The Effect of Prostacyclin and 6-Keto-Prostaglandin $F_{1\alpha}$ on Insulin Secretion and Cyclic Adenosine 3′, 5′-Monophosphate Content in Isolated Rat Islets

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SUMMARY

The effects of prostacyclin (PGI_2) and its degradation product 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) on insulin secretion and cyclic adenosine 3′, 5′-monophosphate (cAMP) production were investigated in isolated rat islets. At a glucose concentration of 5.6 mM, addition of both PGI_2 and 6-keto-$PGF_{1\alpha}$ in a concentration range of 10^{-8}–10^{-4} M significantly stimulated insulin secretion. The effects were concentration dependent, being maximal at an initial PGI_2 concentration of 10^{-6} M (2.7-fold increase, $P < 0.001$) and an initial 6-keto $PGF_{1\alpha}$ concentration of 10^{-7} M (2.1-fold increase, $P < 0.005$). Lesser stimulation was observed at higher concentrations of both agents. In contrast to their relative effects on insulin secretion, PGI_2 was approximately 100-fold more potent at increasing islet cAMP content, and higher concentrations were progressively more effective in raising islet cAMP content.

The effect of PGI_2 on insulin secretion was dependent on the medium glucose concentration. At an initial PGI_2 concentration of 40 μM, significant stimulation of insulin secretion was seen at glucose concentrations of 3.3 and 5.6 mM, but no effect was seen either in the absence of glucose, or at high glucose concentrations (10 and 16.7 mM). In contrast to the glucose dependency of the effect of PGI_2 on insulin secretion, it caused a comparable increase in islet cAMP content at all glucose concentrations tested.

In the presence of 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), PGI_2 inhibited insulin secretion at glucose concentrations of 5.6–16.7 mM. However, in this situation PGI_2 caused a further increase in islet cAMP above that found with IBMX alone.

It is concluded that both PGI_2 and 6-keto-$PGF_{1\alpha}$ affect insulin secretion in vitro, although the effects of PGI_2 may depend on its conversion to 6-keto-$PGF_{1\alpha}$. The effects of PGI_2 can be either stimulatory or inhibitory, depending on the concentrations of glucose and IBMX in the medium, and cannot be explained solely by its effect on islet cyclic AMP content. DIABETES *30*:824–828, October 1981.

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The role of prostaglandins (PGs) in the regulation of insulin secretion has been examined extensively. Although the results from in vivo and in vitro experiments have led to conflicting views on whether the effects of PGs are stimulatory or inhibitory, most studies have suggested that PGs are involved in the regulation of insulin release (for reviews, see refs. 1,2). Recent studies have shown that a major product of arachidonate metabolism in several tissues is prostacyclin (PGI_2).[3,4] PGI_2 is known to be widely distributed, being produced by the lung, pericardium, uterus, and gastric mucosa in addition to vascular tissue throughout the body (for reviews see refs. 3,5). Furthermore, PGI_2 is the most potent PG with respect to inhibition of platelet aggregation and relaxation of vascular smooth muscle.[3] These observations indicate that PGI_2 is potentially a biologically important PG. However, only preliminary studies of the possible effects of this PG on insulin secretion have been performed. One report showed no effect of PGI_2 on insulin secretion by the perfused rat pancreas.[6] The other report came from our own laboratory and described a stimulation of insulin secretion by PGI_2 in a tissue culture of a benign insulin-secreting tumor from a human subject.[7]

Many of the diverse effects of PGs, including modulation of other secretory processes, are thought to be mediated via changes in cellular cyclic adenosine 3′, 5′-monophosphate (cAMP) levels. Several groups, including our own, have shown that PGs stimulate adenylate cyclase activity[7,8] and presumably increases the cAMP content of islets. For these reasons, and in view of the role of cAMP in potentiating glucose-induced insulin secretion (for review, see ref. 9), this study was designed to examine the role of cAMP in the effects of PGI_2 and 6-keto-$PGF_{1\alpha}$ on insulin release.

