

# Enhanced Release of Insulin by Prostaglandins in Isolated Pancreatic Islets

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## SUMMARY

The effects of several prostaglandins on insulin release were studied during incubation of isolated pancreatic islets from the rat. The glucose-stimulated release of insulin into medium containing 300 mg. per cent glucose and 1.0 mM. theophylline was increased approximately twofold by PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub>. The effect of PGE<sub>1</sub> was apparent at doses as low as 10<sup>-8</sup> M. and progressively increased with concentrations up to 10<sup>-5</sup> M. PGA<sub>1</sub> did not affect insulin release. Inhibition of insulin release by norepinephrine (10<sup>-5</sup> M.) was partially reversed by both PGE<sub>1</sub> and PGE<sub>2</sub>. None of the prostaglandins affected the small basal release of insulin into medium containing 30 mg. per cent glucose. PGE<sub>1</sub> (10<sup>-5</sup> M.) increased the accumulation of cyclic AMP formed from labeled precursor by pancreatic islets incubated in media containing 10 mM. theophylline and either 30 mg. per cent or 300 mg. per cent glucose.

These results indicate that prostaglandins can enhance the release of insulin from beta cells in response to glucose. This action is antagonistic to the inhibitory effect of norepinephrine on insulin release and may be mediated through effects on adenylyl cyclase activity. *DIABETES* 22:658-63, September, 1973.

Previous studies suggest that release of insulin from the beta cells of the pancreas involves cyclic AMP.<sup>1-3</sup> Substances, such as glucagon,<sup>4</sup> which activate adenylyl cyclase in other tissues, elevate cyclic AMP levels in pancreatic islet tissue and release insulin.<sup>1</sup> Other agents, like theophylline<sup>5</sup> or tolbutamide,<sup>6</sup> which inhibit cyclic AMP catabolism by phosphodiesterase, similarly augment insulin release. However, glucose-stimulated insulin

release has not been associated with increased cyclic AMP or adenylyl cyclase activity.<sup>1-3</sup>

Since prostaglandins are known to affect many metabolic functions regulated by cyclic AMP,<sup>7,8</sup> the effect of prostaglandins on insulin release has been studied by several investigators. Bressler et al.<sup>9</sup> reported that injection of PGE<sub>1</sub> into mice increased plasma insulin levels. However, Spellacy et al.<sup>10</sup> found no change in plasma glucose or insulin concentrations following infusion of PGE<sub>2</sub> or PGF<sub>2α</sub> into human subjects. In vitro studies of insulin release from isolated rat islets by Vance et al.<sup>11</sup> and Rossini et al.<sup>12</sup> have not demonstrated an effect of either PGE<sub>1</sub> or PGA<sub>1</sub> on insulin release.

The sympathetic neurotransmitter, norepinephrine inhibits insulin release both in vivo<sup>13</sup> and in isolated pancreatic islets.<sup>11</sup> Prostaglandins are released during sympathetic nerve stimulation and have been shown to inhibit the further release of norepinephrine.<sup>14-16</sup> We have therefore investigated the effect of prostaglandins on glucose-stimulated insulin release by isolated rat pancreatic islets in the absence and presence of norepinephrine.

## MATERIALS AND METHODS

### *Isolation of pancreatic islets*

Pancreatic islets were prepared by a modification of the technic of Lacy and Kostianovsky.<sup>17</sup> Male Wistar rats (300 to 400 gm.) were killed by cervical dislocation and the abdomen opened. The pancreases were infused with synthetic interstitial fluid (SIF)<sup>18</sup> containing collagenase (Worthington), 2.0 mg. per milliliter, via the pancreatic duct. The tissues were excised, minced, and incubated for fifteen minutes at 37° C. in Erlenmeyer flasks, and gassed with 95 per cent oxygen—5 per cent carbon dioxide which contained 6 ml. of

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Accepted for publication March 14, 1973.

