Prostaglandin E₂ inhibits phosphoinositide metabolism in isolated pancreatic islets

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Isolated islets of the rat labelled with *myo*-[³H]inositol showed decreased accumulation of total inositol phosphates (InsPs) and [³H]polyphosphoinositide hydrolysis in response to glucose after preincubation with prostaglandin E₂ (PGE₂). The response was concentration-dependent and specific for PGE₂. PGE₂ did not affect basal [³H]phosphoinositide hydrolysis or InsPs accumulation. Pertussis-toxin pretreatment antagonized the response to PGE₂, whereas 8-bromo cyclic AMP was without effect. The PGE₂-induced decrease in InsPs may contribute to the suppression of insulin secretion.

INTRODUCTION

Prostaglandins (PGs) appear to exert an inhibitory effect on insulin secretion from pancreatic islets, in light of the evidence that the inhibition of PG synthesis augments glucose-stimulated insulin release from isolated islets (Robertson, 1983; Turk *et al.*, 1988) and increases plasma insulin in diabetics exposed to a glucose challenge (Robertson & Chen, 1977). Exogenous PGE also inhibits insulin release from dog pancreas (Robertson, 1974) and insulinoma cells (Robertson *et al.*, 1987), although stimulatory effects of PGs on insulin release have also been reported (Pek *et al.*, 1978, 1980; Matsuyama *et al.*, 1983). The cellular mechanisms accounting for the PG effects appear to be complex. Although PGE₂ inhibits adenylate cyclase in insulinoma cells (Robertson *et al.*, 1987), it also inhibits glucose oxidation in islets (Laychock, 1989). The last two reports link the effect of PGE₂ to a transduction mechanism(s) modulated by a guanine-nucleotide-binding regulatory (G) protein which is sensitive to pertussis toxin.

Among its many actions, glucose is known to promote phosphoinositide hydrolysis (Laychock, 1983) and the production of inositol phosphates (InsPs) in pancreatic islets (Best & Malaisse, 1984; Turk *et al.*, 1986), which may participate in Ca²⁺ mobilization and insulin release (Wolf *et al.*, 1985; Wollheim *et al.*, 1986). Moreover, a G-protein mediates the activation of phospholipase C and InsPs production in the islet (Dunlop & Larkins, 1986; Blachier & Malaisse, 1987). The effect of PGE₂ on phosphoinositide metabolism was investigated in order to determine if the activity of this cycle might account for some of the eicosanoid effects on insulin release.

EXPERIMENTAL

Materials

myo-[2-³H(n)]Inositol (10–20 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, U.S.A.). Pertussis toxin was obtained from List Biological Laboratories (Campbell, CA, U.S.A.). Collagenase from *Clostridium histolyticum* was purchased from Serva (Heidelberg, Germany). Prostaglandins were given

by Upjohn (Kalamazoo, MI, U.S.A.). Dowex ion-exchange resin (AG 1-X8, formate form) was purchased from Bio-Rad (Richmond, CA, U.S.A.). All other chemicals were reagent grade.

Tissue preparation and incubation

Pancreatic islets were isolated from excised pancreata of male Sprague–Dawley rats by collagenase digestion, as described previously (Hoffman & Laychock, 1988). Batches of equal numbers of islets were incubated in Krebs–Ringer bicarbonate (KRB) buffer containing 16 mM-Hepes, 2.8 mM-glucose (basal glucose concentration) and 0.01 % bovine serum albumin (fatty-acid-free, fraction V), adjusted to pH 7.4 after equilibration with O₂/CO₂ (19:1) at 37 °C. After a 30 min preincubation in KRB buffer, the islets were resuspended in fresh KRB buffer and prelabelled with *myo*-[³H]inositol for determination of InsPs production. This prelabelling was performed by preincubating islets in KRB buffer (0.1 ml) containing 7.3 mM-glucose and *myo*-[³H]inositol (5–10 µCi) for 60 min. Additional KRB buffer was then added to give a final volume of 0.3 ml and 4.3 mM-glucose. The preincubation was continued for an additional 60 min, after which time the radioactive buffer was removed and the islets were washed three times during 30 min with KRB buffer (1 ml each time) containing 2.8 mM-glucose. When islets were treated with pertussis toxin (1.5 µg/ml), this agent was added to the islets at the same time as the radiolabel.

