Stimulatory Effects of Prostaglandins E-1, E-2, and F-2-Alpha on Glucagon and Insulin Release In Vitro

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SUMMARY

The effects of prostaglandins (PG) E_1, E_2, and F_2 upon the release of glucagon and insulin from the isolated perfused rat pancreas were investigated. In the presence of 5.6 mM glucose the release of both glucagon and insulin was stimulated by $PGF_{2\alpha}$ at several concentrations ranging from 2.8×10^{-8} M to 3.6×10^{-6} M. Ten-minute perfusions of 1.4×10^{-6} M PGE_1 or 2.8×10^{-7} M $PGF_{2\alpha}$ evoked biphasic release of both islet hormones, and they augmented their biphasic release induced by 10 mM L-arginine. The addition of 5 mM fumarate, glutamate, and pyruvate permitted PGE_2 to stimulate insulin release at a concentration as low as 1.1×10^{-9} M. The failure of 10^{-6} M oleic acid to elicit hormone release suggested that these effects of PGs were not nonspecific effects of long-chain fatty acids. In the absence of glucose, in response to 2.8×10^{-7} M PGE_1 or PGE_2 or $PGF_{2\alpha}$, the magnitudes of glucagon release were similar to or slightly greater than those seen with 5.6 mM D-glucose, but the release of insulin failed to occur. In the presence of 16.7 mM glucose, in response to 1.4×10^{-6} M PGE_2 and 2.8×10^{-7} M $PGF_{2\alpha}$, the release of glucagon was blunted and that of insulin unchanged as compared with the secretory responses seen in the presence of 5.6 mM D-glucose.

These data indicate that administered PGs can stimulate acutely the release of glucagon and insulin at pharmacologic concentrations as well as at concentrations similar to those found in pancreatic tissues and effluent.

These observations are in keeping with the hypothesis that PGs of the E and F series may play a role in the regulation of secretion of glucagon and insulin. DIABETES 27:801-09, August, 1978.

The prostaglandins (PG) are a group of biologically active lipids that influence the release of various hormones, including those of the ovary,[2] adrenal gland,[3-5] and the anterior pituitary.[6-13]

The possible role PGs play in regulating the release of pancreatic islet hormones has been studied in vitro and in vivo. Information about the effects of PGs on secretion of glucagon is limited. Employing the method of the isolated perfused rat pancreas we have shown that PGE_2 stimulates the release of glucagon and augments glucagon response to L-arginine.[14] Since our report, Sacca and Penez have demonstrated in vivo that the administration of PGE_1 leads to increases in plasma levels of glucagon in rats.[15] The relationships of PGs and the secretion of insulin have been studied more extensively. In vitro studies of Vance et al.[16] and Rossini et al.[17] did not reveal an effect of PGE_1 or PGA_1 on the release of insulin from isolated rat islets. Saunders and Moser[18] reported that, in the presence of 5.5 mM glucose, PGE_2 or $PGF_{2\alpha}$ had no effect on insulin release from the isolated perfused rat pancreas. Johnson et al.[19] showed that insulin release from isolated islets induced by 16.7 mM glucose was augmented by PGE_1, PGE_2, and $PGF_{2\alpha}$, an effect that was not observed at a glucose concentration of 1.7 mM. PGE_1 and PGE_2 also reversed partially norepinephrine-induced inhibition of insulin release.[19] Burr and Sharp[20] reported that, in

The work was presented in part during the Midwestern Regional Meeting of the American Federation for Clinical Research in Chicago, Illinois, in November 1974.

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Accepted for publication February 6, 1978.

vitro, PGE_1 stimulates insulin release in the presence of 2.8 mM glucose and reverses the inhibition of insulin release by epinephrine, but it inhibits the release evoked by 16.7 mM glucose. In the isolated perfused rat pancreas we have shown that PGE_2 is a potent secretagogue of insulin at physiologic concentrations of glucose, and it augments the release of insulin evoked by arginine.[14] The results of in vivo studies on the effects of PGs on plasma levels of insulin have been variable as well. In the mouse the administration of PGE_1 induced increases in plasma insulin as well as blood glucose.[21] Lefebvre and Luyckx observed in the dog that pancreaticoduodenal venous levels of insulin increased during and after intravenous infusions of PGE_1, while the insulin output increased only after the infusions.[22] These changes in insulin occurred without any concomitant changes in plasma glucose. Spellacy et al. did not observe any changes in plasma insulin or glucose in pregnant women who received infusions of PGE_2 or $PGF_{2\alpha}$.[23] In sheep, intravenous infusion of PGE_1 did not alter plasma levels of insulin or glucose.[24] In the rat, PGE_1 caused no change in plasma insulin despite increases in plasma glucose.[24] Sacca et al. observed in the rat that PGE_1 or PGE_2 lowered plasma insulin and raised plasma glucose; PGE_1 inhibited the increases in plasma insulin evoked by glucagon, aminophylline, or tolbutamide.[25] Robertson et al. reported that in the anesthetized dog, intravenous infusions of PGE_1 lowered basal levels and glucose-induced increases in serum insulin.[26,27] More recently, Robertson and Chen have shown in normal man that intravenous infusions of PGE_2 inhibit glucose-induced but not arginine-induced increases in plasma insulin.[28]

