

STIMULATION BY PROSTAGLANDIN E<sub>2</sub> OF GLUCAGON  
AND INSULIN RELEASE FROM ISOLATED RAT PANCREAS

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ABSTRACT

To ascertain whether prostaglandins (PG) may play a role in the secretion of glucagon and in an attempt to elucidate the conflicting observations on the effects of PG on insulin release, the isolated intact rat pancreas was perfused with solutions containing  $1.1 \times 10^{-9}$  to  $1.8 \times 10^{-5}$  M PGE<sub>2</sub>. In the presence of 5.6 mM glucose significant increments in portal venous effluent levels of glucagon and insulin were observed in response to minimal concentrations of  $2.8 \times 10^{-8}$  and  $1.4 \times 10^{-7}$  M PGE<sub>2</sub>, respectively; a dose-response relationship was evident for both hormones at higher concentrations of PGE<sub>2</sub>. When administered over 60 seconds,  $1.4 \times 10^{-6}$  M PGE<sub>2</sub> resulted in a significant increase in glucagon levels within 24 seconds and in insulin within 48 seconds. Ten-minute perfusions of  $1.4 \times 10^{-6}$  M PGE<sub>2</sub> elicited biphasic release of both islet hormones; Phase I glucagon release preceded that of insulin. Both phases of the biphasic glucagon and insulin release which occurred in response to 15-minute perfusions of 10 mM arginine were augmented by PGE<sub>2</sub>. These observations indicate that PGE<sub>2</sub> can evoke glucagon and insulin release at concentrations close to those observed by others in the extracts of rat pancreas. We conclude that PG may be involved in the regulation of secretion of glucagon and insulin and may mediate and/or modify the pancreatic islet hormone response to other secretagogues.

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## INTRODUCTION

Administered or endogenous prostaglandins (PG) have been shown to affect the release of hormones from various endocrine glands (1). Evidence has been presented that PG produced in situ within the endocrine glands may mediate the effects of certain secretagogues (2). The reports on the effects of PG upon insulin release have been contradictory. Stimulation of insulin secretion or increases in plasma levels of insulin in response to PGE<sub>1</sub> have been observed in vitro and in vivo (3-5). Other investigators could not show any effect of PG on insulin release in vitro or in vivo (6-11). On the other hand Robertson et al. reported that administered PGE<sub>1</sub> inhibits insulin release in dogs (12). No information is available on the effects of PG upon secretion or plasma levels of glucagon. In order to establish whether PG may influence the secretion of glucagon and to elucidate the role of PG in the release of insulin, the isolated intact rat pancreas was perfused in vitro with solutions containing varying concentrations of PGE<sub>2</sub>. The results of our studies indicate that PGE<sub>2</sub> is a potent secretagogue of glucagon as well as of insulin release.

## MATERIALS AND METHODS

Male, albino Sprague-Dawley rats weighing 250-300 g were fed with Purina rat chow. Food was withdrawn 16-18 hours prior to the beginning of the experiments. The animals were anesthetized with sodium pentobarbital, 50 mg/kg body weight, injected intraperitoneally. The surgical technique employed for the removal of the pancreas was a modification of that described by Sussman et al. (13). The pancreas was dissected free from all surrounding structures; its connections to a short segment of the duodenum and its vasculature were left intact. All vessels not supplying the pancreas were ligated. The segment of the abdominal aorta from which the celiac and the superior mesenteric arteries originate was cannulated in a retrograde fashion and perfused with an oxygenated buffer medium. Another cannula was placed in the portal vein to collect the effluent from the pancreas. A third cannula was inserted in the duodenum to ascertain the free flow of exocrine pancreatic secretions and to relieve pressure in the pancreatic duct. To retain correct anatomical position of the vasculature, the pancreas was affixed on a small platform using ligatures which had been placed on the three cannulae. The surgical procedure was completed within 60-80 minutes. During this period the pancreas was being perfused either by the animal's own circulation or by the oxygenated buffer medium so that the oxygen supply was adequate at all times.

