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PROSTAGLANDIN E_2 INHIBITION OF GLUCAGON-INDUCED HEPATIC GLUCONEOGENESIS AND CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE ACCUMULATION

ROBERT A. LEVINE and EDMUND H. SCHWARTZEL, JR.

Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, NY 13210, U.S.A.

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Abstract—This study examines the role of prostaglandin E_2 (PGE_2) in modulating hormonal control of hepatic gluconeogenesis. The effects of PGE_2 (2.8×10^{-6} M), administered alone and in combination with glucagon (1.4×10^{-9} M), on gluconeogenesis from 20 mM sodium lactate and on cyclic AMP (cAMP) concentration were studied in isolated livers of rats fasted for 24 hr and perfused with Krebs–Ringer bicarbonate (KRB) solution containing 3% albumin and 100 mg/dl of glucose. Samples of perfusate and liver were taken at frequent intervals between 1 and 60 min after KRB (control), PGE_2, glucagon, and PGE_2 + glucagon infusion (ten experiments in each group). Glucagon stimulated more glucose production [45–60 min increment = 80 ± 7 mg/100 ml (mean ± standard error)] than did PGE_2 (35 ± 5 mg/100 ml) or KRB (49 ± 8 mg/100 ml) ($P < 0.01$). Concomitant infusion of PGE_2 with glucagon inhibited the glucagon-induced gluconeogenesis (56 ± 6 mg/100 ml) ($P < 0.01$). Glucagon increased hepatic cAMP concentration approximately 4-fold (control = 354 ± 29 pmoles/g, maximal stimulation = 1881 ± 380 pmoles/g). PGE_2 + glucagon reduced cAMP accumulation to approximately 2-fold (control = 365 ± 31 pmoles/g, maximal stimulation = 806 ± 99 pmoles/g) ($P < 0.05$). The data show that PGE_2 inhibits glucagon-mediated gluconeogenesis from lactate. It remains to be determined whether the antigluconeogenic action of PGE_2 is causally related to the inhibition of cAMP content.

Prostaglandins of the E series are of interest in view of their potential role as modulators of hormone action. Many of the diverse metabolic effects of prostaglandins can be attributed to increased adenylate cyclase activity and cyclic AMP (cAMP) formation in tissues. Recent work from this laboratory with isolated rat liver perfusions has focused on the possible role of prostaglandins in hepatic metabolism [1, 2]. These studies showed that prostaglandin E_1 and E_2 (PGE_1, PGE_2) did not promote glucose release from the liver, glycogenolysis, or lipolysis, did not stimulate cyclic nucleotides, and did not prevent epinephrine-induced hyperglycemia.

Hepatic gluconeogenesis is under various hormonal and nutritional influences. Most of the conditions conducive to increased gluconeogenesis are paralleled by increases in intracellular levels of cAMP. For example, in perfused liver glucagon increased cAMP concentration and gluconeogenesis [1, 3, 4], while, conversely, somatostatin reduced hepatic cAMP and the rate of gluconeogenesis in isolated hepatocytes [5, 6] and chlorpropamide inhibited cAMP and gluconeogenesis in perfused liver [7]. There are conflicting data regarding the possibility that prostaglandins play a role in regulating hepatic gluconeogenesis. PGE_1 has been reported to inhibit gluconeogenesis in perfused rat liver [8], but in rat liver slices PGE_2 failed to influence gluconeogenesis when lactate or alanine was used as a substrate [9].

The data reported in this paper show that PGE_2 alone does not influence hepatic gluconeogenesis from lactate but does suppress glucagon-mediated gluconeogenesis and cAMP production in isolated perfused rat liver.

MATERIALS AND METHODS

Experimental design. Isolated rat livers were prepared and perfused with a blood-free recirculating system, as described earlier [10]. The livers, obtained from Sprague–Dawley male rats weighing 250–300 g and fasted for 24 hr, were perfused with Krebs–Ringer bicarbonate (KRB) solution, pH 7.4, containing 3% albumin and 100 mg/dl of glucose. Samples of perfusate and liver tissue were taken, respectively, at zero time, 15, 30, 45 and 60 min, and at zero time, 1, 5, 15 and 30 min after exposure to test substances, as reported previously [10]. Liver samples were quick-frozen until assay, as described [11].

