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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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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 nM 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 E$_2$, and prostaglandin E$_2$ receptors of the EP$_2$ 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$_2$ 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 intracellular 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.

2. Materials and methods

2.1. Animals and chemicals

Cells were prepared from male fed Wistar rats (200-300 g) (Winkelmann, Böchen, Germany). Chemicals were analytical grade and purchased from commercial sources. Collagenase A, culture medium M199, the digoxigenin-labeling mix and the digoxigenin 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 x 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 AM. 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 supernatant [13]. cAMP was determined after extraction with 10 mM HCl containing 100 AM isobutyl methyl xanthine to inhibit phosphodiesterase.

2.3. Tools and assays

Northern blots detecting hepatocyte cytosolic PCK were carried out

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3. Results and discussion

3.1. Inhibition by PGE₂ 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. PGE₂ (10 µM) added concomitantly with glucagon, prevented both, the

Fig. 1. Time course of the increase in PCK mRNA and enzyme activity by glucagon and PGE₂ 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 (Ggn) and/or 10 µM PGE₂ (left panel). Alternatively, induction was started by the addition of fresh medium with or without 0.1 nM glucagon and PGE₂, 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.

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 PGE₂ alone were below control levels. In these cells no transient increase in mRNA was observed. PGE₂ 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 PGE₂ had no effect on basal cAMP levels [1].

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 PGE₂ was added concomitantly with glucagon. In these cells and in cells receiving no hormones or PGE₂ 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 activation. 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 PGE₂.

3.2. Attenuation by pertussis toxin of the inhibition by PGE₂ of glucagon-induced PCK enzyme activity

Previous studies have shown that the inhibition by PGE₂ of the glucagon-stimulated cAMP formation in hepatocytes is
mRNA was nearly doubled (Fig. 1, upper right), cAMP-levels, 2 h after glucagon, the degradation of glucagon-induced PCK transcription had already returned to basal [9]. If PGE2 was added 2 h after glucagon, the degradation of glucagon-induced PCK mRNA was measured as PCK mRNA remaining (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 PGE2 receptors of the EP3 subtype, which are linked to adenylate cyclase via a pertussis toxin sensitive G protein [1]. The inhibition by PGE2 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 PGE2 was mediated via a pertussis toxin sensitive G protein.

3.3. Acceleration by PGE2 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 PGE2 might not only inhibit the glucagon-induced PCK transcription but might also increase PCK mRNA degradation. Hence, PGE2 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 PGE2 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 PGE2 2 h after glucagon administration (not shown). In hepatocytes that received PGE2 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 PGE2 was not abolished by pertussis toxin treatment (Fig. 3). Yet, the inhibition by PGE2 of the glucagon-induced PCK enzyme activity was no longer significant. This result indicates that the reduction of glucagon-induced enzyme activity by PGE2 added 2 h after induction might only partially be due to the G3-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]. PGE2 appears to be a major regulator in mediating inflammatory processes in the liver. PGE2 synthesis in Kupffer cells is increased by tumor necrosis factor α (TNFα) [17]. PGE2 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 PGE2 in combination with TNFα or IL1β increased NO-formation in Kupffer cells, whereas TNFα and IL1β alone were inactive [20].

Thus PGE2 seems to play a dual role in liver during inflammation: First, PGE2 apparently can limit the acute phase response by feedback-inhibiting the production and release of various pro-inflammatory cytokines. Second, PGE2 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.

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