After the wash procedure, the islets were resuspended in KRB buffer in preparation for a 30 min preincubation in the absence or presence of PGE₂, followed by a final 30 min incubation in the presence of various concentrations of glucose as indicated in the text. LiCl (10 mM) was present during the final 30 min incubation to inhibit InsPs metabolism. The reaction was stopped with 4.5 % (w/v) HClO₄, and the InsPs were extracted and isolated by ion-exchange chromatography as described previously (Berridge *et al.*, 1983; Chaudry *et al.*, 1988). Radiolabelled *myo*-inositol 1-phosphate, *myo*-inositol 1,4-bisphosphate, *myo*-inositol 1,4,5-trisphosphate and *myo*-inositol 1,3,4-trisphosphate (InsP₃) and *myo*-inositol 1,3,4,5-tetrakisphosphate (InsP₄) were quantified and the

Abbreviations used: PG, prostaglandin; InsPs, total inositol phosphates; KRB, Krebs–Ringer bicarbonate.

Fig. 1. Effects of glucose and PGE₂ on [³H]InsPs production in islets

Islets were prelabelled with *myo*-[³H]inositol and then incubated in KRB buffer containing 2.8 mM-glucose (basal) for 30 min in the absence or presence of PGE₂ (1 μM). Glucose (Glu; 17 mM) was then added to certain samples as indicated, and the incubation was continued for 30 min. Values are means ± S.E.M. for the radiolabel measured in total combined InsPs (inositol monophosphate, bisphosphate, trisphosphate and tetrakisphosphate). The number of experiments is indicated at the base of each bar. *P* values were determined by one-way analysis of variance: ns, not significant.

values added together for a measure of total InsPs produced by islets in most experiments. Radiolabelled phosphoinositides (phosphatidylinositol 4-phosphate and 4,5-bisphosphate) were extracted from islet membranes after the extraction of InsPs, and isolated by t.l.c., as described previously (Laychock, 1983). Data are expressed as a summation of the two phosphoinositide lipids.

Statistical analysis

Statistical analysis of data was carried out by Student's *t* test or one-way analysis of variance: *P* values less than 0.05 were accepted as significant. Values are means ± S.E.M. for the numbers of different experiments (*n*). When values are expressed as percentages, statistical analysis was performed on real data values.

RESULTS

[³H]InsPs accumulation in islets prelabelled with *myo*-[³H]inositol was increased 2-fold by glucose stimulation (Fig. 1). When islets were pretreated with PGE₂ (1 μM) for 30 min, [³H]InsPs levels in response to a subsequent glucose stimulus were decreased by 25% (Fig. 1). Preincubation with PGE₂, however, did not alter basal levels of [³H]InsPs (Fig. 1). In similar experiments, a lower concentration of PGE₂ (0.1 μM) decreased glucose (17 mM)-stimulated [³H]InsPs accumulation in islets by 15 ± 7% (*P* < 0.05; *n* = 10); however, 0.001 μM- and 0.01 μM-PGE₂ had no effect (results not shown). In addition, the accumulation of [³H]InsPs in glucose (17 mM)-stimulated islets pretreated with 6-oxo-PGF_{1α} (1 μM) or PGD₂ (1 μM) was 90 ± 7 and 94 ± 5%, respectively, of the stimulated control values (*P* > 0.05; *n* = 6).

Table 1. Effects of glucose and PGE₂ on [³H]polyphosphoinositide levels in islets

Islets were incubated as described in the legend to Fig. 1, in the presence of 2.8 mM-glucose, except as indicated. [³H]Polyphosphoinositides (phosphatidylinositol 4-phosphate and 4,5-bisphosphate) were isolated by t.l.c., and the radiolabel content of the phospholipids was determined. Values are means ± S.E.M. of the total c.p.m. in [³H]polyphosphoinositides, calculated as percentages of basal (2.8 mM-glucose; 86 ± 15 c.p.m./50 islets for six experiments). **P* < 0.05 compared with basal as determined by Student's *t* test (paired). The numbers of different experiments are shown in parentheses.