MATERIALS AND METHODS

Islets were isolated from the pancreas of nonfasted, male Sprague-Dawley rats weighing 250–300 g using a modifi-

cation[10] of the collagenase digestion technique of Lacy and Kostianovsky.[11]

Preincubation of the islets was performed using batches of eight islets in 1 ml of Krebs-Ringer bicarbonate buffer (KRB)[12] containing 350 mg bovine serum albumin (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) per 100 ml; 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (Hepes, Sigma Chemical Co., St. Louis, Missouri); gassed with 95% O_2/5% CO_2; pH 7.4] in 4-ml Auto Analyser cups (Medical Plastics, S. Australia). After 20 min at 37°C the islets were washed twice using KRB (3.3 mM D-glucose). The test solutions were then added at appropriate concentrations in 1 ml of KRB. In all experiments, several separate incubations were also performed in the presence of either high or low concentrations of glucose as a control to show normal responsiveness of the islets. In the experiments examining the effects of PGI_2, either 37 μl of PGI_2 (Upjohn Chemical Co., Kalamazoo, Michigan) in carbonate buffer (0.01 M Na_2CO_3, 0.02 M $NaHCO_3$, pH 10.0) or 37 μl of carbonate buffer alone was added immediately before the final incubation. In the experiments studying 6-keto-$PGF_{1\alpha}$ (Upjohn Chemical Co., Kalamazoo), a similar procedure was followed except that the 6-keto-$PGF_{1\alpha}$ was first dissolved in analytical grade 95% ethanol (BDH Chemicals Pty Ltd, Port Fairy, Victoria) at a concentration of 10 μg/μl and then diluted with carbonate buffer. A carbonate buffer/ethanol mixture was added to the controls so that all vials contained equal volumes of carbonate buffer and ethanol. All vials in each experiment were gassed with 95% O_2/5% CO_2 and capped immediately before the final incubation of 20 min at 37°C in a shaking water bath. Following the incubation, a sample of the supernatant was taken for insulin assay, and the islets were left in 100 μl of KRB. Perchloric acid (0.5 ml of 0.8 M) was added to each vial to precipitate protein and the acidified contents were then transferred to Wasserman tubes and centrifuged (2200 × *g*, 10 min at 4°C). The supernatants were neutralized, centrifuged to remove the salt precipitate, and a sample taken immediately for cAMP assay.

The radioimmunoassay for cAMP was performed using antibody raised against O$^{2'}$-mono-succinyl adenosine 3',5'-cyclic monophosphate (Sigma Chemical Co.) in rabbits, tracer prepared by iodination of the tyrosyl methyl ester of O$^{2'}$-mono-succinyl adenosine 3',5'-cyclic monophosphate (Sigma Chemical Co.), and cAMP standards, prepared as described previously.[13,14] Samples and standards were acetylated before being assayed. The sensitivity of the assay was 2 fmol cAMP per tube. The rat insulin radioimmunoassay was based on rat insulin standards (Novo Research Institute, Bagsvaerd, Denmark), guinea pig antibovine insulin antiserum (Wellcome Laboratories, Beckenham, England), and tracer prepared by iodination of monocomponent porcine insulin (Novo). Antibody-bound insulin was precipitated using 85% ethanol.[15]

Results are presented as the mean + standard error. Significance of differences between means was assessed by use of Student's *t* test for unpaired samples.

RESULTS

The effects of different concentrations of exogenous PGI_2 and 6-keto-$PGF_{1\alpha}$ on insulin secretion by isolated rat islets and the cAMP content of the islets is shown in Figure 1. In the presence of 5.6 mM D-glucose, all concentrations of

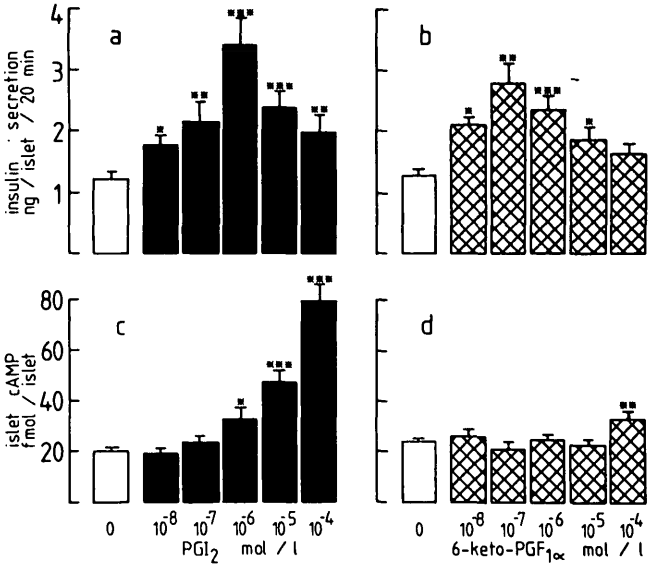


FIGURE 1. The effect of PGI_2 and 6-keto-$PGF_{1\alpha}$ on insulin secretion and cAMP content in isolated rat islets. Islets were incubated in the presence of 5.6 mM D-glucose alone (control, open columns), or in the presence of either 5.6 mM D-glucose plus the concentration of PGI_2 indicated (darkly shaded columns) or 5.6 mM D-glucose plus the concentration of 6-keto-$PGF_{1\alpha}$ indicated (cross-hatched columns). Each column and bar represents the mean + the standard error of 9–34 separate incubations. Significance was evaluated by comparison of the test (glucose + PG) with the control (glucose alone). ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