All agents and substrate (0.5 ml of 40% sodium lactate, final concentration 20 mM) were added to the media at zero time in a dose of 1 ml and then administered by a constant infusion Harvard Apparatus pump at the rate of 2 ml/hr. Livers were perfused during a 30-min equilibration period using an endoportal infusion of KRB prior to the addition of experimental agents at zero time. After zero time, KRB with lactate was infused as the control agents.

PGE_2 (supplied by Dr. John Pike of Upjohn Co., Kalamazoo, MI) was initially added at a dose of 100 μg and then infused at a dose of 200 μg/hr. The final perfusate concentration was 2.8×10^{-6} M. The dose of PGE_2 was chosen after preliminary experiments using 5, 10 and 50 μg failed to alter hepatic cAMP

concentrations significantly after 1–30 min of exposure. In addition, PGE_2 at the lower concentrations did not inhibit glucagon-stimulated gluconeogenesis up to 60 min after administration. PGE_2 was stored in absolute ethanol and diluted just before use. The volume of ethanol infused into the perfusate was negligible (final concentration 0.0009%). Ethanol was also added to KRB and glucagon as a control, since ethanol was used as a diluent of PGE_2. Hepatic viability during PGE_2 infusions, as measured by bile production, appeared intact.

Glucagon was added at zero time using a single dose of 50 μg, and then was subsequently infused at a dose of 100 μg/hr (initial perfusate concentration 1.4×10^{-9} M). PGE_2 and glucagon, when used together, were added at the aforementioned molar concentrations. Ten perfusions were performed for each of the four experimental groups (KRB, glucagon, PGE_2, and PGE_2 + glucagon).

Chemical analysis. cAMP was measured by radioimmunoassay (RIA) on liver samples prepared as described previously [11]. RIA was carried out using kits commercially available from Schwartz–Mann, Orangeburg, NY. The detection limit of the assay (65 per cent of initial bound to free ratio) was routinely 25 fmoles/tube. RIAs were performed in duplicate. The interassay coefficient of variation for an internal standard reading at the midportion of the curve was 5 per cent (N = 25). cAMP recovery (80–85 per cent) was verified by treatment with phosphodiesterase, by recovery of known quantities of cAMP added to the homogenate, and by serial sample dilution [11].

Perfusate glucose and hepatic glycogen samples were taken at the indicated times and assayed as described previously [10]. Perfusate lactate was also determined in the KRB and PGE_2 experiments and was measured by standard enzymatic technique [12].

Statistical evaluation. The statistical significance of the data was evaluated by Student's *t*-test, using paired analysis for evaluation of differences between pre- and post-treatment values of the same liver, or unpaired analysis for comparisons between different populations of livers exposed to different test agents. Results are expressed as means ± standard errors (S.E.M.).

RESULTS

Gluconeogenesis from lactate. Glucose concentration in the perfusate was similar for both the experimental and the KRB control group at zero time before addition of the substrate (116 ± 9 mg/100 ml). The rate of hepatic glucose production, by rat liver perfused with KRB, after the addition of substrate is shown in Fig. 1. There was a progressive increase in glucose formation from lactate over 60 min during the control KRB infusion. PGE_2 also induced glucose production from lactate but to a slightly lesser extent than KRB. The glucose increment at the end of the perfusion (45–60 min period) was not significantly different for the two agents (KRB = 49 ± 8 mg/100 ml; PGE_2 = 35 ± 5 mg/100 ml). Lactate was the apparent major gluconeogenic precursor in these experiments, since the cumulative perfusate glucose production 60 min after KRB or PGE_2 administration could be quantitatively accounted for by the disappearance of lactate (KRB = −209 ± 29 mg/100 ml; PGE_2 = −162 ± 23 mg/100 ml).

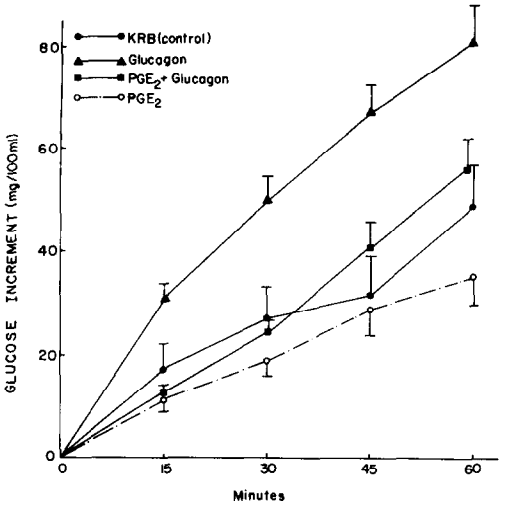

Fig. 1. Effects of Krebs–Ringer bicarbonate (KRB), glucagon (1.4×10^{-9} M), PGE_2 (2.8×10^{-6} M) and PGE_2 + glucagon infusion on perfusate glucose increment in isolated, fasted, donor liver. PGE_2 + glucagon significantly inhibited glucagon-stimulated gluconeogenesis at all sampling periods shown ($P < 0.05$). Substrate (lactate) was added at zero time. Samples were analyzed as described in Materials and Methods. Points represent the means ± S.E.M. for ten experiments, each analyzed in duplicate.