Treatment	[³ H]Polyphosphoinositides (% of basal)
PGE ₂ (1 μM)	93 ± 4 (6)
Glucose (17 mM)	71 ± 6* (6)
Glucose (17 mM) plus PGE ₂ (1 μM)	100 ± 10 (5)

[³H]InsP₃/InsP₄ accumulation in glucose (17 mM)-stimulated islets (73 ± 4 c.p.m./50 islets) was also consistently decreased after exposure to PGE₂ (1 μM) (60 ± 6 c.p.m./50 islets; *P* < 0.05, *n* = 7). During the 30 min preincubation with PGE₂ basal levels of [³H]InsPs were not altered (88 ± 8% of control). Thus the effect of PGE₂ on the response to glucose was not due to eicosanoid-induced hydrolysis and depletion of the phosphoinositide pool. Moreover, without a preincubation period, PGE₂ added to islets together with glucose (17 mM) did not alter InsPs production compared with those values obtained with the glucose stimulus alone (111 ± 12% of glucose-treated values) during a 30 min incubation. Thus PGE₂ exerts a latent effect on islet InsPs production.

In order to determine if changes in [³H]InsPs accumulation correlated with changes in [³H]polyphosphoinositide levels in islets, the phospholipids were extracted and quantified after exposure of islets to PGE₂ and subsequent incubation in the absence and presence of a glucose stimulus. Glucose stimulation alone decreased [³H]polyphosphoinositide levels by 29% (Table 1), which correlates well with the glucose-induced change in [³H]InsPs production. However, pretreatment with PGE₂ antagonized the response to glucose, and [³H]polyphosphoinositide levels in the presence of glucose and PGE₂ did not affect basal [³H]polyphosphoinositide levels (Table 1).

Previous studies have linked the PGE₂ response in insulin-secreting cells to inhibition of adenylate cyclase (Robertson *et al.*, 1987). In order to determine if the suppression of cyclic AMP levels mediated the response to PGE₂, islets were incubated with exogenous cyclic nucleotide. The addition of glucose (17 mM) to islets pretreated with PGE₂ (1 μM) induced a rise in [³H]InsPs production which was 75 (± 5)% of the glucose-stimulated values in the absence of PGE₂ (*P* < 0.02); addition of 8-bromo cyclic AMP (1 mM) to the combination of glucose and PGE₂ resulted in [³H]InsPs levels which were 75 (± 4)% of glucose-stimulated values (*P* < 0.01; *n* = 5). In the absence of PGE₂, [³H]InsPs levels with glucose (17 mM) and 8-bromo cyclic AMP (1 mM) were 112 (± 14)% of glucose-stimulated control values. Thus

Table 2. Effect of pertussis toxin on [³H]InsPs levels in islets

Islets were prelabelled with *myo*-[³H]inositol, and certain samples were pretreated with pertussis toxin (1.5 µg/ml) as indicated. Islets were then incubated in KRB buffer with 2.8 mM-glucose for 30 min in the absence or presence of PGE₂ (1 µM) as indicated. Then glucose (17 mM) was added as indicated, and the incubation continued for 30 min. Mean ± S.E.M. values of radiolabel in total InsPs was determined in three different experiments. *P* values (**P* < 0.05 compared with basal; ***P* < 0.01 compared with pertussis-toxin-treated control) were determined by one-way analysis of variance.

Treatment	[³ H]InsPs (c.p.m./50 islets)
Basal	671 ± 64
Pertussis toxin	576 ± 79
Glucose (17 mM)	1099 ± 179*
Glucose (17 mM) plus pertussis toxin	1112 ± 111
Glucose (17 mM) plus pertussis toxin plus PGE ₂	1204 ± 156**

cyclic AMP did not appear to modulate the PGE₂ response.