PGI_2 tested (10^{-8} M–10^{-4} M) caused a significant stimulation of insulin secretion (Figure 1a). The maximal stimulation of insulin secretion occurred at an initial PGI_2 concentration of 10^{-6} M PGI_2. Higher initial concentrations of PGI_2 were less effective, but still caused a significant stimulation of insulin secretion. Figure 1b shows the stimulation of insulin secretion by 6-keto-$PGF_{1\alpha}$. The stimulation was significant at concentrations of 10^{-8} M–10^{-5} M, with the maximal effect being observed at 10^{-7} M 6-keto-$PGF_{1\alpha}$. Comparison of the effects of PGI_2 and 6-keto-$PGF_{1\alpha}$ on insulin secretion at a glucose concentration of 5.6 mM (Figure 1a and b) shows that the effects of these PGs are almost equivalent, although the maximal effect was noted at an initial PGI_2 concentration of 10^{-6} M compared with 10^{-7} M for 6-keto-$PGF_{1\alpha}$. By contrast, the effects of these two PGs on islet cAMP levels are very different (Figures 1c and d). PGI_2 induced a dose-dependent increase in the islet cAMP levels (Figure 1c) with the effect being significant at initial concentrations of PGI_2 of 10^{-6} M and above. In contrast to its effect on insulin secretion, there was no tendency for the effect of PGI_2 on the islet cAMP levels to decrease at higher initial PGI_2 concentrations. The effect of 6-keto-$PGF_{1\alpha}$ on islet cAMP levels is shown in Figure 1d. Although this PG was apparently as effective as PGI_2 in stimulating insulin secretion, it was about 100 times less effective in raising the islet cAMP levels, with 10^{-4} M 6-keto-$PGF_{1\alpha}$ being only as potent as an initial PGI_2 concentration of 10^{-6} M.

The effect of PGI_2 on insulin release by isolated islets and the cAMP content of the islets in the presence of different concentrations of glucose is shown in Figure 2. It can be seen that in the presence of the lower glucose concentrations (3.3 and 5.6 mM) PGI_2 (4×10^{-5} M) induced a highly significant stimulation of insulin secretion (Figure 2a). The maximum effect of this concentration of PGI_2 was seen in the

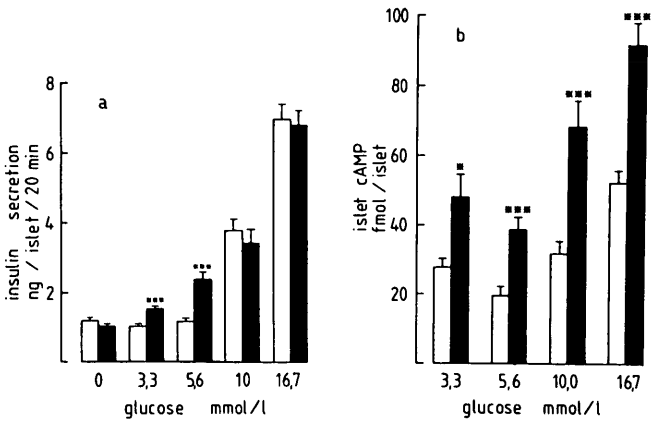
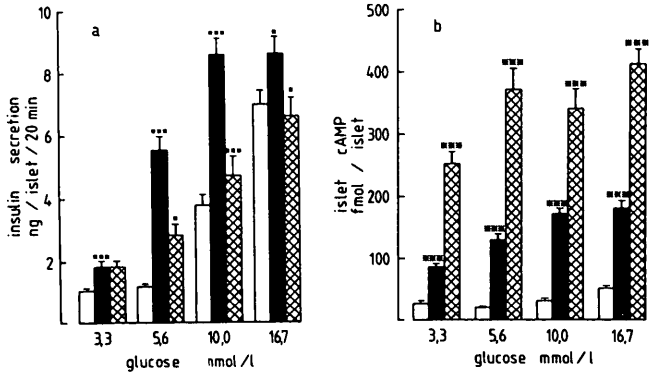