additional collagenase-containing medium. The incubation was terminated by adding 10 ml. of ice-cold SIF and the tissue disrupted by aspiration through a no. 16 gauge needle. The flask contents were transferred to 50 ml. centrifuge tubes. After the tissue had settled, the supernatant was removed to a residual volume of 8.0 ml. and the tubes centrifuged for one minute at 1,000 x g. The supernatant fluid was aspirated and discarded, and fresh cold SIF added to make a total volume of 6.0 ml. The tissue was dispersed again by aspiration and 22.5 ml. of Krebs-Ringer phosphate buffer containing 30 per cent (weight/volume) Ficoll (Pharmacia) added. After the contents were mixed, a discontinuous Ficoll gradient was layered on top of each tube, using 5 ml. layers (specific densities 1.085, 1.075, and 1.045). The tubes were centrifuged for twenty minutes at 1,000 x g. The islets were aspirated from the interphase of 1.085 to 1.075 and placed in ice-cold SIF. They were then transferred individually, using a dissected microscope, with a micropipette to another dish containing fresh medium.

#### Incubation procedure

Incubation baskets were cut from 12 x 75 mm. polystyrene culture tubes (Falcon Plastics) and consisted of a disposable Millipore filter bottom (Millipore NCWG04700) held against the sides of the basket by a removable base support (figure 1). Fifteen islets were placed in each basket, and incubations were carried out subsequently by placing the baskets in individual chambers made of glass scintillation vials with cork lids. The chambers contained 2.0 ml. SIF medium with 0.3 per cent bovine serum albumin gassed with 95 per

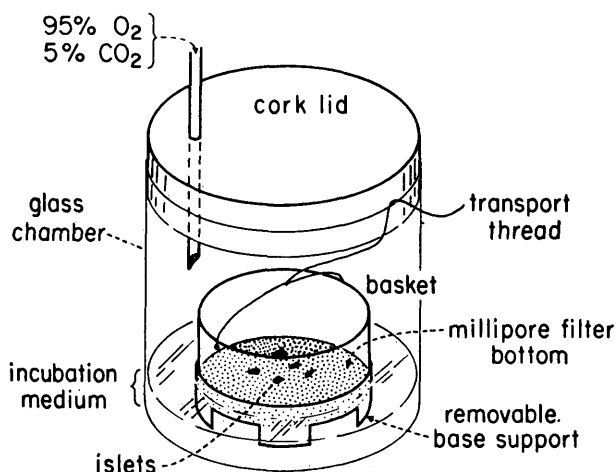


FIG. 1. Incubation apparatus used to study insulin release from isolated islets.

cent oxygen—5 per cent carbon dioxide and maintained at 37° C. After preliminary results indicated that insulin release in response to glucose was quite variable during thirty to sixty minute incubation periods, theophylline (1.0 mM.—final concentration) was added to the medium used in all experiments reported in this study. Glucose-stimulated insulin release in the presence of 1.0 mM. theophylline was easily demonstrable, even during the thirty minute incubation periods, with little variation in release values. Following incubation, the baskets were removed, and the medium frozen until assayed.

#### Assay of insulin

The immunoreactive insulin (IRI) content of the medium was assayed by radioimmunoassay, using cellulose to adsorb the free insulin.<sup>19</sup> Results were compared to a standard curve obtained with rat insulin.

#### Assay for cyclic AMP-14-C accumulation

Pancreatic islets were incubated in a two-step procedure based on a modification of the method of Miller et al.,<sup>20</sup> using adenosine-8-14-C (New England Nuclear Corporation) to label the ATP pool in the islets. The islets were incubated in 1.0 ml. of SIF containing 4 per cent bovine serum albumin with 100 mg. per cent glucose and 10  $\mu$ Ci. of adenosine-8-14-C for sixty minutes at 37° C. In the second step the islets were washed with cold SIF and placed in SIF containing 10 mM. theophylline and appropriate glucose and prostaglandins in a final volume of 0.5 ml. After incubation for thirty minutes at 37° C., unlabeled cyclic AMP (5.0  $\mu$ moles) was added to the tubes, and the reaction was terminated by placing the tubes in a boiling water bath. The islets were broken by sonication and cyclic AMP-14-C was isolated and assayed according to Miller et al.<sup>20</sup>

#### Materials

The prostaglandins were kindly supplied by Dr. John Pike, Upjohn Company, Kalamazoo, Michigan.