To elucidate the effects of prostaglandins on the secretion of pancreatic islet hormones, we have extended our in vitro studies to include PGE_1 and $PGF_{2\alpha}$. The results reported herein demonstrate that, similar to the effect of PGE_2, PGE_1 and $PGF_{2\alpha}$ stimulate the release of glucagon and insulin and that this effect of PGs is modulated by glucose.

MATERIALS AND METHODS

Male albino Sprague-Dawley rats weighing 250 to 300 gm. were fed Purina rat chow. After 15 to 18 hours of fasting, the pancreas was removed surgically together with its intact vasculature and a small segment of the duodenum attached to it. This preparation was placed in a multichannel extracorporeal organ perfusion apparatus. The organ was perfused at 37° C. at a flow rate of 2.5 ml. per minute with Krebs-Ringer bicarbonate buffer containing 40 gm. per liter dextran (average Mol. wt. 72,000; Pharmachem) and 2 gm. per liter bovine serum albumin (Miles Laboratories). The details of the surgical procedure, the perfusion apparatus, and the artificial perfusion media have been reported previously.[14,29,30] Various compounds were added to the perfusion media as indicated in the RESULTS section. Prostaglandins E_1, E_2, and the tromethamine salt of $F_{2\alpha}$ were generous gifts of Dr. John Pike of the Upjohn Company in Kalamazoo, Michigan. Oleic acid was obtained from Sigma Chemical Company. PGE_1 and oleic acid were first dissolved in 50 μl. absolute ethyl alcohol and then diluted further in the perfusion medium; other perfusion solutions used during the PGE_1 experiments also received the same amount of alcohol. The concentration of ethanol in the final perfusion solution did not exceed 1 μl. per liter. In this perfusion system, absolute ethanol at concentrations up to 10 ml. per liter does not affect the basal secretion of glucagon or insulin in the presence of 5.6 mM D-glucose. Prostaglandins E_2 and $F_{2\alpha}$ were dissolved directly in the perfusion medium. D-glucose, fumaric acid, glutamic acid, and sodium pyruvate were of analytic reagent grade (Sigma Chemical). L-arginine monohydrochloride was supplied by ICN.

The experiments were begun 30 minutes after the attachment of the pancreas to the perfusion system. All experiments were completed within 90 minutes. In control experiments, which had been conducted previously, during this time, in the presence of 5.6 mM D-glucose, portal venous effluent levels of glucagon and insulin were stable and devoid of any oscillations or pulsations when measured at one-minute intervals. At the end of each experiment, the functional integrity of the pancreas was ascertained by demonstrating that a one-minute perfusion of 10 mM L-arginine elicited the anticipated glucagon and insulin secretory response. The portal venous effluent was collected in one-minute fractions. The samples were immediately stored at −20° C. until the time they were assayed for hormonal concentrations within two to four weeks.

The portal venous effluent levels of glucagon and insulin were determined by two-antibody radioimmunoassay methods.[31] The antiglucagon serum G9-I (Ann Arbor) reacts with the carboxyl terminal of the glucagon molecule and has negligible cross-reactivity with gut glucagon-like compounds.[31] The affinity of the antiserum to large molecular species of glucagon is about 10 per cent that to 3,500-dalton species. Rat

insulin (Novo, Denmark) was used as standards in the insulin assay.

The results are reported as means ± standard errors of the means. The total cumulative hormonal secretory responses were quantified by computing the sum of the partial areas under the response curve.[29] The incremental response area was computed by subtracting an area that corresponds to the product of the basal value and the elapsed time (basal area) from the total response area. The statistical significance of the differences between sets of values was determined by the two-tailed *t*-test for paired observations or for groups of unequal numbers and by analysis of variance (Anova) for more than two groups.[32] The probability estimates for the incremental response areas of the maximal increments represent paired comparisons between total areas and basal areas and maximal values and basal values, respectively.