FIGURE 2. Comparison of the glucose concentration dependency of the effect of PGI_2 on insulin release to the glucose-independent stimulation of islet cAMP levels by this PG. Islets were incubated in the presence of the glucose concentration indicated in the presence (darkly shaded columns) or absence (open columns) of 4×10^{-5} M PGI_2. Each column and bar represents the mean + the standard error of 15–40 separate incubations. Statistical significance was evaluated by comparison of the control (glucose alone, open columns) with the test (glucose plus PGI_2) at each glucose concentration.

presence of 5.6 mM D-glucose, where it doubled the rate of insulin release. However, PGI_2 had no effect in the absence of added glucose or in the presence of higher glucose concentrations (10, 16.7 mM). In contrast to the glucose dependency of its effect on insulin release (Figure 2a), PGI_2 induced an increase in the cAMP content of the islets at all glucose concentrations (Figure 2b). The extent of the PGI_2-induced increase in islet cAMP levels was approximately the same at all glucose concentrations.

To further investigate the role of cAMP, the effect of PGI_2 on insulin secretion induced by 3-isobutyl-1-methylxanthine (IBMX)-treated islets was examined (Figure 3a). Addition of this phosphodiesterase inhibitor alone to the islets greatly potentiated the glucose-induced insulin secretion (Figure 3a, open and darkly shaded columns). When PGI_2 was added to islets treated with IBMX, it significantly inhibited insulin release (Figure 3a, darkly shaded and cross-hatched colums) at glucose concentrations of 5.6–16.7 mM, reducing to varying extents the stimulatory effect of IBMX. The effect of IBMX and of IBMX plus PGI_2 on the islet cAMP levels is shown in Figure 3b. The addition of IBMX alone to the islets induced a large increase in the cAMP content above basal levels at all glucose concentrations (Figure 3b, open and darkly shaded columns). Addition of PGI_2 to the IBMX-treated islets induced a further increase in the islet cAMP content (Figure 3b, darkly shaded and cross-hatched columns). This maximal stimulation of the islet cAMP content by the combination of PGI_2 plus IBMX (Figure 3b) is in contrast to the inhibition of insulin release caused by the addition of PGI_2 to islets in the presence of IBMX (Figure 3a).

FIGURE 3. Contrast between the inhibition of IBMX-stimulated insulin secretion by PGI_2 plus IBMX and the maximal stimulation of islet cAMP levels by this combination. Islets were incubated at the glucose concentration indicated in the presence of glucose alone (open columns), glucose plus 0.5 mM IBMX (darkly shaded columns), or glucose plus both 0.5 mM IBMX and 4×10^{-5} M PGI_2 (cross-hatched columns). Each column and bar represents the mean + standard error of 10–29 separate incubations. Statistical significance was evaluated by comparison of (a) the control (glucose alone) with glucose plus IBMX, and (b) by comparison of glucose plus IBMX with glucose plus both IBMX and PGI_2 at each glucose concentration.



DISCUSSION

This report describes for the first time a stimulatory effect of PGI_2 and 6-keto-$PGF_{1\alpha}$ on insulin secretion from isolated rat islets and examines the role of cAMP in the effects of these prostaglandins. From the results presented in Figure 1 it is clear that there is not a simple relationship between the effects of PGs on islet cAMP levels and their ability to stimulate insulin secretion. The bell-shaped dose-response curves for the effects of PGI_2 and 6-keto-$PGF_{1\alpha}$ on insulin release are similar to those reported for other PGs.[16,17] Furthermore, the glucose dependency of the effect reported here (Figure 2a) was similar to that previously described for PGE_1, PGE_2, and $PGF_{2\alpha}$.[17–19] That is, the PGs stimulate insulin secretion maximally in the presence of 5.6 mM D-glucose, but have less effect in the presence of higher glucose concentrations. In fact, Burr and Sharp[18] have reported inhibition of insulin secretion by PGE_1 from perifused rat islets in the presence of high glucose concentrations. The observations may explain some of the apparently discrepant results from other studies. In particular, most of the reports on the effects of inhibitors of PG synthesis on insulin secretion in vivo, in which an inhibitory role for PGs in insulin secretion has been postulated, have studied the insulin response to high glucose concentrations.[20,21]