## RESULTS

#### Effect of prostaglandins on glucose-stimulated insulin release

PGE<sub>1</sub> increased the release of insulin into medium containing 300 mg. per cent glucose (figure 2). This effect was evident at concentrations as low as 10<sup>-8</sup> M. and increased in response up to 10<sup>-5</sup> M. Concentrations of PGE<sub>1</sub> higher than 10<sup>-5</sup> M. were less effective than 10<sup>-5</sup> M. PGG<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  were less potent than PGE<sub>1</sub> in enhancing glucose-stimulated insulin release but

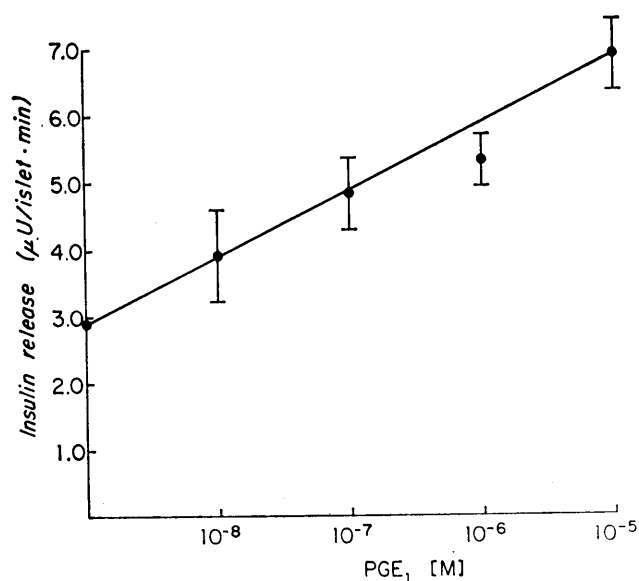


FIG. 2. Effect of PGE<sub>1</sub> concentration on glucose-stimulated release of insulin. Each plotted value represents the mean ( $\pm$  S.E.M.) of four baskets containing fifteen islets each. One hour incubation time in medium containing glucose (300 mg. per cent) and 1.0 mM. theophylline. Correlation coefficient = 0.87;  $P < .001$ .

caused nearly a twofold increase at  $10^{-4}$  M. concentration (figure 3). PGA<sub>1</sub> was ineffective even at  $10^{-4}$  M. concentration.

#### Antagonistic effects of norepinephrine and prostaglandins

Norepinephrine ( $10^{-5}$  M.) caused a marked inhibition of glucose-stimulated insulin release (figure 4). PGE<sub>1</sub> ( $3 \times 10^{-6}$  M.) partially reversed the inhibition produced by norepinephrine, although release in the presence of both agents was much less than that occurring in response to glucose alone. PGE<sub>2</sub> ( $3 \times 10^{-6}$  M.) also partially reversed the inhibition of insulin release caused by norepinephrine (figure 5).

#### Basal insulin release

Most of the insulin released from islets incubated in medium containing 30 mg. per cent glucose was secreted during the first thirty minutes of incubation. After sixty minutes' incubation, insulin release from islets incubated in 30 mg. per cent glucose was approximately one-sixth the release occurring from islets stimulated with 300 mg. per cent glucose (table 1). None of the prostaglandins tested caused any augmentation of the low rate of insulin release into medium containing 30 mg. per cent glucose.