RESULTS

Dose-response Relationships

Solutions of $PGF_{2\alpha}$ were prepared at seven concentrations, ranging from 1.1×10^{-9} M to 1.8×10^{-5} M. Each solution was administered to four pancreases for two minutes separated by a 10-minute rest period, during which the organs were perfused with buffer solution alone. In two pancreases the sequence of administration of solutions of $PGF_{2\alpha}$ was from the lowest to highest concentration; the reverse order was followed in the other two pancreases. Perfusion media contained 5.6 mM D-glucose at all times. The maximal stimulatory effects of $PGF_{2\alpha}$ on glucagon and insulin release were observed at one and two minutes, respectively. The hormonal concentrations then declined rapidly, such that by the fourth or fifth minute after beginning the perfusion of $PGF_{2\alpha}$, both glucagon and insulin levels had returned to their respective basal levels. The initiation and dissipation of glucagon release preceded that of insulin release. For each dose level of $PGF_{2\alpha}$, the means of the maximal increments and of the four-minute incremental response areas in glucagon and insulin are given in table 1. Increases in the levels of each of the hormones reached statistical significance at concentrations of 2.8×10^{-8} M or higher. Hormonal secretory responses were greater at higher concentrations of $PGF_{2\alpha}$. However, unlike our previous observations with PGE_2,[14] where the secretory response was directly related to the dose within the same concentration range used in the present study, the dose-response relationship was not readily discernible beyond 7.1×10^{-7} M for glucagon and 1.4×10^{-7} M for insulin.

We reported previously that, in the presence of 5.6 mM D-glucose, the lowest concentrations of PGE_2 that induced glucagon and insulin release were 2.8×10^{-8} M and 1.4×10^{-7} M, respectively.[14] Six further experiments were performed in which 1.1×10^{-9} M PGE_2 was perfused for one minute in the absence or presence of a 5-mM mixture of fumarate, glutamate, and pyruvate (figure 1). When the perfusion medium was enriched with these energy-yielding substrates of the citric acid cycle, PGE_2 did induce insulin release at the concentration of 1.1×10^{-9} M. The mean of the four-minute incremental areas for the perfusions in the presence of citric acid cycle substrates was 117 ± 31 and in the absence of substrate 3.4 ± 2.2 μU. · min. per milliliter ($p < 0.02$). On the other hand the mixture of fumarate, glutamate, and pyruvate did not appear to facilitate the glucagon secretory response to 1.1×10^{-9} M PGE_2; the incremental areas were 73 ± 30 and 19 ± 29 pg. · min. per milliliter with and without the substrate enrichment, respectively (N.S.).

TABLE 1

Mean ± S.E. incremental areas and maximal increments in glucagon and insulin in response to two-minute perfusions of $PGF_{2\alpha}$ at seven concentrations in the presence of 5.6 mM D-glucose (N = 4)

$PGF_{2\alpha}$ (molar conc'n.)	Incremental response areas (0-4 min.) Glucagon (pg. · min./ml.*)	Insulin (μU. · min./ml.)	Maximal increments above basal levels Glucagon (pg. · min./ml.*)	Insulin (μU. · min./ml.*)
1.1×10^{-9}	395 ± 444	12.1 ± 5.1	209 ± 192	8.5 ± 3.4
5.7×10^{-9}	147 ± 72	0.4 ± 6.8	100 ± 52	1.0 ± 0.6
2.8×10^{-8}	580 ± 181†	23.1 ± 1.9†	355 ± 110†	12.0 ± 1.1†
1.4×10^{-7}	1,941 ± 529†	30.2 ± 6.2†	1,268 ± 346†	15.9 ± 1.9†
7.1×10^{-7}	2,315 ± 541†	21.5 ± 3.6†	1,894 ± 479†	14.5 ± 2.3†
3.5×10^{-6}	2,116 ± 404†	15.9 ± 4.2†	1,885 ± 378†	8.4 ± 2.1†
1.8×10^{-5}	1,373 ± 186†	27.9 ± 15.6	1,050 ± 175†	13.3 ± 6.4

*Anova $p < 0.05$ to 0.001 for the seven dose levels.
†$p < 0.05$ to 0.001 when total response area is compared with corresponding basal area.

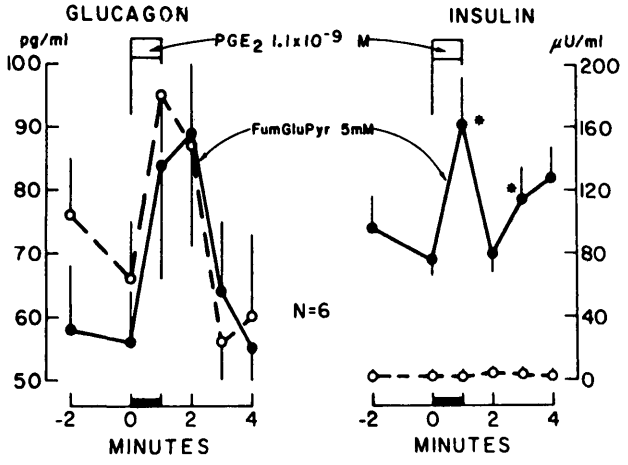

FIG. 1. Mean ± S.E. portal venous effluent levels of immunoreactive glucagon and insulin before, during, and after one-minute perfusions of 1.1 × 10^{-9} M PGE_2. The perfusate concentration of D-glucose was 5.6 mM in all experiments. Solid lines depict the results observed in the presence of and interrupted lines in the absence of a 5-mM mixture of fumarate, glutamate, and pyruvate. Asterisks signify the values that were significantly different from the corresponding basal value (average of values at minus two and zero minutes) ($p < 0.05$ to 0.001).