Compared to KRB or PGE_2, glucagon significantly stimulated glucose production from the substrate at all periods sampled, as illustrated in Fig. 1 ($P < 0.01$). The degree and time course of glucose production which occurred with glucagon at a concentration of 1.4×10^{-9} M is similar to that reported by Conn and Kipnis [4] using the same concentration of lactate and a glucagon concentration (3.3×10^{-8} M), twenty-five times greater than in the present study. Concomitant infusion of PGE_2 with glucagon significantly inhibited the glucagon-induced gluconeogenesis at all time periods ($P < 0.05$), as shown in Fig. 1.

Hepatic glycogen concentration. There were no significant changes in hepatic glycogen content between the basal period and the 60-min termination of the experiment for all agents studied. Control hepatic glycogen values for PGE_2, KRB and glucagon were, respectively, 0.133 ± 0.013, 0.125 ± 0.009 and 0.149 ± 0.021 mg/g compared to post-treatment values at 60 min of 0.171 ± 0.03, 0.163 ± 0.02 and 0.146 ± 0.056 mg/g. Thus, the concentration of liver glycogen was extremely low throughout the perfusion. The increased glucose which appeared in the perfusate medium between zero and 60 min could be accounted for by the concomitant disappearance of lactate from the medium due to gluconeogenesis rather than from concomitant glycogenolysis.

Inhibition of glucagon-stimulated cAMP accumulation by PGE_2. Glucagon was employed to investigate the effects of PGE_2 on agonist-stimulated

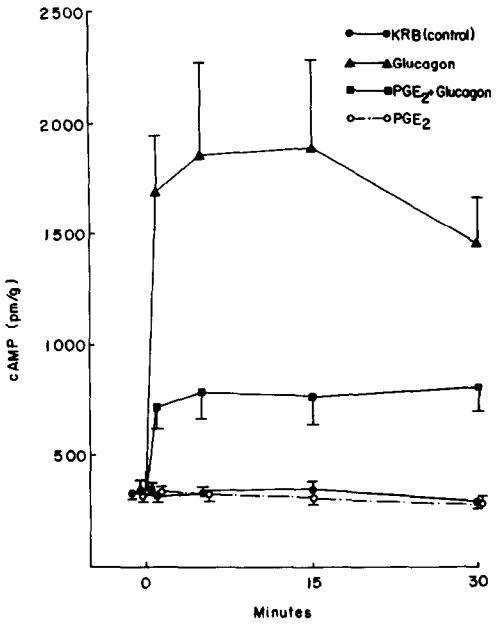

Fig. 2. Effect of PGE_2 on suppression of glucagon-stimulated hepatic cAMP. PGE_2 + glucagon significantly inhibited glucagon-induced cAMP accumulation in liver at all sampling periods shown. Samples were analyzed as described in Materials and Methods. Points represent means ± S.E.M. for ten experiments, each analyzed in duplicate.

cAMP accumulation. Figure 2 compares the hepatic cAMP response following intraportal administration of KRB, PGE_2 and glucagon alone, to that observed when glucagon was administered with PGE_2. Glucagon stimulated cAMP accumulation approximately 4-fold as early as 1 min after its intraportal administration (Fig. 2). This increased hepatic cAMP content persisted throughout the perfusion.

PGE_2 significantly reduced ($P < 0.001$) the magnitude of the increase in hepatic cAMP level observed in response to glucagon to approximately 2-fold, and the reduction in cAMP concentration was sustained between 1 and 30 min throughout the perfusion (Fig. 2). The same concentration of PGE_2 infused alone did not alter hepatic cAMP concentration. The lack of cAMP responsiveness to PGE_2 in rat liver is similar to results observed in the fasted intact dog liver [2].