The isolated pancreas was suspended in a physiological buffer medium contained in a perfusion chamber. The chamber was placed in a perfusion apparatus which was a modification of that reported by Sussman et al. (13). The ambient temperature was 37° C. The system contained four independent perfusion channels which merged into a manifold to which the aortic cannula was connected. With the use of this manifold-regulated multichannel system, within a fraction of a second any one of the four perfusion solutions could be diverted to perfuse

the pancreas, while the remaining three solutions were being recirculated in an "idle" mode to permit continuous oxygenation and to prevent delays in changing buffer solutions. The flow rate was kept constant at 2.5 ml/min in all channels by using a multichannel roller pump. The aortic perfusion pressure was monitored at all times; the pressure ranged between 30-50 mm Hg and had a pulse amplitude of 2-4 mm Hg.

The artificial perfusion medium had the following composition (mmol/l): NaCl 118.5, KCl 3.5,  $MgSO_4 \cdot 7H_2O$  1.0,  $KH_2PO_4$  1.2,  $CaCl_2 \cdot 2H_2O$  1.25, and  $NaHCO_3$  24.9. Dextran, average mol. weight 70,000 (Pharmacia), 40 g/l, and bovine serum albumin (Miles Labs.), 2 g/l, were added to maintain a physiological oncotic pressure and to minimize adhesion of hormones to glassware. After equilibration with 95%  $O_2$  and 5%  $CO_2$  the oxygen tension of the solution was around 400-450 mm Hg and the pH was 7.4. In all experiments the perfusion solutions contained 5.6 mM D-glucose at all times.

L-arginine monohydrochloride (ICN) and  $PGE_2$  (Upjohn Co.) were dissolved directly in the perfusion medium.

Following the attachment of the pancreas to the perfusion system, an equilibration period of 30 minutes preceded the experiments. All experiments were completed within 60-90 minutes. At the end of each experimental period the functional integrity of the pancreas was ascertained by assessing the glucagon and insulin secretory response to 1-minute perfusions of 10 mM arginine.

The portal venous effluent was collected in 12-60 second fractions. The samples were stored at  $-20^\circ C$  until the time of hormone assays.

Portal venous effluent levels of immunoreactive glucagon were measured by a "double-antibody" method using antiglucagon serum G9-I which has a negligible cross-reactivity with glucagon-like materials present in extracts of intestines (14). Immunoreactive insulin was determined by a modification of the method described by Morgan and Lazarow (15); rat insulin (Novo, Denmark) was used as standards.

The statistical significance of differences between observed values was determined by paired t-test (16).

## RESULTS

Solutions of  $PGE_2$  were prepared in seven different concentrations ranging from  $1.1 \times 10^{-9}M$  to  $1.8 \times 10^{-5}M$ . Each solution was administered to 4 pancreases over successive 2-minute periods, separated by 10-minute rest periods during which the organs were perfused with buffer alone. The results are shown in Fig. 1. At  $PGE_2$  concentrations of  $2.8 \times 10^{-8}M$  or greater, the mean maximal effluent level of glucagon attained during the 5 minutes after the beginning of each perfusion period was significantly greater than the level observed during the

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preceding basal period. Increases in insulin levels occurred consistently in response to PGE<sub>2</sub> at 1.4 x 10<sup>-7</sup>M or greater. A dose-response relationship was observed for both islet hormones.

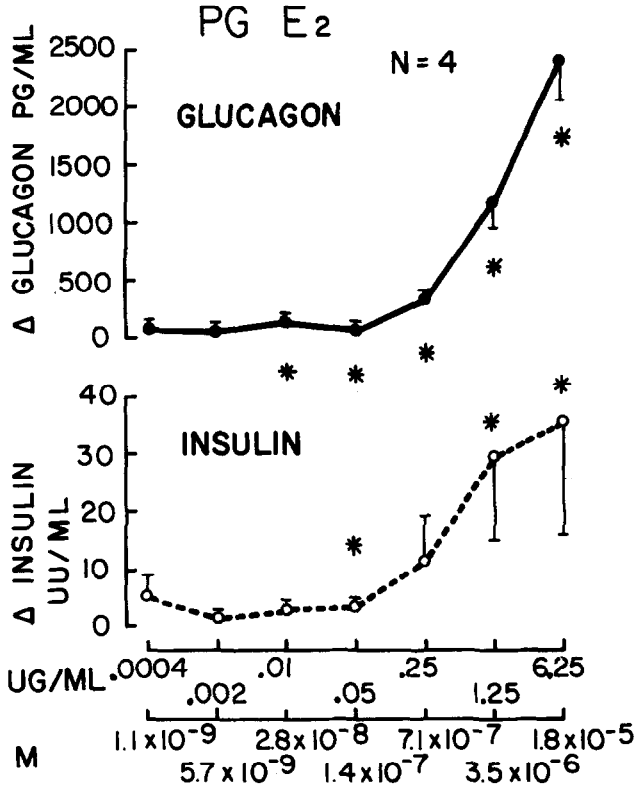


Fig. 1. Mean ± S.E.M. maximal increments in portal venous effluent levels of glucagon and insulin induced by 2-minute perfusions of PGE<sub>2</sub> in the presence of 5.6 mM glucose. Asterisks denote that the increments were statistically significant (p<.05-.001).

