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Prostaglandin E_1: Anomalous Effects on Glucose Production in Rat Liver

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SUMMARY

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Prostaglandin E_1 decreases the basal rate of glucose production and abolishes the increase usually caused by glucagon or cyclic 3',5'-AMP. This effect is mimicked by insulin, indomethacin, and acetylsalicylate, which also decrease prostaglandin levels. Inhibition of the synthesis of a reactive metabolic intermediate in prostaglandin biosynthesis may explain these apparently anomalous effects.

INTRODUCTION

The ubiquitous quasihormones, the prostaglandins, have been credited with numerous biological functions (1), most of which depend on the ability of a particular prostaglandin to either increase or decrease tissue levels of cyclic 3',5'-AMP (2, 3). Mammalian liver, which contains enzymes responsible for both prostaglandin synthesis and degradation (4), also possesses an adenylyl cyclase system which has recently been shown to be stimulated by prostaglandin E_1 (5-7). We wish to report an effect of PGE_1[1] on rat liver not explicable in terms of its effect on adenylyl cyclase activity.

MATERIALS AND METHODS

Livers were obtained from fed male Woodlyn-Wistar rats (150-250 g) and were perfused with Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 4% defatted (8) bovine serum albumin. Slices (70-90 mg) were made using a Stadie-Riggs tissue slicer (A. H. Thomas Company). Prostaglandin E was dissolved in 10 μl of 95% ethanol and then diluted with bicarbonate buffer containing 4% albumin and 10 mM pyruvate at pH 7.4. Bicarbonate-albumin-pyruvate buffer (2 ml), with or without PGE_1, was incubated at 37° (in a reciprocating water bath), and the slices were added. The rate of glucose production in the control was 5.5 ± 0.2 μmoles/g of liver, wet weight/hr. Addition of 10 μg/ml of glucagon resulted in 25-35% stimulation in the rate of glucose production. Substitution of 10 mM lactate for pyruvate reduced both the basal and glucagon-stimulated glucose production to 70% of that given by pyruvate. Glucose was determined on portions of the supernatant, after deproteinization, by the glucose oxidase method. The slices were then removed, blotted, and weighed.

The concentrations of ethanol in the experiment were 0.01% (10 μg/ml of PGE_1), 0.001% (1 μg/ml of PGE_1), and 0.0001% (0.1

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[1] The abbreviations used are: PGE, PGF, and PGA, prostaglandins E, F, and A; cAMP, adenosine cyclic 3',5'-monophosphate; 5'-AMP, adenosine 5'-monophosphate; ADP, adenosine 5'-diphosphate.

μg/ml of PGE_1). Several workers have reported a stimulation of adenylyl cyclase by ethanol (6, 9, 10), but the ethanol concentrations required to give a significant effect are of the order of 1%.

Cyclic nucleotide phosphodiesterase was assayed by the method of Thompson *et al.* (11). A total particulate fraction and a particle-free supernatant were prepared by centrifugation of rat liver homogenates at 6.8×10^6 *g* min. The incubation mixture contained 50 μM cAMP (1.5×10^6 cpm of [^{3}H]cAMP), 5 mM $MgCl_2$, and 20 mM Tris-HCl, pH 8.0, in a final volume of 0.2 ml. Incubation at 30° was terminated after 5 or 15 min by the addition of 50 μl of a mixture containing cAMP, 5'-AMP, adenosine, and ADP, and the reaction mixture was placed in a boiling water bath for 3 min. Then 0.1 ml of reaction mixture was applied to Whatman No. 3MM paper, and the chromatograms were developed overnight with 1 M ammonium acetate–95% ethanol (15:35) (12).

To determine the permeability of rat liver slices to cAMP, the slices were incubated at 37° in 2 ml of bicarbonate-albumin-pyruvate buffer containing 50 μM cAMP (147,000 cpm of [^{3}H]cAMP). Slices were removed at 2, 5, and 15 min, rinsed in nonradioactive medium, and homogenized in 10 ml of 5% trichloracetic acid in an overhead blade homogenizer. The homogenate was centrifuged, and portions of the supernatant were taken for determination of radioactivity. The remainder was extracted three times with diethyl ether to remove the trichloracetic acid, and the neutral solution was evaporated to dryness at 50° in a stream of air. The percentage of radioactivity present as cAMP was then determined as in the phosphodiesterase assay.