Multiphasic Hormonal Release

Six experiments were conducted to ascertain whether the biphasic nature of the secretions of glucagon and insulin we had observed[14] with PGE_2 was applicable to other prostaglandins. $PGF_{2\alpha}$, 2.8 × 10^{-7} M, was perfused for 10 minutes in the presence of 5.6 mM glucose. In response to this stimulus the pattern of release of both glucagon and insulin was biphasic (figure 2). The phase-I release was completed four minutes after the beginning of the perfusion. Around the four-minute time point the levels of glucagon and insulin had decreased, despite the fact that the perfusion of $PGF_{2\alpha}$ was still in progress. Thereafter, glucagon and insulin levels remained above the basal values throughout the remainder of the PG perfusion (phase II). During phase I (zero to four minutes) the mean incremental response area for glucagon was 388 ± 86 pg. · min. per milliliter ($p < 0.01$) and for insulin 107 ± 31 μU. · min. per milliliter ($p < 0.02$). The phase-II (4 to 10 minutes) release of glucagon corresponded to a mean incremental area of 301 ± 124 pg. · min. per milliliter ($p < 0.05$). As depicted in figure 2, although the phase II of insulin release was not prominent, it occurred at a magnitude and consistency sufficient to attain statistical significance (13.8 ± 4.9 μU. · min. per milliliter, $p < 0.02$).

In another series of three experiments, PGE_1, 1.4 × 10^{-6} M, was perfused employing the same protocol. The pattern of secretion of each hormone was again biphasic and followed a sequence similar to that seen with PGE_2 or $PGF_{2\alpha}$. For glucagon and insulin the mean incremental response areas were 1,654 ± 1,349 pg. · min. per milliliter and 21 ± 11 μU. · min. per milliliter, respectively, during phase I, and 814 ± 920 pg. · min. per milliliter and 4.8 ± 2.6 μU. · min. per milliliter, respectively, during phase II.

During these experiments the initiation as well as dissipation of the glucagon secretory process preceded the insulin secretory process during phase I by around one minute (figure 2).

Specificity of the Effect of Prostaglandins

Since PGE_1, E_2, and $F_{2\alpha}$ are 20-carbon unsaturated fatty acids, oleic acid, an 18-carbon unsaturated fatty acid, was selected to establish whether the ability to evoke glucagon and insulin release was characteristic of unsaturated long-chain fatty acids. In four experiments, 10^{-6} M oleic acid was perfused for one minute. Minor and insignificant fluctuations in glucagon levels were observed; insulin levels did not change (figure 3). When 10^{-6} M oleic acid was perfused for 10 minutes, mean portal venous glucagon declined significantly below the basal level at the one-minute time point but otherwise remained unchanged (figure 3). Again no changes were observed in portal venous levels of insulin (figure 3).

Augmentation by Prostaglandins of Arginine-induced Release of Glucagon and Insulin

The effects of 1.4 × 10^{-6} M PGE_1 or 2.8 × 10^{-7} M

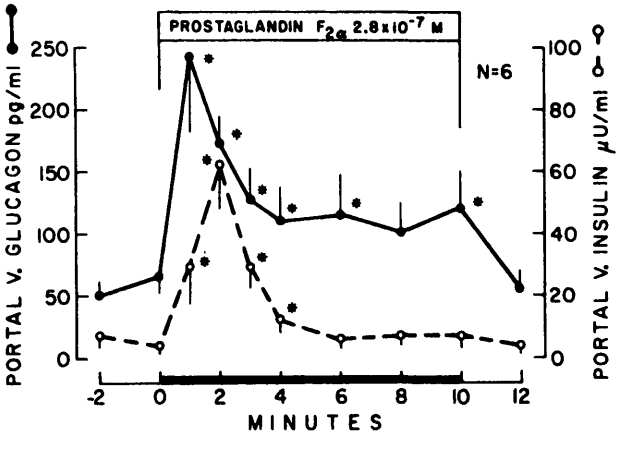

FIG. 2. Mean ± S.E. effluent levels of glucagon and insulin with 10-minute perfusions of 2.8 × 10^{-7} $PGF_{2\alpha}$ in the presence of 5.6 mM D-glucose. Asterisks signify the values that were significantly different from the corresponding basal value ($p < 0.05$ to 0.001).