The rapidity of glucagon and insulin secretory responses was investigated by perfusing 6 pancreases with  $PGE_2$   $1.4 \times 10^{-6}M$  over 60 seconds and collecting 12-second effluent fractions over 180 seconds (Fig. 2). The first significant increase above basal levels in glucagon occurred within 24 seconds at a time when no increases in insulin levels were discernable. If corrected for the 10 seconds which elapsed for the secretagogue to reach the pancreas, this response time would be reduced to 14 seconds. The first significant increment in insulin was observed at 48 seconds. The mean maximal responses in glucagon and insulin were 48 and 72 seconds, respectively.

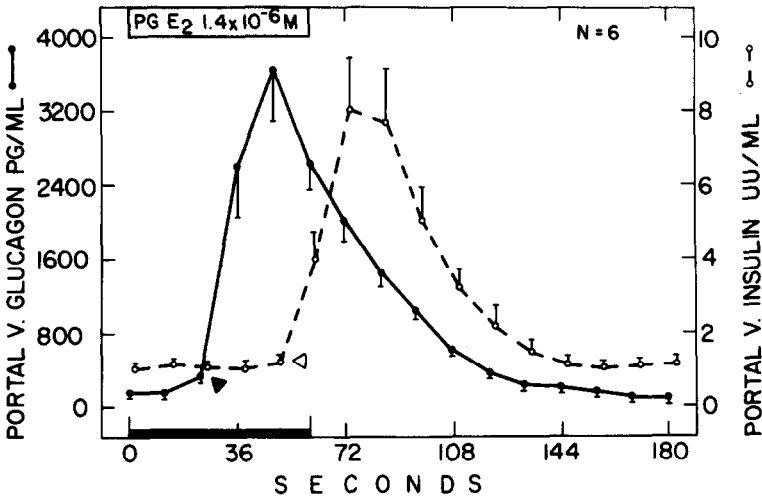


Fig. 2. Mean  $\pm$  S.E.M. portal venous effluent levels of glucagon and insulin before, during and following 60-second perfusions with  $PGE_2$  in the presence of 5.6 mM glucose. The arrow-heads signify the first significant increases above basal levels.

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The patterns of PG-induced secretion of glucagon and insulin were determined by perfusing 5 pancreases with  $1.4 \times 10^{-6}M$  PGE<sub>2</sub> over 10 minutes (Fig. 3). The mean effluent glucagon increased from a basal value of  $91 \pm 12$  pg/ml to a maximal level of  $838 \pm 148$  pg/ml within 1 minute. Mean glucagon decreased to around 300 pg/ml by the third minute and was sustained at this level until the termination of the perfusion of PGE<sub>2</sub>. A similar biphasic release was observed also in insulin. The mean maximal level of  $37 \pm 18$   $\mu$ U/ml was attained at 2 minutes. The Phase I release was completed by the fourth minute. Thereafter insulin levels remained significantly elevated above the basal levels throughout the remainder of the period of PG perfusion.

