FEBS Letters 351 (1994) 353–356

FEBS 14490

Inhibition by PGE_2 of glucagon-induced increase in phosphoenolpyruvate carboxykinase mRNA and acceleration of mRNA degradation in cultured rat hepatocytes

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Received 28 July 1994

Abstract In cultured rat hepatocytes the key gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PCK) is known to be induced by glucagon via an elevation of cAMP. Prostaglandin E_2 has been shown to antagonize the glucagon-activated cAMP formation, glycogen phosphorylase activity and glucose output in hepatocytes. It was the purpose of the current investigation to study the potential of PGE_2 to inhibit the glucagon-induced expression of PCK on the level of mRNA and enzyme activity. PCK mRNA and enzyme activity were increased by 0.1 nM glucagon to a maximum after 2 h and 4 h, respectively. This increase was completely inhibited if 10 μM PGE_2 was added concomitantly with glucagon. This inhibition by PGE_2 of glucagon-induced PCK activity was abolished by pertussis toxin treatment. When added at the maximum of PCK mRNA at 2 h, PGE_2 accelerated the decay of mRNA and reduced enzyme activity. This effect was not reversed by pertussis toxin treatment. Since in liver PGE_2 is derived from Kupffer cells, which play a key role in the local inflammatory response, the present data imply that during inflammation PGE_2 may reduce the hepatic gluconeogenic capacity via a G_i-linked signal chain.

Key words: Prostaglandin E_2; Glucagon; Phosphoenolpyruvate carboxykinase; Inflammation; mRNA degradation

1. Introduction

Prostaglandins are involved in short-term regulation of liver carbohydrate metabolism. They are produced by non-parenchymal liver cells and seem to mediate the hepatic glucose mobilization in response to inflammatory stimuli by e.g. zymosan, immune complexes, platelet activating factor and anaphylatoxins ([1] and references therein). Prostaglandins increased $InsP_3$ formation, glycogen phosphorylase activity and glucose output in hepatocyte cultures and suspensions [1–3]. This glycogenolytic effect was mediated by prostaglandin $F_{2\alpha}$ and prostaglandin E_2 receptors of the EP_1 subtype that are linked to phospholipase C by a pertussis toxin-insensitive G-protein [1]. In addition, prostaglandin E_2 inhibited the glucagon-stimulated cAMP formation [4], glycogen phosphorylase activity [1] and glucose mobilization from glycogen [5,6] and gluconeogenesis [1] in hepatocytes. This glucagon-antagonistic effect was mediated by prostaglandin E_2 receptors of the EP_3 subtype [1], that are linked to adenylate cyclase by a pertussis toxin-sensitive G_i-protein [7]. Since glucagon also stimulated prostaglandin synthesis in Kupffer cells [8], it seems to be possible, that the prostaglandin E_2-mediated inhibition of the glucagon-stimulated cAMP formation not only operates within the short-term regulation of metabolism but also within the control of gene expression and thus represents a general intrahepatic feedback loop for the limitation of glucagon action.

In cultured rat hepatocytes the expression of the key regulatory enzyme of hepatic gluconeogenesis, phosphoenolpyruvate carboxykinase (PCK), is increased by glucagon on the transcriptional level [9]. It had been shown in rat hepatoma cells that the activation of the PCK gene is mediated by the promoter-bound transcription factor cAMP regulatory element binding protein (CREB), which upon cAMP-dependent phosphorylation confers transcriptional activation to the PCK gene [10,11]. Therefore, prostaglandin E_2 might also attenuate the glucagon-induced activation of PCK gene expression. The current investigation supports this hypothesis, since the glucagon-induced increase in PCK mRNA level and enzyme activity was totally abolished, if prostaglandin E_2 was added concomitantly with glucagon.

