Effect of Prostaglandins on Hepatic Cyclic Nucleotide Concentration, Carbohydrate and Lipid Metabolism

ROBERT A. LEVINE, M.D.

State University of New York, Upstate Medical Center
Syracuse, New York

Received June 13, 1978

The effects of exogenous prostaglandin E₁ (PGE₁) or prostaglandin E₂ (PGE₂) were studied in the isolated perfused rat liver and in the intact canine liver in order to determine the possible physiological role of prostaglandins on hepatic carbohydrate and lipid metabolism. The data indicate that PGE₁ and PGE₂ did not stimulate cyclic AMP (cAMP) and cyclic GMP (cGMP) concentrations in intact dog liver and PGE₁ failed to stimulate cAMP or cGMP in fed or fasted perfused rat liver. PGE₂ did not promote hyperglycemia, glycogenolysis, lipolysis, or prevent epinephrine-induced hyperglycemia in the isolated perfused rat liver. Other known glycogenolytic agents including glucagon and epinephrine increased cAMP and glycogenolysis in the same perfusion system. This study does not support a physiologic role for PGE₁ on hepatic glycogenolysis or lipolysis. If PGE₁ subsequently is found to influence other metabolic parameters such as lipogenesis, gluconeogenesis, ureogenesis or amino acid transport in isolated perfused liver, such alterations would probably occur independent of changes in cyclic nucleotide activity.

INTRODUCTION

Our prior studies in isolated perfused rat liver demonstrated the effect of cyclic AMP (cAMP), glucagon, epinephrine and serotonin or its endogenous precursor, 5-hydroxytryptophan, on glycogen metabolism [1,2]. These agents produced glycogenolysis, hyperglycemia, and stimulation of hepatic phosphorylase activity in isolated perfused rat liver [1,2]. The glycemic effect of serotonin observed in vitro was also noted in vivo. Serotonin may produce these physiological actions by activation of hepatic cAMP concentrations [1].

There is increasing evidence to show that E-type prostaglandins regulate intracellular cAMP levels but their complex interrelationships have not been clarified. It remains unclear whether the prostaglandin effect is related solely to adenylate cyclase activity or alteration in phosphodiesterase, adenylate kinase [3], and ATPase.

Although prostaglandins are rapidly metabolized by the liver, there is conflicting data regarding their specific actions on hepatic carbohydrate and lipid metabolism or on the adenylate cyclase-cAMP system in vitro or in vivo. In vitro experiments in adipose tissue demonstrate that prostaglandin E₁ (PGE₁) stimulates glucose uptake, glucose oxidation, and glycogen and triglyceride synthesis from labeled glucose [4].

Adenylate cyclase is stimulated in liver broken-cell homogenates from rat, mouse, 107
rabbit, and dog by PGE₁ in the presence of purine nucleotides, including guanosine triphosphate [5,6]. PGE₁ alone also increased adenylate cyclase activity in liver homogenates and in isolated rat liver cells [7–11]. Curnow and Nuttall [12] observed that PGE₁ decreased hepatic glycogen synthetase without altering glycogen concentrations. This change is compatible with increased hepatic cAMP and suggested possible direct effects on hepatic glycogen levels. Nevertheless, the actions of prostaglandins on glucose metabolism are variable. PGE₁ has been reported to cause hyperglycemia [13–15] or to inhibit glucose metabolism in liver [16].

Prior studies have not clarified the possible in situ role of PGE₁ on hepatic glycogenolysis. Exton et al. [17] concluded that PGE₁ perfusion in rat liver failed to alter cAMP levels or glucose production but detailed results were never presented. Others have reported a glycosenolytic effect of PGE₁ in rat liver slices [18]. In perfused rat liver a variable effect was noted in fed and fasted animals by Wilson and Handewych [19]. Only in the fasted state could they observe PGE₁-induced glycogenolysis. Zenser et al. have shown transient modest increases in cAMP in perfused rat liver 1 to 3 min after PGE₁ administration [10]. There is only a single published report concerning the effects of PGE₁ on gluconeogenesis in perfused rat liver [20]. This study showed that PGE₁ inhibited gluconeogenesis, possibly between the glyceraldehyde and glucose steps [20].