The effects of exposure to pertussis toxin on the islet [³H]InsPs response to glucose and PGE₂ were also determined. After exposure of islets to pertussis toxin, basal values of [³H]InsPs were not altered, and [³H]InsPs levels after a glucose challenge were not different from control values (Table 2). In pertussis-toxin-treated islets, PGE₂ did not affect glucose-stimulated [³H]InsPs accumulation (Table 2), whereas PGE₂ decreased [³H]InsPs accumulation in the absence of pertussis toxin in similar experiments (Fig. 1).

DISCUSSION

The importance of phosphoinositide hydrolysis and the accumulation of InsPs as a cellular messenger for glucose stimulation has been established in pancreatic islets. This study demonstrates that PGE₂ inhibits in a concentration-dependent and specific manner the accumulation of [³H]InsPs in islets. Levels of [³H]InsP₃/InsP₄, which are eluted in the same chromatographic fraction, parallel the accumulation of total InsPs in these studies. The inhibitory response appears to be modulated by a G-protein which is pertussis-toxin sensitive. These studies also indicate that membrane polyphosphoinositide hydrolysis is retarded by PGE₂. It is plausible that PGE₂ exerts an inhibitory effect on phospholipase C activity in islets. Phospholipase C is regulated by a G-protein in the islet and certain other cell types (Blachier & Malaisse, 1987; Cockcroft & Stutchfield, 1988). In islets, the action of pertussis toxin may be in ADP-ribosylating and inhibiting an inhibitory G-protein affecting phospholipase C. These data are the first to suggest the presence of an inhibitory G-protein affecting phospholipase C. Alternatively, some other PGE₂-inhibitable process in islets, such as altered glucose metabolism, may be affected by pertussis toxin and be responsible for indirect effects of PGE₂ on phosphoinositide hydrolysis.

Insulin release from pancreatic islets is antagonized by endogenous PG production and by exogenous PGE₂

(Robertson, 1974, 1983; Turk *et al.*, 1988; Laychock & Bilgin, 1989). The negative influence of PGE₂ on insulin release appears to be modulated in part by the receptor-mediated inhibition of adenylate cyclase (Robertson *et al.*, 1987). PGE₂ (1 µM) causes maximum inhibition of cyclic AMP production (Robertson *et al.*, 1987). An inhibitory G-protein mediates the inhibitory effects of PGE on insulin release, since pertussis toxin reverses the inhibitory response in islets and insulinoma cells (Robertson *et al.*, 1987). However, PGE₂ also inhibits glucose oxidation, and a pertussis-toxin-inhibitable process modulates the glucose-oxidation response (Laychock, 1989; Laychock & Bilgin, 1989). Since glucose utilization in islets is not modulated by cyclic AMP (Laychock, 1987), PGE₂ may have an effect on a transduction mechanism(s) other than adenylate cyclase which modulates metabolism and insulin release.

The responses of [³H]InsPs production, glucose utilization and insulin secretion to PGE₂ have certain characteristics in common. PGE₂ synthesis increases gradually after a glucose stimulus (Kelly & Laychock, 1984; Robertson *et al.*, 1987), and suppresses the secondary phase of insulin secretion (Robertson & Chen, 1977; Evans *et al.*, 1983), suggesting that PGE₂ may act as a negative-feedback inhibitor of insulin secretion. It is not known why the inhibitory effects of PGE₂ on [³H]InsPs accumulation and glucose utilization (Laychock, 1989) require a period of pre-exposure to PGE₂. Perhaps the binding of PGE₂ to its receptor is rate-limiting, or PGE is metabolized to prostaglandyl-inositol cyclic phosphate (Partke & Wasner, 1986), which inhibits protein phosphorylation in islets. The latent response to PGE₂ supports its role as a negative-feedback modulator of the insulin-secretory response. The relationship between glucose utilization and [³H]InsPs levels has not been defined, except that polyphosphoinositide hydrolysis is dependent on glucose metabolism (Laychock, 1983). The inhibitory effects of PGE₂ on [³H]InsPs accumulation may be indirectly mediated by changes in glucose metabolism. Alternatively, polyphosphoinositide hydrolysis to diacylglycerol and InsPs stimulates protein kinase C and Ca²⁺ mobilization, respectively, which may modulate directly or indirectly the activity of glucose-metabolic pathways and insulin release.

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