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2. Materials and methods

2.1. Animals and chemicals

Cells were prepared from male fed Wistar rats (200–300 g) (Winkelmann, Borchen, Germany). Chemicals were analytical grade and purchased from commercial sources. Collagenase A, culture medium M199, the digoxygenin-labeling mix and the digoxygenin detection kit were from Boehringer (Mannheim, Germany). Hormones were supplied by Serva (Heidelberg, Germany) and molecular biology products by Life Technologies (Eggenstein, Germany). Supported nitrocellulose (BA 85) was from Schleicher & Schüll (Dassel, Germany) and culture dishes from Greiner (Nürtingen, Germany). PGE_2 was a product of Cascade (Reading, UK). The cAMP radioimmunoassay was purchased from Amersham Buchler (Braunschweig, Germany).

2.2. Experimental design

Hepatocytes were prepared by the collagenase perfusion technique [12] and cultured on plastic dishes (1×10^6 cells/dish; 60 mm diameter) for 48 h with 2.5 ml medium/dish in the presence of 100 nM dexamethasone and 0.5 nM insulin added as a 'growth factor' for culture maintenance. During the first 4 h of culture 4% fetal calf serum was present, which was then removed by a medium change. The cells were supplied with fresh medium after another 20 h of culture. After 48 h of culture induction experiments were started by washing the cells twice with insulin-free, but dexamethasone-containing medium. The culture was then continued in fresh insulin-free, dexamethasone-containing medium for the times indicated in the presence of 0.1 nM glucagon. PGE_2 was added either simultaneously with or 2 h after glucagon to a final concentration of 10 μM. Cells were harvested at the time points indicated. Total RNA was prepared for Northern blot analysis by phenol extraction. PCK enzyme activity was determined in postmitochondrial supernatants [13]. cAMP was determined after extraction with 10 mM HCl containing 100 μM isobutyl methyl xanthine to inhibit phosphodiesterase.

2.3. Tools and assays

Northern blots detecting hepatocyte cytosolic PCK were carried out

SSDI 0014-5793(94)00877-9

with digoxygenin-labelled antisense cRNA probes. These were prepared with the DIG-labelling kit according to the supplier's manual (Boehringer) by transcription with DNA-dependent RNA T3 polymerase. PCK cRNA was synthesized from the *Hin*dIII-linearized plasmid pBS-PCK, which contained a *Pst*I 1.2 kb cDNA fragment coding for rat PCK [14]. Hybrids were detected according to the protocol of Boehringer and were quantified by video densitometry. For detection of PCK mRNA 20 μg of total RNA were applied to electrophoresis.

cAMP was determined with a ^{125}I-radioimmunoassay kit according to the instructions of the manufacturer.

3. Results and discussion

3.1. Inhibition by PGE_2 of the glucagon-induced increase in PCK mRNA and enzyme activity

Glucagon (0.1 nM) added with fresh medium after 48 h of culture increased PCK mRNA levels about 6-fold (Fig. 1, upper left). This increase was transient with a maximum after 2 h. It reached nearly pre-stimulatory levels again after another 4 h. The increase in mRNA was preceded by a sharp increase in cAMP within 2 min reaching a maximum 2-fold over basal after 5 min (Fig. 2). cAMP remained elevated at about 1.5-fold over basal for 6 h (not shown). Probably due to the medium change, mRNA levels also increased slightly and transiently in control hepatocytes which were not treated with glucagon. PGE_2 (10 μM) added concomitantly with glucagon, prevented both, the glucagon-induced rise in cAMP (Fig. 2) and mRNA (Fig. 1, upper left). Under these conditions cAMP concentrations and PCK mRNA levels were in the range of control cells. PCK mRNA levels in hepatocytes receiving PGE_2 alone were below control levels. In these cells no transient increase in mRNA was observed. PGE_2 alone caused only a slight transient increase in cAMP with a maximum after 2 min, which returned to basal after 5 min (Fig. 2). This is in contrast to previous findings with freshly isolated hepatocytes or hepatocytes after 24 h in primary culture, where PGE_2 had no effect on basal cAMP levels [1].