To determine prostaglandin levels, 4 ml of methylal–ethanol (3:1, v/v) at −25° were added to a liver slice which had been incubated for various periods of time under the appropriate conditions. The mixture was homogenized for 1 min at full speed using an overhead blade homogenizer. The levels of prostaglandins E plus A were determined by radioimmunoassay, using a commercially available kit (Clinical Assays, Inc., Cambridge, Mass.).

RESULTS AND DISCUSSION

PGE_1 (28–0.28 μM) decreased glucose production from rat liver slices (Fig. 1), with 36% inhibition observed after 1 hr. Exton *et al.* (13) reported that in perfused livers PGE_1 lowered glucose output from 164 ± 19 to 125 ± 13 μmoles/100 g of body

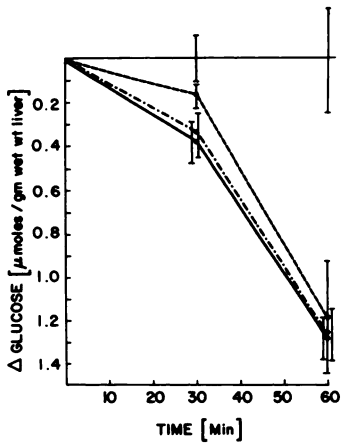

FIG. 1. *Ability of PGE_1 to decrease rate of glucose production from rat liver*

Results are plotted as change in glucose production over control values; values are the means of six determinations and are shown ± one standard deviation. ●- - -●, 0.1 μg/ml of PGE_1; ●·—·●, 1 μg/ml of PGE_1; ●——●, 10 μg/ml of PGE_1.

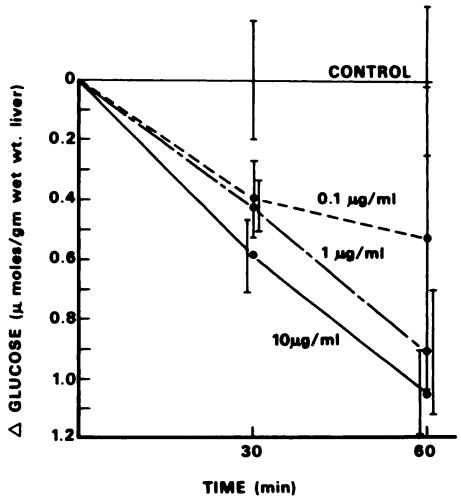

FIG. 2. *Ability of PGE_2 to decrease rate of glucose production from rat liver*

Results are plotted as change in glucose production over control values; values are the means of six determinations and are shown ± one standard deviation. ●- - -●, 0.1 μg/ml of PGE_2; ●·—·●, 1 μg/ml of PGE_2; ●——●, 10 μg/ml of PGE_2.

weight in 30 min, and Wimhurst and Harris (14) demonstrated that prostaglandin $F_{2\alpha}$ reduced perfusate glucose levels from fasted rat livers. We found similar effects with prostaglandin E_2 (Fig. 2). The changes in mitochondrial function and enzyme activities responsible for the effect of $PGF_{2\alpha}$ have been delineated (14).

The threshold concentration of PGE_1 required to stimulate the production of cAMP in perfused rat liver is 20 μM [2 μM is ineffective (6)], and in isolated membrane preparations it is 1.4 μM [optimal stimulation at 7 μM (5)], approximately 10 times higher than the concentration required to produce a significant effect on glucose production. It appears then that exogenous PGE_1 in rat liver has an insulin-like effect, such as that demonstrated in rat adipocytes (15). PGE_1 also abolishes the 25–40% stimulation of glucose production given by glucagon (10 μg/ml) (Fig. 3A). Somewhat more surprisingly, PGE_1 abolishes completely any stimulation (normally 20%) elicited by exogenous cAMP (50 μM) (Fig. 3B) and markedly reduces the stimulation (from 42% to 14%) elicited by higher concentrations of cAMP (1 mM) (Fig. 3C).