Since notable species differences have been found in the hepatic adenylate cyclase-cAMP response to prostaglandins, we have investigated the possible interaction of prostaglandins on hepatic cyclic nucleotide concentration in both rats and dogs. There are no published data on the hepatic cyclic GMP (cGMP) concentration after treatment with prostaglandins. Thus we have studied the response of this nucleotide to 2 types of E prostaglandins, PGE₁ and prostaglandin E₂ (PGE₂). We also have evaluated the effects of PGE₁ on hepatic glycogenolysis, and lipolysis. These studies extend out preliminary report [21].

METHODS

The effects of PGE₁ or PGE₂ were studied in the isolated perfused rat liver (3.3 μg/min) and in the intact canine liver (25 μg/kg bolus then 20 μg/min). Because PGE₁ and PGE₂ are rapidly metabolized by the liver and most of it removed during a single passage, they were administered continuously [22].

The effects of PGE₁ in isolated perfused rat liver on both liver and perfusate cAMP content were compared to that induced by dibutyryl cAMP (DBC), glucagon, epinephrine, and a control infusion of a Ringer solution containing an appropriate amount of ethanol added to the perfusion. Ethanol was used as a control since ethanol is used as a diluent of PGE₁, since extremely small amounts of ethanol can influence carbohydrate and lipid metabolism in the perfused liver, and since certain short chain alcohols, including ethanol, have been shown to activate adenylate cyclase in vitro [23,24].

Isolated male fed or fasted donor Sprague-Dawley rat livers (mean body weight = 297 ± (S.E.M.) 25 g, mean liver weight = 6.4 ± 1.8 g) were perfused with 85 ml Krebs-Ringer bicarbonate solution containing 3% albumin and 0.95% ethanol, as previously described [25]. Samples of perfusate glucose, cAMP, cGMP and free fatty acids (FFA), hepatic glycogen, triglyceride (TG), and cAMP, and bile were taken during a control period of 45 min and at 2, 3, 5, 10, 15, 30, or 60 min intervals and after exposure to PGE₁, epinephrine, glucagon, or DBC (see Table 1 for concentrations

1 Kindly supplied by J.E. Pike, The Upjohn Company, Kalamazoo, Michigan.
used. Since PGE₁ may influence some of the metabolic effects of catecholamines in certain other tissues, the possible interaction between epinephrine and PGE₁ was also studied in the perfusion experiments.

Studies in the 18 hour-fasted intact canine liver were carried out using direct intraarterial infusion of PGE₁, PGE₂, or indomethacin, a prostaglandin synthetase inhibitor (5 mg/kg for 5 min then 5 mg/min), into the hepatic artery via a catheter placed in the celiac axis. All studies were done in the acute dog anesthetized with intravenous sodium pentobarbital, 30 mg/kg. Infusions were given after a 30 min control period with saline or indomethacin. Approximately 25% of the infused radiopaque agent went directly into the hepatic artery, the remainder into the arterial blood supply to the stomach. Samples of dog liver were obtained for cAMP and cGMP at frequent intervals during a 30 min exposure to exogenous PGE₁ or PGE₂ or indomethacin.

cAMP and cGMP were measured by radioimmunoassay [26]. Other biochemical determinations were performed as described [25] or by standard techniques. All results are given as mean ± S.E.M. and were compared using the Student’s t test for mean differences of paired samples.

RESULTS

During the 45 min period of equilibration there was a moderate uptake of fat as determined by a fall in perfusate FFA. Heparinization during the removal of fed donor livers accounted for initially higher levels of FFA. However, during the study period, PGE₁ failed to alter FFA or cAMP levels in the perfusate, bile flow or induce hyperglycemia compared to control perfusions (Fig. 1).

That this system responded to known glycogenolytic agents which increase hepatic cAMP content is shown in Fig. 2. Epinephrine and glucagon increased hepatic cAMP content an average of 36% and 770%, respectively, with maximal responses 15 min after their administration. PGE₁ did not significantly influence hepatic cAMP levels compared to control infusions. DBC caused extraordinarily high cAMP levels in the few livers analyzed, as well as in the perfusate and bile.