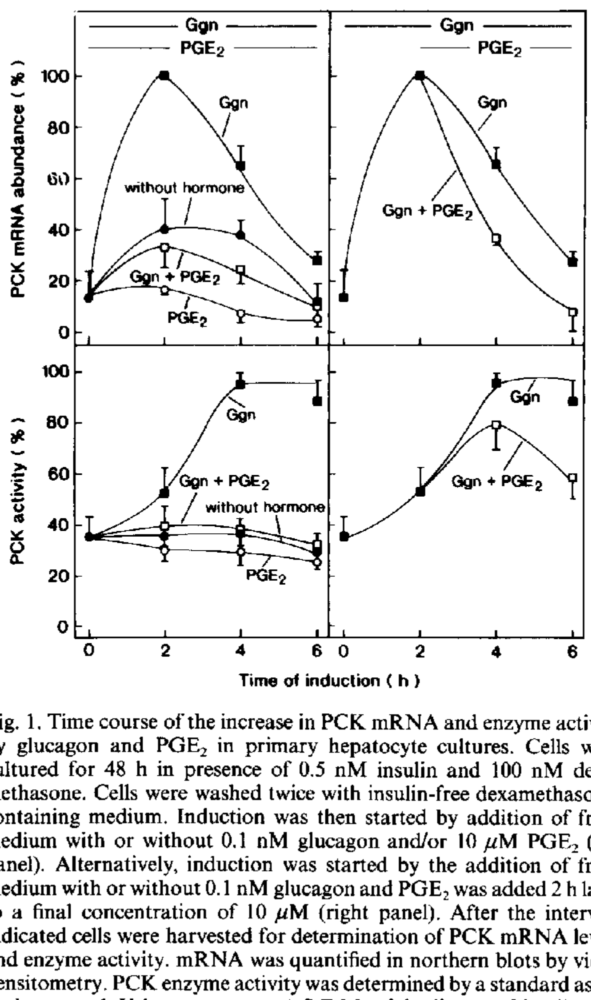

Fig. 1. Time course of the increase in PCK mRNA and enzyme activity by glucagon and PGE_2 in primary hepatocyte cultures. Cells were cultured for 48 h in presence of 0.5 nM insulin and 100 nM dexamethasone. Cells were washed twice with insulin-free dexamethasone-containing medium. Induction was then started by addition of fresh medium with or without 0.1 nM glucagon and/or 10 μM PGE_2 (left panel). Alternatively, induction was started by the addition of fresh medium with or without 0.1 nM glucagon and PGE_2 was added 2 h later to a final concentration of 10 μM (right panel). After the intervals indicated cells were harvested for determination of PCK mRNA levels and enzyme activity. mRNA was quantified in northern blots by video densitometry. PCK enzyme activity was determined by a standard assay in the cytosol. Values are means ± S.E.M. of duplicates of 3 cell preparations.