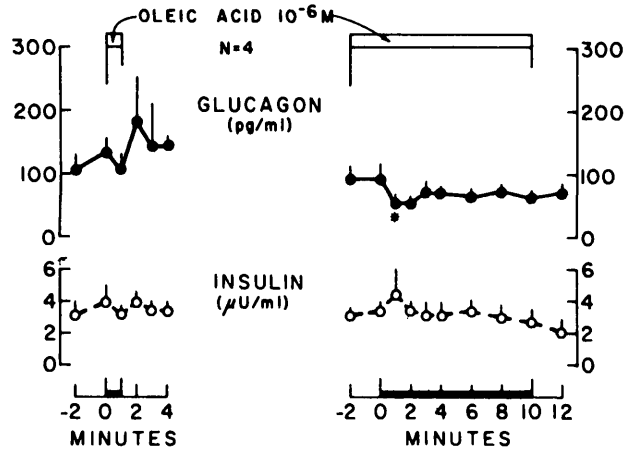

FIG. 3. Mean ± S.E. effluent levels of glucagon and insulin during one-minute or 10-minute perfusions of 10^{-6} M oleic acid in the presence of 5.6 mM D-glucose. The asterisk denotes a value that was significantly less than the corresponding basal value ($p < 0.05$).

$PGF_{2\alpha}$ on arginine-induced release of pancreatic islet hormones were ascertained in 10 experiments. Each pancreas was perfused with 10-mM solutions of L-arginine over two 15-minute periods. For the 10 minutes preceding and during one of these periods of arginine administration, the perfusion media contained either PGE_1 or $PGF_{2\alpha}$. The test periods were separated by 15-minute rest periods and were arranged randomly. As shown in figure 4 for PGE_1 and in table 2 for both PGE_1 and $PGF_{2\alpha}$, both phases of arginine-induced release of glucagon and insulin were augmented by PGE_1. $PGF_{2\alpha}$ augmented both phases of the glucagon secretory response to arginine and phase II of the insulin response.

Modulation of the Effects of Prostaglandins by Glucose

The effects of prevailing concentrations of glucose upon PG-induced release of glucagon and insulin were investigated in two series of experiments.

In seven experiments, the perfusion solution contained no glucose at any time. Each pancreas was perfused randomly with 2.8×10^{-7} M PGE_1, PGE_2, or $PGF_{2\alpha}$ for two minutes separated by a 10-minute rest period (figure 5). The basal levels of glucagon were five to six times those observed in the presence of 5.6 mM D-glucose. Among the PGs tested, $PGF_{2\alpha}$ was the strongest stimulus for glucagon release. The four-minute glucagon incremental response area was 5,672 ± 1,491 pg. · min. per milliliter ($p < 0.01$) for $PGF_{2\alpha}$, followed by PGE_1, 1,860 ± 640 pg. · min. per milliliter ($p < 0.05$), and by PGE_2, 1,605 ± 720 pg. · min. per milliliter ($p < 0.05$). The Anova of the glucagon secretory responses indicated that the variations in the potencies of $PGF_{2\alpha}$, PGE_1, and PGE_2 were significant ($p < 0.02$). In the absence of glucose, insulin release did not occur in response to any of the prostaglandins at the concentrations tested (figure 5).

FIGURE 4

Mean ± S.E. portal venous effluent levels of glucagon and insulin during 15-minute perfusions of 10 mM L-arginine in the presence of 5.6 mM D-glucose. In the experiments depicted by solid lines, 1.4×10^{-6} M PGE_1 was perfused for 10 minutes before and during the administration of L-arginine. The asterisks signify pairs of values with and without PGE_1 that differed significantly ($p < 0.05$ to 0.001).

