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Regulation of Adenylyl Cyclase from Isolated Pancreatic Islets by Prostaglandins and Guanosine 5′-Triphosphate†

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ABSTRACT: Adenylyl cyclase activity of homogenates or membrane preparations from isolated rat pancreatic islets was slightly activated by 10 μM prostaglandin E_1 (PGE_1) or GTP. However, when both PGE_1 and GTP were used in combination, adenylyl cyclase activity was increased twofold to a level that was 70% of that obtained with 10 mM sodium fluoride. In the presence of GTP (20 μM) PGE_1 stimulation was evident at 0.2 μM and maximal at 10 μM. Prostaglandins E_2, F_2, and A_1 (0.1 or 10 μM) had no effect on basal enzyme activity, but PGE_2 or PGA_1 (10 μM) increased activity slightly in the presence of GTP (20 μM). Kinetic analysis indicated that PGE_1 plus GTP increased both the apparent Michaelis constant for ATP and the maximum velocity of adenylyl cyclase. Neither compound had any effect on the activity of cyclic nucleotide phosphodiesterase in pancreatic islet homogenates.

Considerable evidence has accumulated in recent years to suggest that release of insulin from the β cells of the pancreas involves cAMP[1] (Turtle and Kipnis, 1967; Montague and Cook, 1971; Atkins and Matty, 1971). Substances that activate adenylyl cyclase in other tissues, such as glucagon (Sutherland and Robison, 1966), elevate cAMP levels in pancreatic islet tissue and release insulin (Turtle and Kipnis, 1967). Other agents that are thought to act by inhibiting cAMP catabolism by cyclic nucleotide phosphodiesterase, like theophylline (Turtle *et al.*, 1967) or tolbutamide (Lacy *et al.*, 1968), similarly augment insulin release.

Previous studies in our laboratory (Johnson *et al.*, 1973) indicated that prostaglandins, particularly PGE_1, increased glucose-stimulated release of insulin. PGE_1 also increased the accumulation of cAMP formed from ^{14}C-labeled precursor by incubated pancreatic islets. These results suggested that PGE_1 might affect insulin secretion by stimulating adenylyl cyclase activity or by inhibiting cyclic nucleotide phosphodiesterase. Therefore, we have investigated the effects of PGE_1 on adenylyl cyclase and cyclic nucleotide phosphodiesterase activities of pancreatic islet tissue from the rat. Since GTP is required for the prostaglandin activation of adenylyl cyclase in platelet membranes (Krishna *et al.*, 1972) and thyroid plasma membranes (Wolff and Cook, 1973), we also studied the effects of GTP on the pancreatic islet enzymes either alone or in combination with PGE_1.

Experimental Section

Materials. Male Wistar rats (300–400 g) were purchased from Simonson Laboratories, Gilroy, Calif. Chemicals were

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[1] Abbreviations used are: cAMP, adenosine 3′,5′-monophosphate; PGE_1, prostaglandin E_1.

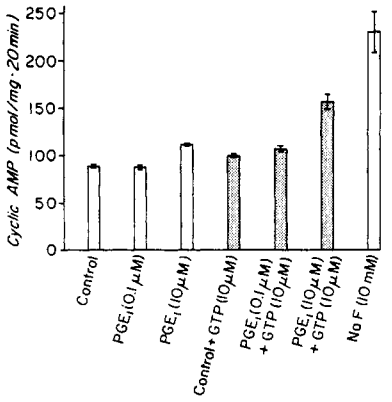

FIGURE 1: Effect of PGE_1, GTP, and NaF on adenylyl cyclase activity of pancreatic islet homogenates. Pancreatic islets were isolated from rats by the method of Lacy and Kostianovsky (1967) as modified by Johnson *et al.* (1973). After homogenization aliquots were assayed for adenylyl cyclase activity in the presence of PGE_1, GTP, or NaF. Results are expressed as the mean ± standard error of triplicate determinations made with the same homogenate.