Figure 3 illustrates the failure of PGE₁ to significantly alter hepatic TG concentration after 60 min infusion. These studies were carried out in only 6 of the 9 experiments, respectively, in the control and PGE₁-treated groups.

Table 1 summarizes the increment changes observed in perfusate glucose and cAMP and hepatic glycogen, TG and cAMP content during the 60 min period of
FIG. 1. Effect of PGE$_1$ or Ringer-ethanol (ETOH) infusion on mean perfusate FFA, cAMP, glucose increment change, and bile flow in isolated perfused fed rat liver. Zero time value in this and Figs. 2 and 3 represents time after 45 minute period of equilibration. Concentrations of drugs in this and Figs. 2 and 3 are given in Table 1. From Levine[21].

FIG. 2. Effect of control infusion (Ringer-ETOH), PGE$_1$, glucagon, and epinephrine on hepatic cAMP content (mean ± S.E.M.) at time intervals shown in abscissa. From Levine [21].
infusion. Epinephrine, glucagon, and DBC caused significant glycemia. Glycogen content in the liver decreased after 60 min of perfusion in control livers. The decrease was significantly greater when epinephrine, glucagon, or DBC was added to the perfusate, but unchanged by PGE₁.

Epinephrine-induced glycogenolysis and augmentation of hepatic cAMP were not affected by pretreatment or concomitant administration of PGE₁, as shown in a representative example of three such studies (Fig. 4).

The effects of PGE₁ or Ringer solution on certain biochemical parameters in the fasted perfused rat liver are shown in Fig. 5. PGE₁ did not significantly alter perfusate glucose or cAMP, or hepatic cAMP, glycogen, or TG as shown in Fig. 5. Note that the perfusate cAMP concentration appeared to be greater in the fasted than in the fed perfused liver (Fig. 1).

The effects of PGE₁ or Ringer solution on hepatic cAMP and cGMP concentrations in fasted rat liver, as early as 2,3,5,10,15 and 30 min after the onset of perfusion, are shown in Fig. 6. There was no significant difference in the response of the hepatic cyclic nucleotides to either PGE₁ or Ringer solution. Moreover, hepatic glycogen levels did not change significantly before and 30 min after perfusion. Mean control liver glycogen for Ringer solution and PGE₁ were, respectively, 0.199 ± 0.62 and 0.170 ± 0.039 mg/g before and 0.190 ± 0.073 and 0.142 ± 0.030 mg/g 30 min after infusion. Thus, in the fasted rat liver PGE₁ failed to significantly alter cyclic nucleotide, TG, and glycogen content.

The effects of both PGE₁ and PGE₂ on canine hepatic cyclic nucleotide concentrations are shown in Fig. 7. PGE₁ or PGE₂ failed to significantly alter cyclic nucleotide content for 30 min after the onset of arterial infusion.
FIG. 4. Effect of pre-treatment with PGE₁ or concomitant PGE₁ infusion on epinephrine-induced changes in perfusate cAMP and glucose and on hepatic cAMP and glycogen content. From Levine [21].

FIG. 5. Effect of PGE₁ and Ringer-ETOH infusion on mean perfusate glucose and cAMP, and mean hepatic cAMP, glycogen, and TG in the fasted perfused liver. From Levine [21].
FIG. 6. Effect of Ringer-ETOH or PGE₁ infusion on mean (± S.E.M.) hepatic cAMP and cGMP concentrations in fasted perfused rats. As noted in the text, hepatic glycogen levels did not significantly change compared to the control (zero time) value.