DISCUSSION

The results indicate that PGE_2 suppresses stimulation of hepatic gluconeogenesis by glucagon by approximately 50 per cent. The concentrations of PGE_2 and glucagon used in these studies were supraphysiologic. However, our preliminary studies showed that lesser concentrations of PGE_2 did not affect glucagon-induced gluconeogenesis. Furthermore, we have shown previously that the same concentrations of PGE_1 and PGE_2 as utilized in the present study do not alter glycogenolysis or lipolysis in the perfused liver [1, 2]. Moreover, ureogenesis, in the absence of glucagon, is also unaffected by various types of prostaglandins studied in this same isolated system at doses 1/4 to 1/40 as large as the dose used in our study (L. L. Miller, personal communication). Thus, the only metabolic function that appears to be influenced by prostaglandins in intact liver is hormone-induced gluconeogenesis.

Is there physiological relevance to the interaction of PGE_2 and glucagon with hepatic gluconeogenesis? A possible role for prostaglandins in modulating the action of glucagon on the liver appears reasonable, since rat liver perfused with glucagon has been reported to release prostaglandins [13]. PGE_1 has been shown to inhibit glucagon induction of the cAMP-dependent tyrosine aminotransferase activity *in vitro* [14]. Prostaglandins also play a key role as modulators of hormonal action in non-hepatic tissues. Inhibition of cAMP-mediated hormonal effects by certain prostaglandins has been well documented in a number of systems, including adipose, corpora luteal and parathyroid tissues [15–20].

Our data suggest that the antigluconeogenic action of PGE_2 on glucagon-mediated hepatic gluconeogenesis may be related to inhibition of cAMP metabolism in the cell, resulting in suppression of the key gluconeogenic enzymes. However, the interference by PGE_2 with the action of glucagon may not be based solely on reduced hepatic cAMP concentration and might even be an entirely independent effect. The nucleotide levels in the liver after PGE_2 treatment were still increased twice above the pre-treatment value (Fig. 2), while PGE_2 completely blocked the gluconeogenic effect of glucagon, returning glucose production to control levels. If prostaglandins of the E type act by a cAMP-dependent mechanism, one would expect that conditions would be found where this agent would affect other metabolic processes as well, such as glycogenolysis, yet this is not the case in perfused rat liver [2].

Our findings confirm a previous report by DeRubertis *et al.* [21], which demonstrated that in perfused rat liver PGE_1 and PGE_2 inhibited glucagon-induced increases in hepatic cAMP levels, but had no effect on adenylate cyclase activity in liver homogenates [22]. The locus of the inhibition of glucagon-stimulated cAMP concentration by prostaglandins was not apparent in their study and cannot be inferred further from our data. Thomas *et al.* [19] recently showed that prostaglandins rapidly inhibit luteinizing hormone [LH]-stimulated progesterone production in cultured luteal cells. The antigonadotropic action of prostaglandins was due to a block in intracellular cAMP accumulation and not to phosphodiesterase stimulation of cAMP degradation or to inhibition of LH binding to its receptors [19].

Our study does not show a significant effect of PGE_2 alone on the rate of hepatic gluconeogenesis from lactate, although a trend toward decreased glucose production, compared to KRB, was observed. In preliminary experiments we were unable to show inhibition of gluconeogenesis by PGE_1 or PGE_2 in livers perfused for as long as 120 min.

The interacting effects of PGE_2 and glucagon in perfused liver are comparable to the inhibitory effects of somatostatin and chlorpropamide on the

adenylate cyclase-cAMP system and on gluconeogenesis in glucagon-treated rat liver *in vitro* [5–7, 23]. The ability of prostaglandins to modulate hormone action in perfused rat liver appears specific for gluconeogenesis, since epinephrine-induced glycogenolysis and augmentation of cAMP production are unaffected by either pre-treatment or concomitant administration of PGE_1 [2]. This latter situation is similar to the failure of somatostatin to influence epinephrine-induced glycogenolysis in isolated rat hepatocytes, despite its suppression of glucagon-stimulated glycogenolysis in this same liver preparation [6].

It appears likely that PGE_2, like chlorpropamide, somatostatin, insulin and a number of polypeptides, modulates hormone or drug-induced gluconeogenesis at the cellular level by affecting cAMP concentration or tissue susceptibility to the nucleotide. However, unlike certain drugs of hormones, PGE_2 does not appear capable of altering normal hepatic cellular processes, such as glycogenolysis, lipolysis, ureogenesis and gluconeogenesis.

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