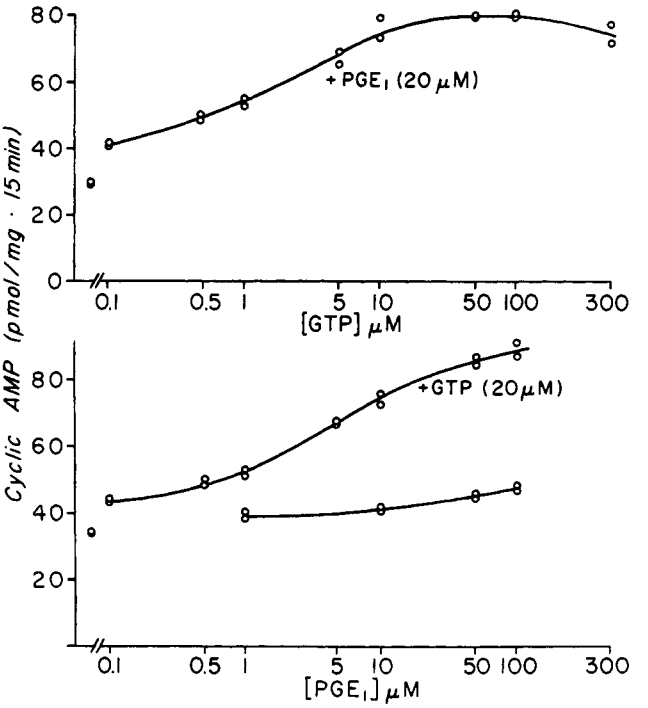

FIGURE 2: Effect of different concentrations of GTP or PGE_1 on adenylyl cyclase activity of membranes prepared from isolated rat pancreatic islets. The upper plot shows the effect of GTP on PGE_1 stimulation of adenylyl cyclase activity. Enzyme activity in the absence of GTP is represented by the point to the left of the discontinuation marks on the abscissa. The lower plot shows the effect of PGE_1 on adenylyl cyclaase activity either alone (lower curve) or in combination with GTP.

obtained through the following sources: collagenase, Class 229A (Worthington); Ficoll (Pharmacia); [α-^{32}P]ATP (5–15 Ci/mmol) (New England Nuclear); [8-^{3}H]cAMP (28 Ci/mmol) (Schwarz/Mann) purified according to Thompson and Appleman (1971); creatine phosphokinase, phosphocreatine, ATP, GTP, cAMP (Sigma); neutral aluminum oxide, Activity I (E. Merck, Darmstadt, Germany); manganese dioxide (Matheson Coleman and Bell); enzyme-grade sucrose (Mann); snake (Ophiophagus hannah) venom (Sigma); Dowex 1-X8 (200–400 mesh, chloride form) (Bio-Rad Laboratories) prepared according to Thompson and Appleman (1971); bovine serum albumin (Fraction V) (Nutritional Biochemical Corp.). The prostaglandins were kindly supplied by Dr. John Pike, Upjohn Co., Kalamazoo, Mich. Prostaglandins were dissolved in ethanol, and 10^{-2} M stock solutions made with nitrogen-gassed Krebs-Ringer bicarbonate (Umbreit *et al.*, 1945) were stored at −20°.

Methods. Isolation of Pancreatic Islets. Pancreatic islets were prepared by the technique of Lacy and Kostianovsky (1967), using discontinuous Ficoll gradients (Lindall *et al.*, 1969) as modified by Johnson *et al.* (1973). All adenylyl cyclase activities were measured using freshly prepared pancreatic islets (average isolation time was 3 hr). Two hundred pancreatic islets (sufficient for 50 assays) isolated in each experiment from four rats was collected by sedimentation at 1000*g* for 1 min in the final preparation medium of synthetic interstitial fluid (Bretag, 1969) at 4°, and resuspension in 1.2 ml of 0.32 M sucrose at 4°. The islets were homogenized by hand, using 6–8 strokes in a Duall-type glass homogenizer fitted with a Teflon plunger. For some experiments islet cell membranes were prepared by transferring the homogenate with a cold Pasteur pipet to a Nalgene test tube (15 × 100 mm), centrifuging (4°) at 13,000*g* for 10 min, and resuspending the resultant pellet in 0.3 M sucrose with a cold, smooth glass rod after discarding the supernatant. A homogeneous membrane preparation was ensured by gentle rehomogenization with 1–2 strokes of a Teflon plunger in the original vessel.