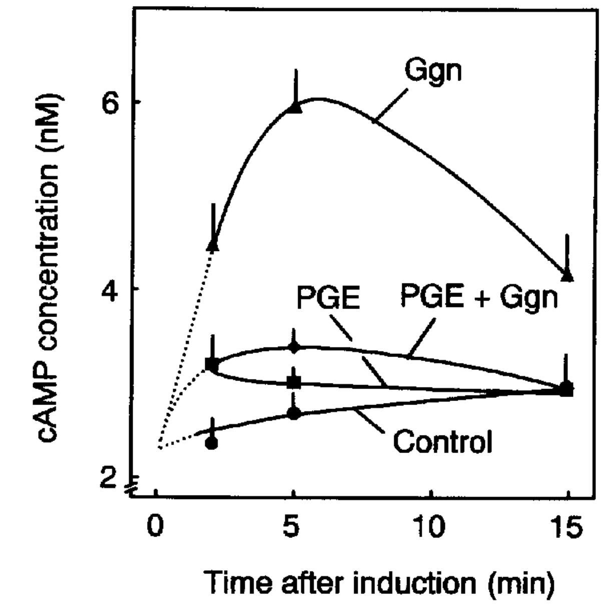

Fig. 2. Inhibition by PGE_2 of the glucagon-induced cAMP formation in hepatocytes. Hepatocytes were cultured for 48 h in presence of 0.5 nM insulin and 100 nM dexamethasone. Cells were washed twice with insulin-free dexamethasone-containing medium. Induction was then started by addition of fresh medium with or without 0.1 nM glucagon (Ggn) and/or 10 μM PGE_2. At the times indicated medium was removed, cells were lysed in liquid nitrogen and then homogenized in 10 mM HCl containing 1 mM IBMX. The homogenate was centrifuged and cAMP was determined in the supernatant by radio immunoassay. Values are means ± S.E.M. of 6 experiments of 2 cell preparations.

Following the increase in mRNA levels PCK enzyme activity increased in glucagon-stimulated cells to a maximum between 4 and 6 h. This increase in enzyme activity was completely abolished if PGE_2 was added concomitantly with glucagon. In these cells and in cells receiving no hormones or PGE_2 alone enzyme activity remained constant at basal levels throughout 6 h (Fig. 1, lower left).

The glucagon-induced increase in PCK mRNA has previously been shown to be due to transcriptional activaton. The 5′-promoter of the PKC gene contains a cAMP responsive element (CRE). A regulatory protein, CREB, binds to the CRE and stimulates transcription after cAMP-dependent phosphorylation [10,11]. Thus, glucagon induced PCK mRNA and enzyme activity by an increase in cAMP which was prevented by simultaneous application of PGE_2.

3.2. Attenuation by pertussis toxin of the inhibition by PGE_2 of glucagon-induced PCK enzyme activity

Previous studies have shown that the inhibition by PGE_2 of the glucagon-stimulated cAMP formation in hepatocytes is

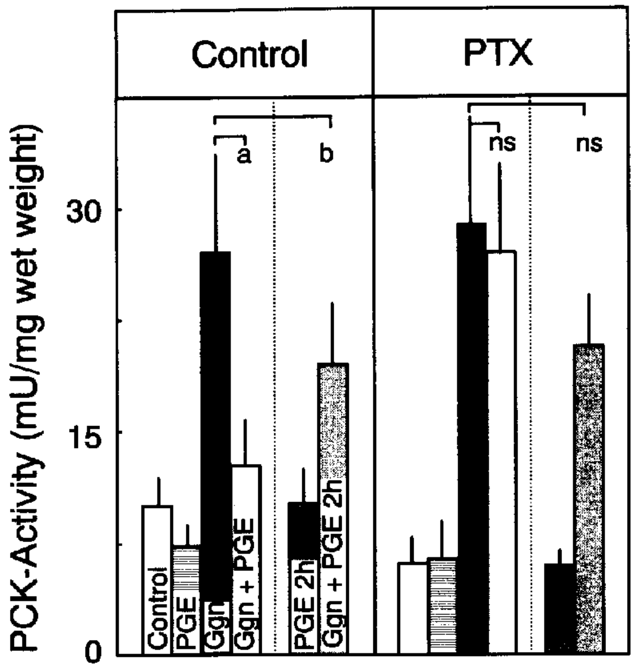

Fig. 3. Attenuation by pertussis toxin of the inhibition by PGE_2 of glucagon-induced PCK enzyme activity. Hepatocytes were cultured for 48 h in presence of 0.5 nM insulin and 100 nM dexamethasone and, where indicated, 100 ng/ml pertussis toxin for the last 18 h. Cells were washed twice with insulin-free dexamethasone-containing medium. Induction was then started by addition of fresh medium with or without 0.1 nM glucagon and/or 10 μM PGE_2 (control, glucagon (Ggn), PGE_2 (PGE) and glucagon + PGE_2 (Ggn + PGE)). Alternatively, induction was started by addition of fresh medium with or without 0.1 nM glucagon; PGE_2 was then added 2 h later to a final concentration of 10 μM (PGE 2 h, Ggn + PGE 2 h). After 6 h of induction cells were harvested and PCK enzyme activity was determined in the cytosol by a standard assay. Values are means ± S.E.M. of three cell preparations. Statistics: Students t-test for paired samples: a, $P < 0.01$; b, $P < 0.05$.