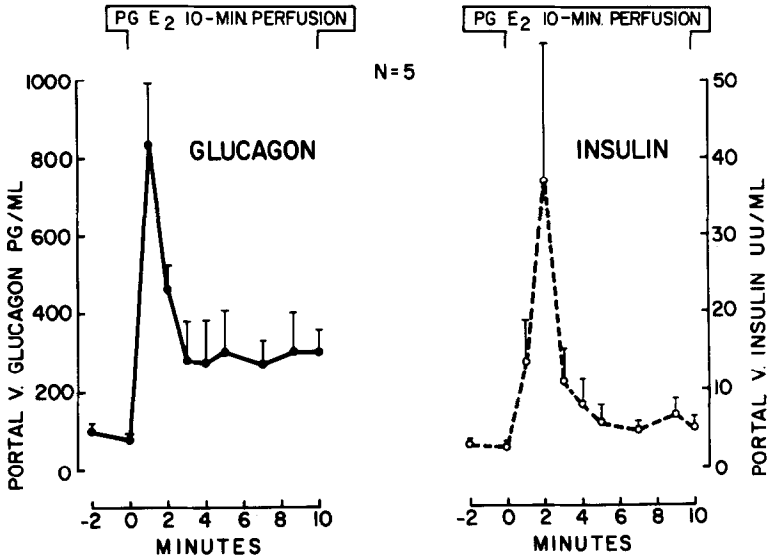


Fig. 3. Mean  $\pm$  S.E.M. portal venous effluent levels of glucagon and insulin before and during 10-minute perfusions with  $1.4 \times 10^{-6}M$  PGE<sub>2</sub> in the presence of 5.6 mM glucose.

The effects of PG upon the release of pancreatic hormones in response to another secretagogue were examined. Each pancreas was perfused with 10 mM solutions of arginine over two 15-minute periods. For 10 minutes preceding and during one of these periods of arginine administration the perfusate contained PGE<sub>2</sub>, 2.8 x 10<sup>-7</sup>M. The test periods were separated by 15-minute rest periods and were arranged randomly. Arginine evoked the expected biphasic increases in the levels of glucagon and insulin; Phase I release dissipated within 4-5 minutes. Phase I and Phase II hormonal responses were computed as incremental areas under the response curve (Table 1). PGE<sub>2</sub> augmented both phases of glucagon and of insulin release induced by arginine.

Table 1

Mean  $\pm$  S.E.M. incremental response areas in glucagon and insulin to 15-minute perfusions of 10 mM arginine with and without 2.8 x 10<sup>-7</sup>M PGE<sub>2</sub> (N=6)

	Glucagon, pg·min/ml		Insulin, $\mu$ U·min/ml	
	Phase I (0-4 min)	Phase II (4-15 min)	Phase I (0-4 min)	Phase II (4-15 min)
Arginine	1859 $\pm$ 427	2957 $\pm$ 675	617 $\pm$ 76	978 $\pm$ 123
PGE <sub>2</sub> + Arginine	4312 $\pm$ 930	7306 $\pm$ 1859	904 $\pm$ 102	1415 $\pm$ 160
p <	0.05	0.05	0.05	0.01

DISCUSSION

The results of our studies indicate that administered PGE<sub>2</sub> is a potent secretagogue of glucagon as well as of insulin in the isolated rat pancreas.

The physiological significance of these observations remains to be established. Karim et al. have reported that the concentration of PGE<sub>2</sub> in the rat pancreas is 18.2 ng/g wet tissue (17). The lowest perfusate concentrations of PGE<sub>2</sub> at which we have observed significant increases in glucagon and insulin release approach those tissue levels. These findings are consistent with the hypothesis that prostaglandin synthesis within the pancreas may be one of the mechanisms regulating the secretion of glucagon and insulin.

As demonstrated in the studies reported here for arginine, the

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secretion of islet hormones in response to a variety of stimuli is biphasic. The release of glucagon and insulin induced by administered  $PGE_2$  was also biphasic. Thus endogenous PG may play a role as mediators of biphasic islet hormone release in response to certain stimuli.

PG-induced glucagon release preceded that of insulin. We have reported that such sequential release of glucagon and insulin occurs in response to a variety of stimuli common to both hormones (18). Glucagon stimulates insulin release when administered in pharmacological amounts (19). Thus prior release of glucagon observed in our studies may have a physiological significance in the secretion of insulin which follows. However, the analysis of our results failed to reveal any correlation between the dynamics or the magnitudes of glucagon release (18). Therefore, under the conditions which prevailed in our studies, the antecedent release of glucagon does not appear to be the principal mediator of insulin release. On the other hand, at times when both islet hormones are being secreted, a role for endogenous glucagon as a modifier of insulin release has not been ruled out.

Among prostaglandins,  $PGE_2$  does not appear to be unique in stimulating the release of glucagon and insulin. Our preliminary studies indicate that  $PGE_1$  and  $PGF_{2\alpha}$  have similar effects on both islet hormones (20). The observations made by others (3-5) on the stimulation of insulin release by  $PGE_1$  are in support of our findings.

In conclusion, prostaglandins may be involved in the regulation of secretion of glucagon and insulin and may mediate and/or modify the pancreatic islet hormone response to other secretagogues.



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