Adenylyl Cyclase Assay. Adenylyl cyclase activity was measured by the method of Ramachandran (1971) as modified by Thompson *et al.* (1973). Each assay contained [α-^{32}P]ATP (10–200 μM; approximately 2.4 × 10^6 cpm), 0.2 mg of creatine phosphokinase (5–15 units), 15.6 mM phosphocreatine, 7.5 mM theophylline, 5 mM magnesium chloride, 0.5 mM cAMP, 0.09% bovine serum albumin, 8 mM Tris-Cl (pH 7.4), and 25 μl of the enzyme preparation in a final volume of 0.10 ml. Fresh solutions of ATP and prostaglandins were prepared for each experiment. Assays were incubated at 30° for 20 min, terminated by Dry Ice–acetone freezing, and then immersed in boiling water (3 min). The reaction mixture was cooled and diluted to 1.0 ml with 50 mM Tris-Cl (pH 7.4) containing approximately 10,000 cpm of tracer [^{3}H]cAMP to test recovery. After adding 1 g of manganese dioxide, the mixture was thoroughly mixed and centrifuged (4000*g*) for 8 min. The clear supernatant was applied to 2 g of a neutral alumina–lead sulfate (3:1) mixture packed in a Pasteur pipet column (6 × 70 mm). After adsorption to alumina, [^{32}P]cAMP and [^{3}H]cAMP were eluted with 3 ml of 50 mM Tris-Cl (pH 7.4) into polyethylene scintillation vials to which were added 15 ml of scintillation fluid consisting of naphthalene (125 g), 2,5-diphenyloxazole (7.5 g), 1,4-bis-(2,5-phenyloxazolyl)benzene (0.382 g), and 1,4-diethylene dioxide (1.0 l.) Radioactivity of ^{3}H and ^{32}P was measured by liquid scintillation counting techniques.

Phosphodiesterase Activity. Cyclic nucleotide phosphodiesterase activity was measured according to Thompson and Appleman (1971). One hundred pancreatic islets were homogenized in 2.0 ml of 0.32 M sucrose and 40 μl used for each assay. cAMP substrate concentration was 0.4 μM.

Results

Effects of PGE_1 and GTP on Adenylyl Cyclase Activity of Pancreatic Islet Homogenates. PGE_1 and GTP were first tested on crude homogenate enzyme preparations. Adenylyl cyclase activity was shown to be linear for 20 min using 50 μM ATP under the conditions of assay as described in Methods. Basal activity varied slightly in different preparations, but the effects of GTP, PGE_1, or sodium fluoride were similar in all

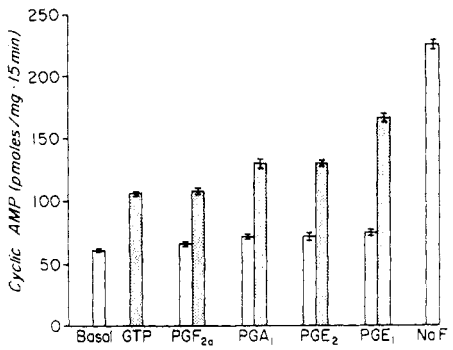

FIGURE 3: Effect of different prostaglandins on adenylyl cyclase activity of membranes prepared from isolated rat pancreatic islets. Shaded bars represent enzyme activity in the presence of GTP. Results are expressed as the mean ± standard error of triplicate determinations made with the same membrane preparations.

experiments. Figure 1 shows the results obtained with a representative islet homogenate. PGE_1 at 0.1 μM or GTP at 10 μM had no effect on the formation of cAMP from ATP, but 10 μM PGE_1 did slightly stimulate adenylyl cyclase activity. However, when PGE_1 (10 μM) and GTP (10 μM) were added together, adenylyl cyclase activity was increased nearly twofold or 70% of the maximal stimulation caused by 10 mM sodium fluoride. GTP (1–500 μM) had only a slight inhibitory effect on sodium fluoride stimulated enzyme activity. Maximum inhibition in the presence of 500 μM GTP was 20%.

Adenylyl Cyclase Activity of Pancreatic Islet Membranes. Adenylyl cyclase activity was also demonstrable in the plasma membranes prepared from pancreatic islets as described in Methods and was linear for at least 20 min at either 20 or 200 μM ATP. Sodium fluoride produced a nearly fourfold increase in enzyme activity, similar to its stimulation of islet homogenates. GTP had a variable effect on different membrane preparations, causing small increases in adenylyl cyclase activity in some experiments (Figure 2 and Figure 4), but increases of up to 75% in other preparations (Figure 3). The different responses to GTP could not be correlated with minor changes in technique. In the absence of GTP, PGE_1 (20 μM) produced little change over basal activity, whereas in the presence of GTP (20 μM) PGE_1 markedly increased adenylyl cyclase activity (Figure 2). The PGE_1 activation of adenylyl cyclase was evident at 0.1 μM GTP and became maximal at 10 μM GTP (Figure 2). In the presence of 20 μM GTP, stimulation of adenylyl cyclase activity by PGE_1 was detectable at 0.1 μM hormone, maximal at 10 μM, and indicated a half-maximal stimulating concentration of 4 μM.