FIG. 7. Effect of Ringer-ETOH, PGE₁, or PGE₂ infusion (± S.E.M.) on cAMP and cGMP concentrations in fasted intact dog liver. Thirteen experiments were performed, 7 with PGE₁ and 6 with PGE₂.
The effects of either PGE₁ or PGE₂ on canine hepatic cyclic AMP concentration are shown in Table 2. Neither PGE₁ or PGE₂ showed a differential effect on hepatic cAMP concentration during the 30 min perfusion. The effects of PGE₂ on cGMP concentration in 12 fasted dog livers are shown in Table 3. There were no significant differences between the treatment periods compared to the basal period (zero time value). Moreover, PGE₂ administered in conjunction with indomethacin failed to potentiate the action of PGE₂ on liver cAMP (Table 2) or cGMP (Table 3) content. Pretreatment with indomethacin for 30 min before the zero time showed values essentially similar to the basal level (789 ± 62 pmol/g and 2.4 ± 0.5 pmol/g, respectively, for cAMP and cGMP).

DISCUSSION

These studies indicate that PGE₁ does not stimulate the rat liver adenylate cyclase-cAMP system even though the same system responds to known agents which activate cAMP (glucagon and epinephrine) and responds to exogenous DBC. Moreover, cGMP is not stimulated by PGE₁ in the same perfusion model. In another species, the dog, PGE₁ and PGE₂ failed to activate hepatic cAMP or cGMP. Thus, it appears that hepatic cyclic nucleotides fail to respond to intraportal infusion of PGE₁ and PGE₂.

Our findings fail to confirm the report of Zenser et al. [10] who did not transient stimulation of rat liver cAMP after PGE₁ administration. The apparently conflicting observations may be reconciled in part by differences between their assay techniques, which may account for spurious cAMP values, and our own. First, cross reactivity and nonspecific reactivity producing an artificial elevation of assayable cAMP is less common with the use of radioimmunoassay compared to the protein binding technique [26], as was utilized by Zenser et al. [10]. Second, pigments, including hemoglobin, can be another source of falsely elevated assayable cyclic nucleotides. Blood pigments are acid extractable during purification procedures. Unless great effort is made to avoid any tissue contamination by blood prior to processing, such pigments may significantly alter cAMP values. The use of a neutral alumina column

**TABLE 2**

| Agent   | No. | 0     | 5     | 10    | 15    | 30 min |
|---------|-----|-------|-------|-------|-------|--------|
| PGE₁    | 7   | 799 ± 81| 893 ± 141| 900 ± 195| 924 ± 195 | 612 ± 213 |
| PGE₂    | 6   | 873 ± 71| 861 ± 72 | 982 ± 193| 1114 ± 189| 1039 ± 121 |
| PGE₂ +  | 3   | 791 ± 26| 879 ± 117| 1172 ± 180| 590 ± 209 | 1046 ± 139 |
| Indomethacin | |       |       |       |       |        |

**TABLE 3**

| Agent   | No. | 0     | 5     | 10    | 15    | 30 min |
|---------|-----|-------|-------|-------|-------|--------|
| PGE₁    | 12  | 4.4 ± 0.7| 4.9 ± 0.8| 4.3 ± 0.8| 4.8 ± 0.6 | 4.4 ± 0.7 |
| PGE₂    | 12  | 4.0 ± 2.3| 2.6 ± 0.5| 2.6 ± 0.8| 2.3 ± 0.6 | —      |
| Indomethacin | |       |       |       |       |        |
EFFECTS OF PROSTAGLANDINS ON HEPATIC METABOLISM

in sample preparation effectively removes hemoglobin from contaminated liver samples [26]. Zenser et al. [10] failed to employ such an alumina precolumn.

The possibility exists that continuous infusion of PGE1 or PGE2 did not increase intracellular prostaglandin levels. However, in perfused rat liver PGE1 (6.7 μg/min) increased prostaglandin concentrations in portal vein, perfusate, and liver as measured by radioimmunoassay (D.E. Wilson, personal communication). The initial perfusate concentration was 4.9 \times 10^{-9} M before PGE1 infusion, 1.9 \times 10^{-7} M during infusion, and 6.2 \times 10^{-8} M 30 min after discontinuing PGE1 administration. Thus PGE1 infusion, which resulted in a portal vein concentration of 6.4 \times 10^{-7} M during prostaglandin infusion, markedly increased perfusate concentrations. PGE concentration in liver rose from 20.9 ± 0.8 to 1091 ± 89 pg/mg tissue and decreased to 362 ± 30 pg/mg 30 min after discontinuing the infusion. From this data, it appears that prostaglandin infusion in rat liver is capable of increasing hepatic concentrations of prostaglandins even though approximately 70% of the infused PGE1 is metabolized during a single passage through the liver (D.E. Wilson, personal communication).