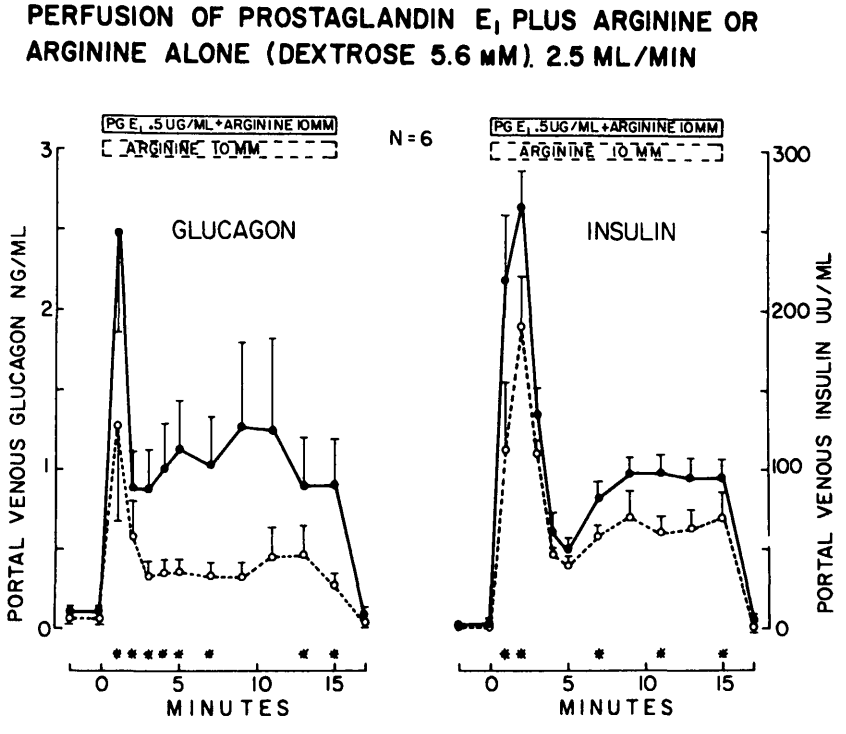

TABLE 2

Mean ± S.E. incremental response areas in glucagon and insulin to 15-minute perfusions of 10 mM L-arginine in the presence or absence of 1.4×10^{-6} M PGE_1 (N = 6) or 2.8×10^{-7} M $PGF_{2\alpha}$ (D-glucose 5.6 mM) (N = 4)

Perfusate contents	Glucagon (pg. · min./ml.) Phase I (0-4 min.)	Glucagon (pg. · min./ml.) Phase II (4-15 min.)	Insulin (μU. · min./ml.) Phase I (0-4 min.)	Insulin (μU. · min./ml.) Phase II (4-15 min.)
Arginine	2,120 ± 940*	3,269 ± 1,097*	430 ± 74*	627 ± 62*
PGE_2 + arginine	4,309 ± 1,309*	10,462 ± 3,794*	638 ± 113	929 ± 62*
p†	< 0.02	< 0.05	< 0.05	< 0.05
Arginine	1,480 ± 449*	3,638 ± 2,008*	514 ± 153*	758 ± 91*
$PGF_{2\alpha}$ + arginine	3,979 ± 444*	7,304 ± 1,918*	472 ± 84*	963 ± 111*
p†	< 0.05	< 0.001	N.S.	< 0.05

*When total response area is compared with corresponding basal area, $p < 0.05$ to 0.001.
†Paired *t*-tests, responses to arginine alone vs. PGE_2 + arginine or $PGF_{2\alpha}$ + arginine.

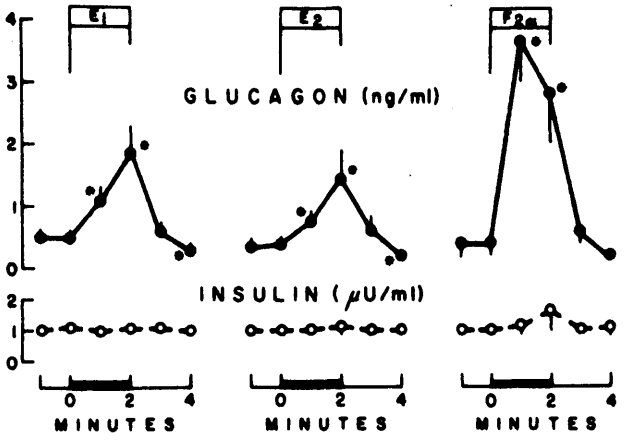

FIG. 5. Mean ± S.E. effluent levels of glucagon and insulin when 2.8×10^{-7} M PGE_1, PGE_2, and $PGF_{2\alpha}$ were administered to each of seven pancreases for two-minute periods in random sequence. Perfusion media did not contain glucose. Asterisks signify the values that differed significantly ($p < 0.05$ to 0.001) from the corresponding basal value (average of values at minus two and zero minutes).

Seventeen experiments were carried out to ascertain the effects on PG-induced hormone release of glucose at a high concentration. Each pancreas was perfused with perfusion media containing either 5.6 mM or 16.7 mM D-glucose in random sequence. During those periods of exposure to physiologic or high concentrations of glucose, either 1.4×10^{-6} M PGE_2 or 2.8×10^{-7} M $PGF_{2\alpha}$ were administered for one minute. In the presence of 16.7 mM D-glucose, the release of glucagon in response to PGE_2 (figure 6) or $PGF_{2\alpha}$ (figure 7) was markedly blunted. When the concentration of D-glucose was 16.7 mM, the four-minute incremental response areas were 13 ± 18 pg. · min. per milliliter (N.S.) with PGE_2 and 6 ± 15 pg. · min. per milliliter (N.S.) with $PGF_{2\alpha}$. These incremental responses in glucagon were significantly ($p < 0.01$) less than those seen when the glucose concentration was 5.6 mM [822 ± 230 ($p < 0.01$) with PGE_2, and 838 ± 289 pg. · min. per milliliter ($p < 0.05$) with $PGF_{2\alpha}$]. On the other hand the "basal" levels of insulin were magnified greatly in the presence of 16.7 mM D-glucose (figures 6 and 7). At high concentrations of glucose, the four-minute incremental response areas were 59 ± 19 μU. · min. per milliliter ($p < 0.02$) with PGE_2 and 104 ± 21 μU. · min. per milliliter ($p < 0.005$) with $PGF_{2\alpha}$. These responses were greater than but statistically not different from those that occurred in the presence of 5.6 mM D-glucose: 31 ± 11 ($p < 0.05$) with PGE_2 and 58 ± 24 ($p = 0.05$) μU. · min. per milliliter with $PGF_{2\alpha}$.

