepatocytes to avoid indirect or secondary effects possible in vivo, (2) the study of stimulated rates of glycogenolysis, and (3) the addition of PGE in a manner that circumvented the rapid degradation of PGE_1 and PGE_2 by liver.[7,8]

The inhibition of glucagon-stimulated, but not dibutyryl-cAMP-stimulated or 8-bromo-cAMP-stimulated, glycogenolysis observed in the present studies is consistent with inhibition of stimulated adenylate cyclase activity. This would also agree with PGEs' reported inhibition of glucagon-stimulated cAMP accumulation in hepatocytes[9,10] and the observation that PGE exposure results in a desensitization of liver adenylate cyclase to subsequent glucagon stimulation.[11] The differentiation between glucagon and cAMP analogue stimulation as representing a simple bypass of the adenylate cyclase step is based on: (1) the observation that the two cAMP analogues used behaved in a qualitatively similar manner (Table 1), (2) that even high concentrations of butyric acid could not reproduce the effects of dibutyryl-cAMP, and (3) the report that glucagon and dibutyryl-cAMP cause identical patterns of protein phosphorylation in hepatocytes.[19] Inhibition of hormone-stimulated adenylate cyclase activity by prostaglandins has also been reported in other tissues.[20,21] In a series of studies analogous to those reported here, Dominguez and Puschett[22] have found that PGE_2 inhibits the renal effects of parathyroid hormone, but not the effects of 8-bromo-cAMP.

An effect of PGE on hepatocyte cAMP metabolism does not explain PGEs' inhibition of hepatocyte glycogenolysis stimulated by cAMP-independent mechanisms (phenylephrine, angiotensin II, and A23187). The effects of these agonists are clearly cAMP independent and calcium dependent.[15-18] In the adult, male rat, epinephrine also stimulates glycogenolysis primarily by an alpha-adrenergic, cAMP-independent mechanism with only a minor beta-adrenergic contribution. Since cAMP-dependent stimulation of glycogen phosphorylase activity appears to be calcium independent,[23] there appear to be no common components of the two systems before phosphorylase kinase.[17,19,24] Both

TABLE 2
Effect of PGE on cAMP-independent stimulation of glycogenolysis

Conditions	Rate of glycogenolysis (μg/10^6 cells/min)
Group 1 (N = 4)	
Basal	1.93 ± 0.28
8 × 10^{-6} M Phenylephrine	3.45 ± 0.30
8 × 10^{-6} M Phenylephrine + 1.7 × 10^{-6} M 16,16-dimethyl-PGE_2	2.27 ± 0.92*
Group 2 (N = 5)	
Basal	1.83 ± 0.23
8 × 10^{-6} M Phenylephrine	2.71 ± 0.28
8 × 10^{-6} M Phenylephrine + PGE_2	2.03 ± 0.17*
Group 3 (N = 4)	
Basal	1.63 ± 0.30
1.6 × 10^{-6} M Angiotensin II	2.33 ± 0.18
1.6 × 10^{-6} M Angiotensin II + 1.7 × 10^{-6} M 16,16-dimethyl-PGE_2	1.73 ± 0.19*
Group 4 (N = 7)	
Basal	1.58 ± 0.25
0.55 μg/ml A23187	2.60 ± 0.34
0.55 μg/ml A23187 + 1.7 × 10^{-6} M 16,16-dimethyl-PGE_2	2.20 ± 0.34*
Group 5 (N = 5)	
Basal	1.36 ± 0.26
0.55 μg/ml A23187	2.36 ± 0.31
0.55 μg/ml A23187 + PGE_2	1.68 ± 0.11*

Incubations were conducted as detailed in the text. Additions were made at time zero except for PGE_2, which was added at 10^{-6} M at 0, 10, and 20 min. Values are mean ± SEM, *$P < 0.05$, agonist versus agonist + PGE.

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cAMP and calcium activate the same phosphorylase kinase.[19,24] Since dibutyryl cAMP-stimulated glycogenolysis is mediated via this kinase,[19] but was not inhibited by PGE (Table 1), an action of PGE at, or distal to, the phosphorylase kinase is unlikely.

The inhibition by PGE of A23187-stimulated glycogenolysis implies that, at least in part, PGE acts to inhibit glycogenolysis at a level after the hormone-induced increase in cytoplasmic calcium. That PGE does not affect the hormone-induced increase in calcium is supported by the report that epinephrine-induced alterations in hepatic cytoplasmic calcium concentrations are not affected by indomethacin,[25] which should alter PGE concentrations by inhibiting PGE production. Also supporting an effect of PGE on calcium-mediated cellular events is the observation that PGE inhibits phosphoinositide breakdown in platelets.[26] In tracheal smooth muscle, PGE_2 has been found to inhibit, and indomethacin to potentiate, the contractile response to calcium in the presence of histamine.[27] A possible mechanism by which a single effect of PGE on cAMP generation might affect calcium-mediated processes is suggested by a recent report by Morgan et al.[28] They report that exposure of hepatocytes to cAMP-generating agents increased the calcium response to alpha-adrenergic or angiotensin II stimulation. Since no cAMP agonist was present during our stimulation with cAMP-independent agonists, such a mechanism in our studies would involve changes in basal cAMP metabolism.

Thus, while the available data do not permit a definitive conclusion to be drawn, it appears that PGE inhibits cAMP-dependent hormone stimulation of glycogenolysis at the level of cAMP generation and, in addition, PGE also inhibits cAMP-independent hormone stimulation of glycogenolysis by an unknown mechanism. While these results demonstrate the potential for a regulatory effect of PGE on hepatic glucose metabolism in agreement with in vivo studies,[29] the specific physiologic role of this PGE effect is as yet undefined. Further work will be required to further define the role of PGEs' modulation of hepatic hormone actions on glucose homeostasis.

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