Effects of Different Prostaglandins on Adenylyl Cyclase Activity. The effects of $PGF_{2\alpha}$, PGA_1, PGE_2, and PGE_1 in the presence or absence of GTP were compared in three different membrane preparations. Similar responses were obtained in all preparations. PGE_1 was the most potent of the prostaglandins tested (Figure 3). $PGF_{2\alpha}$ had no effect on enzyme activity in the presence or absence of GTP. At 100 μM concentrations, PGA_1 and PGE_2 had a very slight effect on adenylyl cyclase astivity. In the presence of GTP, PGA_1 and PGE_2 stimulated adenylyl cyclase activity approximately half as much as PGE_1.

Kinetics of Adenylyl Cyclase Activity. The differences in reaction velocities between basal and PGE_1 or GTP additions alone were usually slight at all substrate concentrations tested (Figure 4) and did not allow any statistically significant

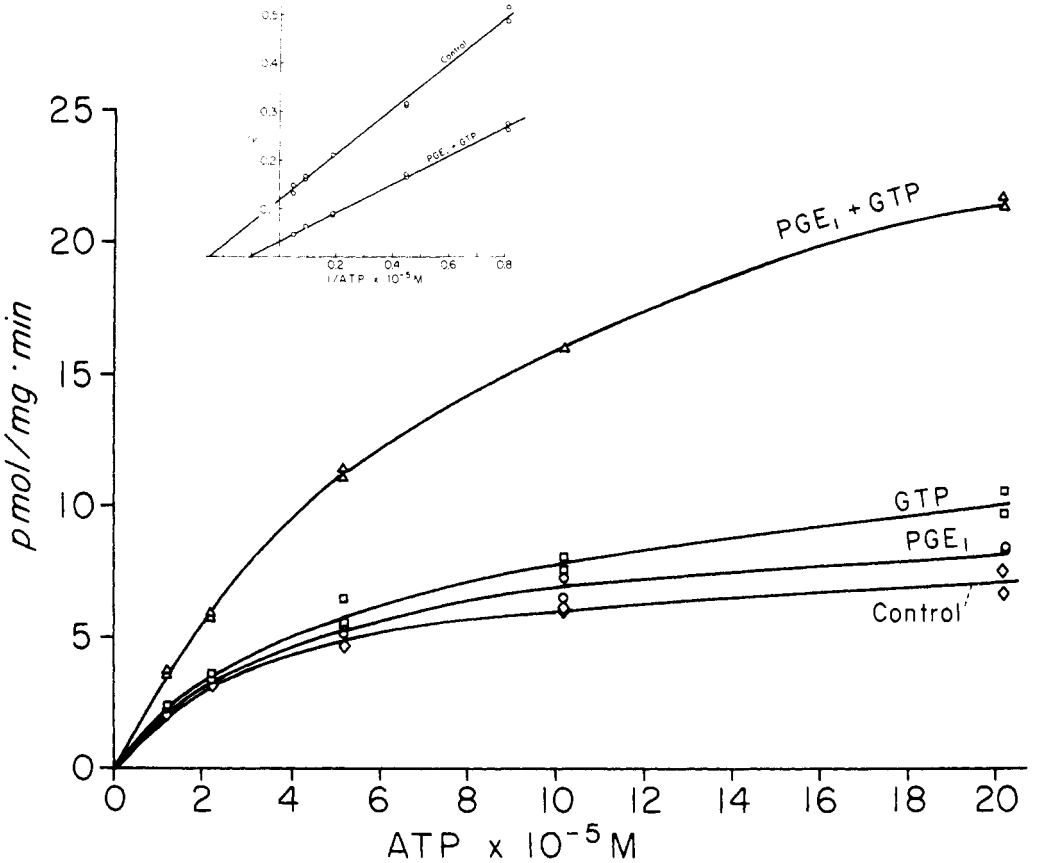

FIGURE 4: Effect of varying ATP concentration on the activatien of pancreatic islet adenylyl cyclase by PGE_1 (10 μM) and GTP (10 μM). The membranes were prepared and assayed for enzyme activity as described in the Methods. All duplicate determinations are plotted.The insert shows a double reciprocal plot of adenylyl cyclase activity. The lower curve shows the effect of PGE_1 (10 μM) and GTP (20 μM) on enzyme activity.

kinetic parameters to be determined. The increase in activity produced by PGE_1 and GTP was evident at all concentrations of ATP tested (10–200 μM). When plotting the data according to Lineweaver–Burk (Figure 4, insert), enzyme activity in the presense of PGE_1 and GTP compared to basal activity demonstrated the anticipated increase in the maximum velocity from 11 to 36 pmol min^{-1} mg of $protein^{-1}$ but also showed a change in the apparent K_m of the reaction from 42 to 87 μM. The kinetics of the adenylyl cyclase reaction were also studied using pancreatic islet homogenates, which demonstrated a shift in the apparent K_m cause by PGE_1 and GTP in addition to increasing the V_{max} similar to those seen with membrane preparations.