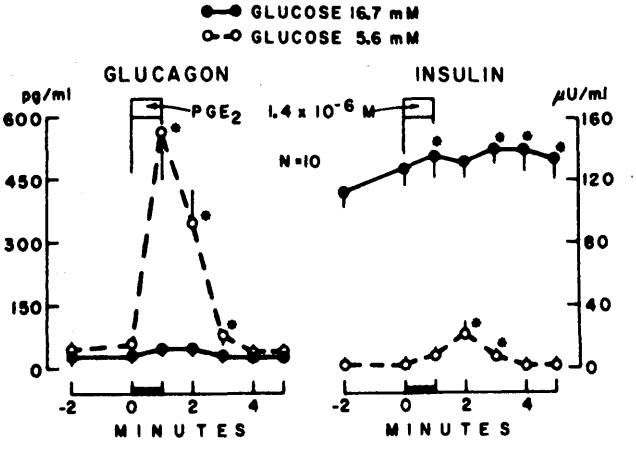

FIG. 6. Mean ± S.E. effluent levels of glucagon and insulin during one-minute perfusions of 1.4×10^{-6} M PGE_2. In the experiments depicted by the solid lines, the concentration of D-glucose in the perfusion media was 16.7 mM and in those depicted by the interrupted lines, 5.6 mM. Asterisks signify the values that differed significantly ($p < 0.05$ to 0.001) from the corresponding basal values.

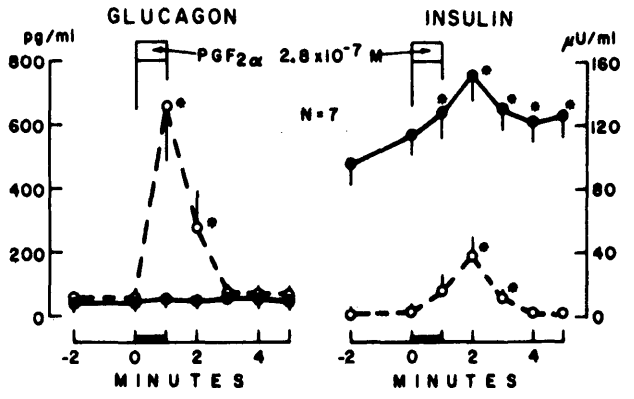

FIG. 7. Mean ± S.E. portal venous effluent levels of glucagon and insulin during one-minute perfusions of 2.8×10^{-7} M $PGF_{2\alpha}$. In the experiments depicted by the solid lines, the concentration of D-glucose in the perfusion media was 16.7 mM and in those depicted by the interrupted lines, 5.6 mM. Asterisks signify the values that differed significantly ($p < 0.05$ to 0.001) from the corresponding basal value.

DISCUSSION

The results of our previous study[14] and those reported herein demonstrate that prostaglandins E_1, E_2, and $F_{2\alpha}$, when administered in vitro to the intact rat pancreas, evoke the secretions of glucagon and insulin under a variety of conditions.

The secretory responses of glucagon and insulin to $PGF_{2\alpha}$ were related to dose up to the concentrations of 7.1×10^{-7} M and 1.4×10^{-7} M, respectively. The lack of a discernible dose-response relationship at higher concentrations may reflect a limited capacity of the secretory mechanism that is activated by $PGF_{2\alpha}$. With PGE_2 in the same range of concentrations, the secretion of both hormones increased progressively up to a concentration of 1.8×10^{-5} M.[14] These apparent differences in the dose-response patterns for $PGF_{2\alpha}$ and PGE_2 may be due to differences in the mechanisms by which these prostaglandins activate the release of the islet hormones. Alternatively, at the high concentrations, $PGF_{2\alpha}$ may have exerted a partial antagonistic effect, an effect that has been ascribed by others[15,33] to these prostaglandins in different biologic systems.