PGE_1 does not affect the ability of glucagon to stimulate adenylyl cyclase in membrane preparations from rat liver (5), although it has recently been reported to affect the glucagon-stimulated rise in cAMP levels in perfused liver at high prostaglandin to glucagon ratios (16). However, $PGF_{2\alpha}$, which has effects similar to those of PGE_1 on glucose metabolism (14), does not lower glucagon-stimulated levels of cAMP even in intact perfused liver (16). In view of this and the action of PGE_1 on the stimulation of glucose production by exogenous cAMP, it seemed a reasonable assumption that PGE_1 was affecting either cyclic nucleotide phosphodiesterase activity (the enzyme responsible for the hydrolysis of cAMP) or the permeability of hepatocytes to cAMP. However, PGE_1 does not significantly affect either of these two parameters (Tables 1 and 2). A similar conclusion regarding the effect of PGE_1 on phosphodiesterase activities has recently appeared (6, 16). Thus, if PGE_1 does influence the glucagon-stimulated rise in intracellular cAMP, it is not via changes in the activity of adenylyl cyclase or phosphodiesterase. In addition, the observation that exogenous PGE_1 can inhibit hepatic glucose production, even in the presence of cAMP which has permeated into the tissue slice, makes it even more likely that this effect is independent of intracellular levels of cAMP.

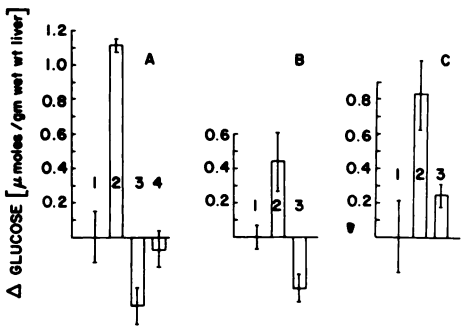

FIG. 3.

A. Effect of PGE_1 on glucagon-stimulated glucose production. *1*, control; *2*, 10 μg/ml of glucagon; *3*, 10 μg/ml of PGE_1; *4*, 10 μg/ml of glucagon + 10 μg/ml of PGE_1. Incubation time was 40 min. Results are shown as the means of 12 determinations ± one standard deviation. B and C. Effect of PGE_1 on production of glucose stimulated by 50 μM and 1 mM cAMP, respectively. *1*, control; *2*, cAMP; *3*, cAMP + 10 μg/ml of PGE_1. Incubation time was 30 min. Results are shown as the means of six determinations ± one standard deviation.

TABLE 1

Phosphodiesterase activity

Results are given as percentage of recovered radioactivity not migrating as cAMP (i.e., percentage of cAMP hydrolyzed) and are the means and standard deviations of duplicate determinations. There is no significant difference between runs with and without PGE_1 either in the percentage of cAMP recovered or in the relative concentrations of the metabolites of cAMP.

Phosphodiesterase	Time	PGE_1, 10 μg/ml	cAMP destroyed
	min		%
Particulate	5	–	26 ± 1.2
		+	30 ± 1.8
	15	–	57 ± 0.2
		+	55 ± 0.4
Soluble	5	–	16 ± 1.8
		+	15 ± 0.3
	15	–	49 ± 1.7
		+	48 ± 5.4

TABLE 2

Permeability of rat liver slices to exogenous cAMP

Time	PGE_1, 10 μg/ml	^3H[a]	cAMP destroyed[b]
min		*cpm*	%
2	–	268 ± 40	72[b]
	+	320 ± 32	68[b]
5	–	1535 ± 447	72
	+	1464 ± 583	75
15	–	1173 ± 34	78
	+	1546 ± 517	79

[a] Total radioactivity which entered the cell from 147,000 cpm (50 μM) of extracellular [^{3}H]cAMP.

[b] Results determined as for Table 1.

Physiological concentrations (33–100 μ units/ml) of insulin have been shown to reduce glucose production in rat liver (17), and therefore it is possible that the mode of action of insulin could be via an increased synthesis of prostaglandin. However, the presence of insulin significantly reduces the amount of immunoreactive PGE plus PGA in rat liver slices (Fig. 4). This inhibition is not immediate, and there may even be a slight rise in prostaglandin levels during the first 10 min of incubation. Dawson and Ramwell (See ref. 1) using perfused rat liver, found that glucagon, which has opposite metabolic actions to insulin, stimulated the release of prostaglandins (A, E, and F) into the perfusate. Insulin also lowers prostaglandin levels in rat adipose tissue (1). The opposing effects of insulin and glucagon on prostaglandin levels could be explained as a result of their opposing actions on cAMP levels, assuming that this nucleotide is involved in the regulation of prostaglandin levels in the liver as it is in fat cells (18).