The interaction of PGE1 with catecholamines in liver was of particular interest since prostaglandins antagonize cAMP-induced lipolysis in fat cells caused by epinephrine [27] and inhibit hormonally-induced responses in several other tissues [28,29]. Although PGE1 was reported not to affect glucagon-stimulated increases in hepatic cAMP in vitro, when injected intraortally PGE1 inhibited glucagon stimulation of cAMP and stimulated cAMP itself [11]. In contrast, our studies would support the concept that PGE1 fails to alter the effects of another hepatic glycogenolytic agent, epinephrine, in rat liver.

Our data do not support a physiologic role for PGE1 on hepatic glycogenolysis or lipolysis. Preliminary studies in our laboratory, using lactate as a substrate for gluconeogenesis in the isolated perfused rat liver, have shown that PGE1 failed to induce a greater gluconeogenic effect compared to control infusion with Ringer solution. Glucagon, added at the same time as lactate, induced a further increased glucose level than either a control or PGE1 infusion. However, it was of interest that when glucagon was given simultaneously with PGE1, there appeared to be inhibition of the glucagon-induced hepatic gluconeogenesis. This particular observation would support the findings of Imesch and Rous [20] and indicate that PGE1 may have inhibitory effects on hormone-mediated hepatic gluconeogenesis.

Other preliminary observations in our laboratory concerning the effects of PGE1 on liver glycogen synthetase indicate that PGE1 and PGE2 failed to alter glycogen synthetase in either fed or fasted perfused rat or fasted intact dog liver. These findings contrast with the report of Curnow and Nuttall [12], who showed a decrease in glycogen synthetase in the fed and fasted intact rat liver 5 and 30 min, respectively, after PGE1 administration.

Although we have not yet investigated the effects of PGE1 on lipogenesis, ureogenesis, or amino acid transport, if effects are subsequently found on these metabolic parameters they probably would occur independent of a change in hepatic cAMP or cGMP activity. It should be pointed out that our conclusions are limited to the perfused rat liver and intact dog liver. Further studies are indicated before one can conclude that other types of prostaglandins fail to stimulate the hepatic cyclic nucleotides in other species.