When administered in artificial buffer media containing physiologic concentrations of glucose as the sole source of energy, the lowest concentrations of prostaglandins that elicited a hormonal response were higher than those observed in circulating blood[34] or in extracts of pancreas.[35] Upon enrichment of the media with three intermediate substrates of the citric acid energy cycle, insulin release occurred in response to 1.1×10^{-9} M PGE_2. This concentration is lower than the concentration found in the rat pancreas, 52×10^{-9} mol per gram wet tissue.[35] Hamamdzic and Malik,[36] who used a similar system of isolated perfused rat pancreas, reported that the level of PGE_2 in the portal venous effluent increased from 2×10^{-9} M to 6.4×10^{-9} M upon stimulation of the periarterial nerves. Thus, in our experiments, the release of insulin was evoked when PGE_2 was administered at a concentration lower than that found in the pancreatic effluent in vitro. The permissive effect of the intermediaries of the citric acid cycle on prostaglandin-induced insulin release may be analogous to those of energy-yielding substrates that are in abundance in vivo. Although the citric acid intermediaries did not appear to facilitate glucagon secretory response to physiologic concentrations of PGE_2, other in vivo conditions (e.g., amino acids) are likely to have such an effect on the response of the pancreatic alpha cells to prostaglandins.

All three prostaglandins we employed elicited the secretions of glucagon and insulin in a biphasic fashion. This pattern of secretion is characteristic of pancreatic islet hormones and does not represent a unique way by which prostaglandins activate the release of glucagon and insulin. Thus, the stereotypic response of the islet hormones to administered prostaglandins would be consistent with a mediating or modulating role of endogenous prostaglandins in the secretion of glucagon and/or insulin evoked by a variety of secretagogues.

The secretion of glucagon, which occurred in response to prostaglandins, preceded that of insulin when the experiments were conducted under conditions suitable for the release of either hormone (figure 2). We have defined the sequence of prostaglandin-induced release of glucagon and insulin more precisely and reported our observations previously.[30]

We considered the possibility that the glucagon and insulin stimulatory effects of prostaglandins may be inherent to their chemical nature as long-chain, polyunsaturated, hydroxy-fatty acids. Our failure to demonstrate a glucagon or insulin secretory response to the administration of oleic acid makes this possibility unlikely. The cyclo-oxygenation of the lipid structure, which gives the unique biologic potencies to the prostaglandins and related lipids, is likely to be responsible for the stimulatory effects of PGE_1, PGE_2, and $PGF_{2\alpha}$ upon the pancreatic islet hormones.

All three prostaglandins we employed augmented

the secretions of glucagon and insulin that occurred in response to the administration of L-arginine. We selected arginine as a model nutrient in order to establish whether prostaglandins modify the secretory response to such a nutrient. Our findings answer this question affirmatively and provide further support for a possible role for endogenous prostaglandins as modulators of the secretions of glucagon and insulin.

The results of our studies, which were conducted in the absence of or at physiologic or high concentrations of D-glucose, are consistent with the well-established dominant effect of this substrate upon the glucagon and insulin secretory process. As anticipated, the lack of glucose facilitated the secretion of glucagon and abolished the secretion of insulin in response to all prostaglandins tested. Conversely, high concentrations of glucose markedly attenuated the glucagon secretory response. Our inability to demonstrate an augmentation by a high concentration of glucose on prostaglandin-induced insulin release may be explained by the near maximally stimulated state at the time of the administration of the prostaglandins. Alternatively, prostaglandins and glucose may act upon certain steps of the insulin secretory mechanism in such a way that a summation of their individual effects cannot occur. The observations made by Burr and Sharp[20] that insulin release is stimulated by PGE_1 at low concentrations and inhibited at high concentrations of glucose suggest further that prostaglandins and glucose interact in a complex manner to modify the beta-cell secretory process.

Prostaglandins have been identified in the extracts of pancreas from a variety of animals including man.[35,37] Recent observations by Hamamdzic and Malik[36] document that, as in many other tissues, the products of the arachidonic-acid metabolism can be generated in the pancreas and can exert biologic effects locally. Besides prostaglandins, many other products of this metabolic cascade have been identified, the biologic effectiveness of which far exceed those of the prostaglandins themselves. Our finding that PGE_1, PGE_2, and $PGF_{2\alpha}$, when administered in vitro, can stimulate the release of glucagon and insulin could be representative of the effects of the endogenous products of the arachidonate metabolism in vivo.

ACKNOWLEDGMENTS

These studies were supported in part by USPHS grants AM-02244 and TI-AM-05110 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and by a grant from the Michigan State Department of Public Health. Funds were also provided by the Michigan Affiliate of the American Diabetes Association, Detroit, Michigan, the Upjohn Company, Kalamazoo, Michigan, and the Pfizer Company, New York, New York. Dr. T.-Y. Tai was a research scholar sponsored by the China Medical Board, New York, New York. Radioimmunoassays were performed by Mrs. S. Grauds and Messrs. G. Burkheiser and P. Hossler.

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