While both PGI_2 and 6-keto-$PGF_{1\alpha}$ were apparently similar in the extent to which they stimulated insulin secretion (Figure 1a and b), with each being effective at similar initial concentrations, they clearly differed in their effects on islet cAMP levels. The observation that PGI_2 is about 100 times more effective than 6-keto-$PGF_{1\alpha}$ in increasing islet cAMP levels (Figure 1c versus 1d) is consistent with results obtained in platelets in other laboratories.[5] Most agents that increase islet cyclic AMP levels potentiate insulin secretion to an extent which is proportional to their effect on islet cAMP levels [e.g., glucagon,[9] IBMX (Figure 3), theophylline[22]]. Furthermore, most of these agents are more effective at higher glucose concentrations. In contrast, the effectiveness of PGI_2 in stimulating insulin secretion was decreased at concentrations above 10^{-6} M (Figure 1a) despite the continually increasing effectiveness of the highest concentrations of PGI_2 in raising the islet cAMP levels (Figure 1c). The stimulation of insulin secretion by 6-keto-$PGF_{1\alpha}$ (Figure 1b) in the absence of any measurable effect of this PG on islet cAMP levels (Figure 1d) further indicates a dissociation between the effects of PGs on insulin release and their effects on cAMP levels. The lack of correlation between the maximal stimulation of islet cAMP levels by the combination of

PGI_2 plus the phosphodiesterase inhibitor IBMX (Figure 3b) and the inhibition of insulin secretion by this same combination (Figure 3a) provides additional evidence that the effect of PGI_2 on insulin secretion cannot be attributed solely to its effect on total islet cAMP. The results presented here suggest either that (1) the mechanism of PG-induced effects on insulin secretion is not medited via cAMP, or (2) cAMP is involved in PG-modulated insulin secretion, but only a small, nonmeasurable increase is needed, and the large increase in cAMP induced by PGI_2 is superfluous. However, possible compartmentalization of cAMP within the beta-cells, or contribution by non-beta-cells to the islet content of cAMP, cannot be excluded as confounding factors. Additional effects that may explain the inhibition of insulin secretion in the presence of IBMX at high glucose concentrations despite its stimulation of cAMP content include possible effects of PGI_2 on calcium fluxes and cGMP concentrations,[23–25] or possible effects on somatostatin secretion. Our results do not allow us to differentiate these possibilities.

Before interpreting the potential roles of PGI_2 and 6-keto-$PGF_{1\alpha}$ in the stimulation of insulin secretion, the instability of PGI_2 at pH 7.4 must be taken into account. Although PGI_2 is stable in the carbonate buffer (pH 10) used to dissolve this PG, it has a half-life of only 1.5–2 min[5,26,27] at pH 7.4, the pH at which the final incubation was performed. This means that during most of the 20 min of the incubation, the concentration of PGI_2 in the medium would have been much less than the initial concentration, and much of it would have been converted to 6-keto-$PGF_{1\alpha}$. It cannot be determined whether the insulin secretion observed in incubations with PGI_2 was actually due to 6-keto-$PGF_{1\alpha}$. Perifusion experiments would be required to clarify this possibility. In contrast, as PGI_2 was much more potent than 6-keto-$PGF_{1\alpha}$ in increasing islet cAMP content, it is clear that this action could not have been due to the conversion of PGI_2 to 6-keto-$PGF_{1\alpha}$. The finding that 6-keto-$PGF_{1\alpha}$ appears to be a potent insulin secretagogue contrasts with its relative lack of activity in other tissues.[28–30]

The ability of PGI_2 and presumably other PGs to stimulate insulin secretion when the islets are exposed to low glucose concentrations, but to inhibit insulin secretion from stimulated islets, suggests that PGs may play a role as local modulators of insulin secretion. For example, under fasting conditions they may maintain a basal level of insulin secretion, preventing the adverse effects of total insulin lack, and under stimulated conditions they may buffer excessive and prolonged elevations of insulin, thus preventing rebound hypoglycemia. Concurrent effects of PGs on glucagon secretion[17,31,32] may aid the buffering capacity of the islet PGs. A possible physiologic role for local PG production is supported by the recent demonstration of the presence of PGs in isolated guinea pig islets.[31]

Although many questions about the physiologic role of PGs in the islet remain to be clarified, the present study, taken together with previous reports, supports the concept that PGs are modulators of insulin secretion. They show that the apparently conflicting results from previous studies can be explained in many cases by the conditions under which the studies were performed. The present study also indicates that actions in addition to elevation of cAMP must be considered in the mechanism of PG effects of insulin secretion. It supports the concept that the importance of PGI_2 and 6-keto-$PGF_{1\alpha}$ may not be limited to effects on platelet aggregation and vascular smooth muscle relaxation.

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