mediated via PGE_2 receptors of the EP_3 subtype, which are linked to adenylate cyclase via a pertussis toxin sensitive G_i protein [1]. The inhibition by PGE_2 of glucagon-induced PCK activity was abolished in hepatocytes treated with pertussis toxin for 18 h prior to the experiment (Fig. 3). Therefore, the inhibition of glucagon-induced PCK activity by PGE_2 was also mediated via a pertussis toxin sensitive inhibitory G protein.

3.3. Acceleration by PGE_2 of the degradation of glucagon-induced PCK mRNA

IL6, the major acute phase mediator in the liver, accelerated the degradation of PCK mRNA [15]. It seemed, therefore, possible, that PGE_2 might not only inhibit the glucagon-induced PCK transcription but might also increase PCK mRNA degradation. Hence, PGE_2 was added at the maximum of glucagon-induced PCK mRNA 2 h after induction. The degradation of PCK mRNA was measured as PCK mRNA remaining in the absence of a transcriptional inhibitor. This was mandatory because the use of inhibitors of transcription stabilized PCK mRNA [9]. Moreover, this was permissive, since after 2 h of glucagon treatment the transient increase in gene transcription had already returned to basal [9]. If PGE_2 was added 2 h after glucagon, the degradation of glucagon-induced PCK mRNA was nearly doubled (Fig. 1, upper right). cAMP-levels, that had remained elevated were slightly reduced by addition of PGE_2 2 h after glucagon administration (not shown). In hepatocytes that received PGE_2 2 h after glucagon the glucagon-induced enzyme activity continued to increase until 4 h, reaching a maximum about 25% below the maximum with glucagon alone and then started to decline between 4 and 6 h (Fig. 1, lower right). The glucagon-induced PCK-activity at 6 h was only 60% of the activity in presence of glucagon alone. This effect of PGE_2 was not abolished by pertussis toxin treatment (Fig. 3). Yet, the inhibition by PGE_2 of the glucagon-induced PCK enzyme activity was no longer significant. This result indicates, that the reduction of glucagon-induced enzyme activity by PGE_2 added 2 h after induction might only partially be due to the G_i-decrease in cAMP and that in addition, a different signal path might also be involved.

3.4. Pathophysiological significance

IL6 [15], IL1β and TNFα [16] have previously been shown to antagonize the glucagon-mediated induction of PCK in hepatocytes. It has been discussed that this downregulation of the expression of the key gluconeogenic enzyme may represent a molecular economy which could provide additional biosynthetic capacity for acute phase protein synthesis [15]. PGE_2 appears to be a major regulatory mediator in inflammatory processes in the liver. PGE_2 synthesis in Kupffer cells is increased by tumor necrosis factor α (TNFα) [17]. PGE_2 has been implicated in the feedback inhibition of TNFα [17] as well as the IL6 [18,19] and IL1 [19] production by Kupffer cells. On the other hand PGE_2 in combination with TNFα or IL1β increased NO-formation in Kupffer cells, whereas TNFα and IL1β alone were inactive [20].

Thus PGE_2 seems to play a dual role in liver during inflammation: First, PGE_2 apparently can limit the acute phase response by feedback-inhibiting the production and release of various pro-inflammatory cytokines. Second, PGE_2 can enhance inflammatory responses like NO production or, as described here, downregulation of gluconeogenic enzymes in order to liberate additional synthetic capacity for acute phase protein production.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft through the SFB 236, Teilprojekt A11. We wish to thank Prof. Dr. K. Jungermann for his invaluable advice and critical reading of the manuscript.

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