#### Accumulation of cyclic AMP-14-C

PGE<sub>1</sub> ( $10^{-5}$  M.) caused a twofold increase in cyclic

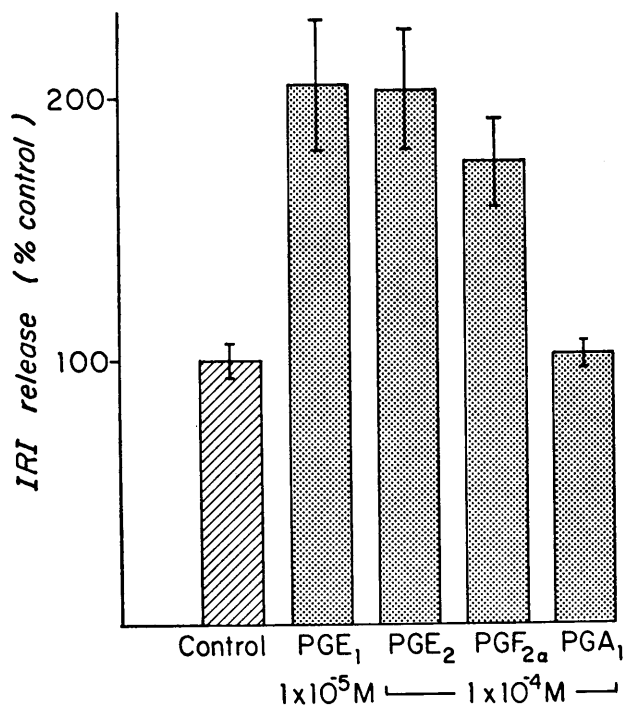


FIG. 3. Effect of different prostaglandins on glucose-stimulated insulin release. Each bar represents the mean ( $\pm$  S.E.M.) of eight baskets containing fifteen islets each. One hour incubation in medium of 300 mg. per cent glucose and 1.0 mM. theophylline.  $P < 0.001$ , for PGE<sub>1</sub>, PGE<sub>2</sub> and PGF<sub>2α</sub> versus control group.

AMP-14-C accumulation in islets incubated in medium containing 300 mg. per cent glucose and 10 mM. theophylline (table 2). In contrast to the lack of effect on insulin secretion, PGE<sub>1</sub> produced nearly a threefold increase in cyclic AMP-14-C accumulation in the presence of 30 mg. per cent glucose.

#### DISCUSSION

The augmentation of insulin release by PGE<sub>1</sub> found in this study is similar to the enhancement by PGE<sub>1</sub> of the hormonal responses of several other endocrine glands, including the pituitary,<sup>21</sup> thyroid,<sup>22</sup> adrenal cortex,<sup>23</sup> and ovary.<sup>24</sup> Since cyclic AMP has been implicated as a second messenger in the release of hormone from these glands, it is possible that PGE<sub>1</sub> exerts its stimulatory effect by a similar action on cyclic AMP metabolism in all these organs. The marked increase in cyclic AMP-14-C accumulation produced by PGE<sub>1</sub> (table 2) is in agreement with this concept. The greater potency of PGE<sub>1</sub>, compared with PGE<sub>2</sub> and PGF<sub>2α</sub>, in enhancing insulin release corresponds with its greater