REFERENCES

1. Levine RA, Pesch LA, Klatskin G, et al: Effect of serotonin on glycogen metabolism in isolated rat liver. J Clin Invest 43:797–809, 1964
2. Levine RA: Effect of glycolytic agents on phosphorylase activity of perfused rat liver. Am J Physiol 208:317–323, 1965
3. Pradhan TK, Criss WE: Prostaglandin regulation of adenylate kinases purified from liver, skeletal muscle, and hepatoma. Oncology 33:15–16, 1976
4. Bergstrom S, Carlson LA, Weeks JR: The prostaglandins: A family of biologically active lipids. Pharmacol Rev 20:1–48, 1968
5. Sweat RW, Wineck TS: The stimulation of hepatic adenylate cyclase by prostaglandin E1. Biochem Biophys Res Comm 55:522–529, 1973
6. Yamashita L, Sweat FW: The stimulation of rat liver adenylate cyclase by prostaglandin E1 and E2. Biochem Biophys Res Comm 70:438–444, 1976
7. Tomasi V, Poll A, Ferretti E, et al: Hormone and prostaglandin E1 control of potassium and cyclic AMP levels in isolated rat liver cells. Adv Enzyme Regul 13:189–199, 1975
8. Tomasi V: Prostaglandin E1 as an intercellular regulator of cyclic AMP levels. Exper Cell Biol 44:260–277, 1976
9. Ferretti E, Biondi C, Tomasi V: Cyclic AMP control by prostaglandin E1 in non-parenchymal liver cells. FEBS Letters 69:70–74, 1976
10. Zenser TV, DeRubertis FR, Curnow RT: Effect of prostaglandins on hepatic adenylate activity and hepatic cAMP content. Endocrinology 94:1404–1410, 1974
11. DeRubertis FR, Zenser TV, Curnow RT: Inhibition of glucagon-mediated increases in hepatic cyclic adenosine 3',5'-monophosphate by prostaglandin E1 and E2. Endocrinology 94:93–101, 1974
12. Curnow RT, Nuttall FQ: Effect of prostaglandin E1 administration on the liver glycogen synthetase and phosphorylase systems. J Biol Chem 247:1892–1898, 1972
13. Bohle E, May B: Metabolic Effects of Prostaglandin E1 Upon Lipid and Carbohydrate Metabolism, Prostaglandin Symposium of the Worcester Conference for Experimental Biology. Edited by PW Ramwell and JE Shaw. New York, Interscience, 1968, pp 115–129
14. Lemberg A, Wikinski R, Izurieta EM, et al: Effects of prostaglandin E1 and norepinephrine on glucose and lipid metabolism in isolated perfused rat liver. Biochem Biophys Acta 248:198–204, 1971
15. Sacca L, Perez G, Rengo F, et al: Effects of different prostaglandins on glucose kinetics in the rat. Diabetes 23:532–535, 1974
16. Wheeler GE, Epand RM: Prostaglandin E2: Anomalous effects on glucose production in rat liver. Molec Pharmacol 11:335–339, 1975
17. Exton JH, Robinson GS, Sutherland EW, et al: Studies on the role of adenosine 3',5'-monophosphate in the hepatic action of glucagon and catecholamines. J Biol Chem 246: 6166–6177, 1971
18. Berti F, Lentati R, Usardi MM: La prostaglandina E1 (PGE1) spiega attivita ipoglicemizzante. Boll Soc Ital Sper 41:1327–1329, 1965
19. Wilson DE, Hankewych M: Prostaglandin E1 effects on hepatic glycogenolysis. Gastroenterology 65:576, 1973
20. Imesch E, Rous S: Effect of PGE, on gluconeogenesis and glyceral esterification in perfused liver of fasted rats. Prostaglandins 9:945–957, 1975
21. Levine RA: Effect of prostaglandin E1 on hepatic cyclic AMP activity, carbohydrate and lipid metabolism. Prostaglandins 6:509–521, 1974
22. Dawson W, Jessup SJ, McDonald-Gibson W, et al: Prostaglandin uptake and metabolism by the perfused rat liver. Br J Pharmacol 39:585–598, 1970
23. Gorman RE, Bitensky MW: Selective activation by short chain alcohols of glucagon responsive adenyl cyclase in liver. Endocrinology 87:1073–1081, 1970
24. Greene HL, Herman RH, Kraemer S: Stimulation of jejunal adenyl cyclase by ethanol. J Lab Clin Med 78:336–342, 1971
25. Levine RA, Washington A: Glycogenolytic activity of cyclic 3',5'-monophosphates in perfused rat liver. Endocrinology 87:377–382, 1970
26. Schwartzel EH, Bachman S, Levine RA: Cyclic nucleotide activity in gastrointestinal tissues. Anal Biochem 78:395–405, 1977
27. Butcher RW, Baird CE: Effect of lipolytic and antilipolytic substances on adenosine 3',5'-monophosphate levels in isolated fat cells. J Biol Chem 243:1705–1712, 1968
28. Ramwell PW, Rabinowitz I: Interaction of Prostaglandins and Cyclic AMP, Effects of Drugs on Cellular Control Mechanisms. Edited by BR Rabin and RB Freedman. Baltimore, University Park Press, 1972, pp 207–235
29. Lemberg A, Wikinski R, Izurieta EM, et al: Effect of prostaglandin E1 and norepinephrine on lipid and glucose metabolism in isolated perfused rat livers overloaded with a lipid substrate. Biochim Biophys Acta 280:458–465, 1972