Vane (19, 20) suggested that the mode of action of certain anti-inflammatory drugs, such as aspirin and indomethacin, is to inhibit the biosynthesis of prostaglandins. If this was true for rat liver, the action of insulin, aspirin, and indomethacin on glucose production should be similar. Insulin (100 μunits/ml), indomethacin (10 μg/ml), and aspirin (100 μg/ml) all significantly lower the glucagon stimulation of glucose production (Fig. 5), this effect being duplicated by exogenous PGE_1. The physiological importance of these effects, and the possible relationship between prosta-

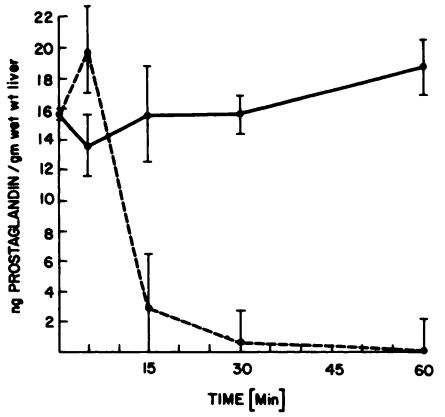

FIG. 4. *Effect of insulin on endogenous prostaglandin (PGE and PGA) level in rat liver*

Results are shown as the means of four determinations ± one standard deviation. Insulin (100 μunits/ml) produced 20% inhibition of glucose production after 1 hr of incubation. ●——●, control; ●- - -●, 100 μunits/ml of insulin.

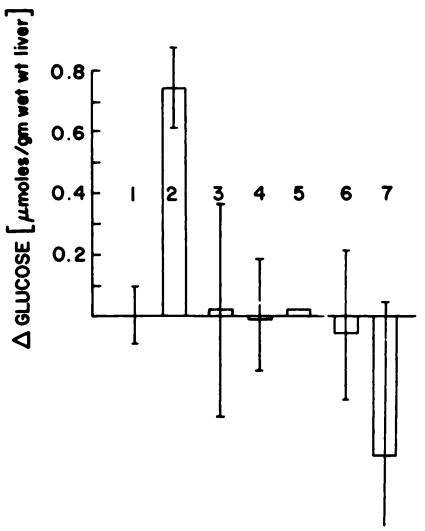

FIG. 5. *Effect of insulin, prostaglandin E_1, indomethacin, and aspirin on production of glucose by rat liver*

1, control; *2*, 10 μg/ml of glucagon; *3*, 10 μg/ml of glucagon + 100 μunits/ml of insulin; *4*, 100 μunits/ml of insulin + 10 μg/ml of PGE_1; *5*, 100 μunits/ml of insulin; *6*, 10 μg/ml of glucagon + 10 μg/ml of indomethacin; *7*, 10 μg/ml of glucagon + 100 μg/ml of aspirin. Results are shown as the means ± one standard deviation of 12 (*1*, *2*, *6*), six (*3*, *4*, *7*), or one (*5*) determinations.

glandin levels and hormone action, remain to be established.

There is an apparent anomaly in that agents which lower prostaglandin levels lower glucose production, while at the same time raising prostaglandin levels by the addition of exogenous PGE_1 also lowers glucose production. PGE_1 also has opposite effects on the incorporation of acetate into cholesterol, fatty acids, and carbon dioxide in liver slices, which is dependent on its concentration in the liver tissue (21).

There are several possible explanations of this apparently anomalous behavior of exogenous PGE_1. One is that stimulation of glucose production by cAMP is dependent on a prostaglandin similar to but not identical with PGE_1; PGE_1 may then act as a competitive inhibitor for this PGE_1-related endogenous prostaglandin. Another explanation is that the reactive prostaglandin in the production of glucose is a metabolic intermediate of prostaglandin biosynthesis, such as the efficacious peroxide intermediates (20), the levels of which are decreased by aspirin or indomethacin through inhibition of synthesis (19, 20), by insulin via an unknown mechanism, and by exogenous PGE_1 through feedback inhibition of prostaglandin synthesis.

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