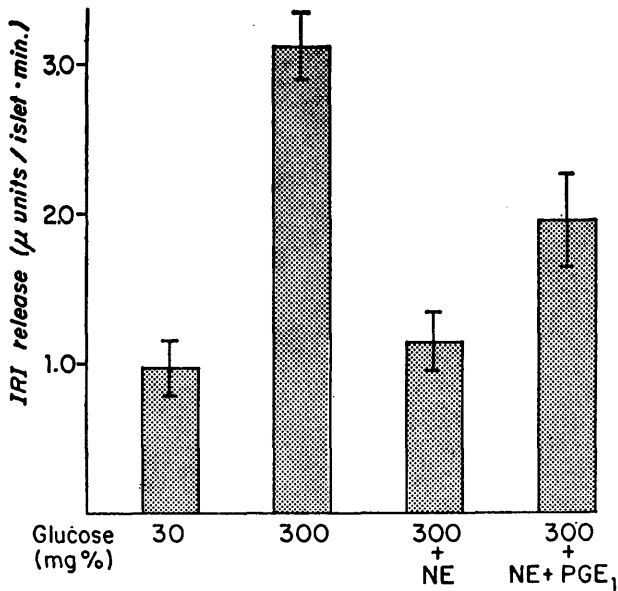


FIG. 4. Effect of norepinephrine ( $10^{-5}$  M.) and PGE<sub>1</sub> ( $3 \times 10^{-6}$  M.) on glucose-stimulated insulin release in medium containing 1.0 mM. theophylline. Each bar represents the mean ( $\pm$  S.E.M.) of ten baskets containing fifteen islets each, thirty minute incubation.  $P < 0.05$ , for NE + PGE<sub>1</sub> group versus NE group.  $P < 0.01$ , for NE or NE + PGE<sub>1</sub> groups versus 300 mg. per cent glucose.

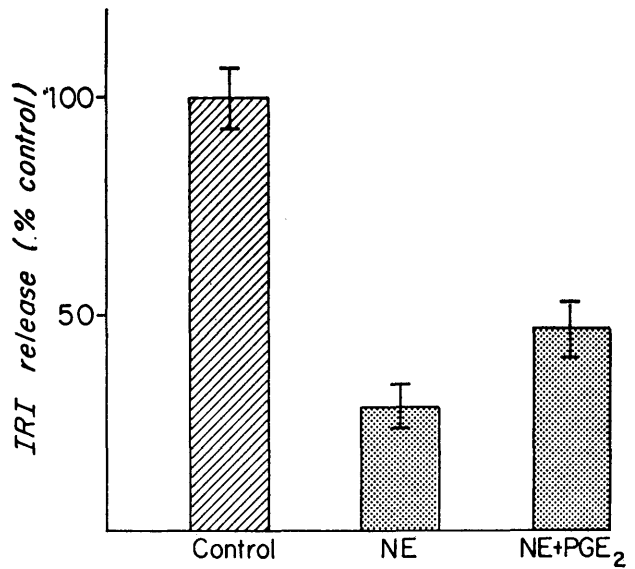


FIG. 5. Effect of norepinephrine ( $10^{-5}$  M.) and PGE<sub>2</sub> ( $3 \times 10^{-6}$  M.) on glucose-stimulated insulin release in medium containing 1.0 mM. theophylline. Each bar represents the mean ( $\pm$  S.E.M.). Thirty minute incubation in medium of 300 mg. per cent glucose.  $P < 0.05$ , for NE + PGE<sub>2</sub> group versus control.  $P < 0.01$  for NE or NE + PGE<sub>2</sub> group versus control.

effect on several other metabolic responses mediated by cyclic AMP.<sup>7,8,26</sup> Thus, these findings provide further indirect evidence that cyclic AMP is involved in the release of insulin from pancreatic islets.

The augmentation of insulin release by PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub> reported in this study contrasts with the lack of response to prostaglandins in the studies with isolated pancreatic islets reported by Vance et al.<sup>11</sup> and Rossini et al.<sup>12</sup> This could be due to several factors. First, PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub> had no direct stimulatory effect on insulin release in medium containing 30 mg.

per cent glucose (table 1) but specifically enhanced the release of insulin which occurred in response to 300 mg. per cent glucose (figures 2 and 3). Furthermore, all our studies were done in the presence of 1.0 mM. theophylline. Our results also indicated that at concen-

TABLE 1

Insulin release in medium containing 30 mg. per cent glucose and 1.0 mM. theophylline in presence of prostaglandins E<sub>1</sub>, E<sub>2</sub>, and F<sub>2α</sub>

	μU. insulin/islet · min.
Control	0.39 ± 0.04
PGE <sub>1</sub>	0.41 ± 0.03
PGE <sub>2</sub>	0.41 ± 0.06
PGF <sub>2α</sub>	0.52 ± 0.04

Values represent the means ( $\pm$  S.E.M.) for groups of eight baskets containing fifteen islets each.

TABLE 2

Effect of prostaglandin E<sub>1</sub> on cyclic AMP accumulation in pancreatic islets incubated in vitro

Glucose concentration (mg. per cent)	Hormone	Cyclic AMP-14-C accumulation (pg./10 islets; 30 min.)
30	Control	215 ± 39
30	PGE <sub>1</sub> ( $10^{-5}$ M.)	632 ± 94*
300	Control	365 ± 27
300	PGE <sub>1</sub> ( $10^{-5}$ M.)	696 ± 94†

Values expressed as the means ( $\pm$  S.E.M.) for groups of five baskets containing ten islets each. Medium contained 10 mM. theophylline.

\* $P < 0.005$ , for PGE<sub>1</sub> group versus corresponding control.

† $P < 0.01$ , for PGE<sub>1</sub> group versus corresponding control.

trations higher than  $10^{-5}$  M., as used by previous workers, PGE<sub>1</sub> was actually less effective. The lack of effect seen with PGA<sub>1</sub> agrees with the findings of Rossini et al.<sup>12</sup> It also suggests that the response noted with the other prostaglandins was not simply a nonspecific effect of all compounds with a prostaglandin-like chemical structure. The marked decrease in pancreatic blood flow caused by PGE<sub>1</sub><sup>26,27</sup> may explain the lack of effect<sup>10,26</sup> on insulin release noted after infusion of PGE<sub>1</sub>. Lefebvre and Luyckx<sup>27</sup> have recently reported an increase in insulin release immediately following infusion of PGE<sub>1</sub>, when pancreatic blood flow was restored.

The inhibition of insulin release caused by norepinephrine (figures 4 and 5) confirms the results obtained in both in vivo<sup>13</sup> and in vitro studies.<sup>11</sup> The partial reversal of norepinephrine inhibition by PGE<sub>1</sub> and PGE<sub>2</sub> appears to represent the net effect of two opposing agents on insulin release. Since epinephrine decreases cyclic AMP concentrations<sup>1</sup> and PGE<sub>1</sub> increases cyclic AMP formation (table 2), opposite effects of catecholamines and prostaglandins on insulin release may be due to antagonistic actions on the adenylyl cyclase system.

PGE<sub>1</sub> increased the accumulation of cyclic AMP-14-C in pancreatic islets incubated in medium containing 30 mg. per cent glucose and theophylline (table 2), although this did not result in increased secretion of insulin (table 1). These results are similar to the finding of Malaisse et al.<sup>28</sup> that theophylline stimulates insulin secretion only in the presence of glucose. Therefore, insulin secretion is dependent on the glucose concentration by some mechanism which is independent of cyclic AMP concentration in pancreatic islet cells.

A role for prostaglandins as circulating hormones has not yet been established. Except for reproductive physiology, most available evidence indicates that prostaglandins are synthesized in the tissues in which they exert their effects.<sup>7</sup> Nevertheless, the effects of prostaglandins on both insulin release and insulin action in peripheral tissues are complementary. The inhibition of norepinephrine release from sympathetic nerves<sup>15,16</sup> could both enhance the secretion of insulin from pancreatic beta cells and interfere with catecholamine-induced lipolysis in adipose tissue.<sup>25</sup> In addition, the results presented in this study demonstrate a direct stimulatory effect of prostaglandins on glucose-induced insulin release.

#### ACKNOWLEDGMENT

These investigations were supported by USPHS Grants AM-02456, AM-05020, and AM-15312 from the National Institute of Arthritis, Metabolism and Di-

gestive Diseases and the McAbee Research Grant. Dr. Johnson was supported by USPHS Fellowship 5F03 AM50646-02 from the National Institute of Arthritis, Metabolism and Digestive Diseases. Dr. Fujimoto was supported by Research Career Development Award 1 K04 AM-47142-01 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

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