Phosphodiesterase Activity. cAMP phosphodiesterase activity of pancreatic islet homogenates was measured using 0.4 μM cAMP. There was no stimulation of enzyme activity measurable at this substrate concentration (approximately 30 pmol of 5′-AMP formed mg of $protein^{-2}$ min^{-1}) by either PGE_1 or GTP alone or in combination.

Discussion

Stimulation of glucose-induced insulin secretion by PGE_1 is accompanied by elevated levels of cAMP (Johnson *et al.*, 1973). The *in vitro* studies with pancreatic islet adenylyl cyclase and cyclic nucleotide phosphodiesterase presented here suggest that the increase in cAMP may be a consequence of activation of islet membrane adenylyl cyclase. Since GTP was required before the effects of PGE_1 on adenylyl cyclase were evident *in vitro*, it is possible that GTP or other guanine nucleotides exert an important modulatory role on adenylyl cyclase activation by substances such as PGE_1 in intact pancreatic β cells. Kuo *et al.* (1973) recently reported that PGE_1 or GTP stimulated adenylyl cyclase activity of rat pancreatic islet homogenates. The method of isolating islets used by Kuo *et al.* included pretreatment of the animals with pilocarpine, which may affect either the metabolism of many regulatory substances such as PGE_1 or GTP in the pancreatic islets or the hormonal responsiveness of the islet adenylyl cyclase system. This is suggested by the many agents with questionable ability to release insulin that affected adenylyl cyclase activity as measured by their procedures. Furthermore, Krishna *et al.* (1972) found that GTP was not required for the PGE_1 activation of platelet adenylyl cyclase at high ATP substrate concentrations such as those used by Kuo *et al.* in their islet study.

The requirements for GTP in the prostaglandin activation of adenylyl cyclase in pancreatic islet membranes shown by our studies appear similar to the action of these two substances on the adenylyl cyclase of human platelet membranes reported by Krishna *et al.* (1972). These activations, however, differ in several respects. Although Krishna *et al.* did not report detailed kinetic studies, they found no evidence for a change in the apparent affinity for ATP of platelet adenylyl cyclase. This contrasts with the apparent shift in K_m found in both membrane preparations (Figure 4) and islet homogenates prepared from pancreatic islets using more detailed kinetic analysis. GTP markedly inhibited fluoride-stimulated adenylyl cyclase in platelet membranes (Krishna *et al.*, 1972), whereas it had very slight effect on fluoride-stimulated adenylyl cyclase from pancreatic islets. No evidence for negative control by GTP was apparent with islet membrane adenylyl cyclase, as was reported by Birnbaumer (1973) using beef renal medullary membranes.

The finding that PGE_1 was the most potent of the prostaglandins tested for activation of pancreatic islet membrane adenylyl cyclase agrees with the results of Kuo *et al.* (1973) in which they found lesser activation of homogenate activities with PGE_2, PGA_1, and $PGF_{1\alpha}$. PGE_1 is also the most potent stimulator of glucose-induced insulin secretion (Johnson *et al.*, 1973).

The establishment of kinetic parameters for the effect of hormones in altering adenylyl cyclase activity has been difficult for a variety of reasons, even in systems that have been studied as extensively as the glucagon-stimulated enzyme of rat liver membranes (Pohl *et al.*, 1971; Birnbaumer *et al.*, 1971; Rodbell *et al.*, 1971a,b,c). Rodbell and coworkers reported that GTP exerted an important allosteric modulatory role on liver adenylyl cyclase by decreasing the binding affinity of the membranes for glucagon (Rodbell *et al.*, 1971b). This resulted in an increase in adenylyl cyclase activity (Rodbell *et al.*, 1971c). Similar results were obtained in the glucagon stimulation of adenylyl cyclase from islet cell tumor (Goldfine *et al.*, 1972). However, more recent studies of GTP dissociation of glucagon bound to liver membranes suggested that because of the redundancy of glucagon binding sites these effects may not be related to hormonal stimulation of catalytic activity (Birnbaumer and Pohl, 1973). It is possible that the decrease in apparent affinity which we found in pancreatic islet adenylyl cyclase is also due to a GTP-induced change in the binding of PGE_1 to islet membranes with a subsequent alteration of substrate binding. Our kinetic analyses of the effects of GTP were conducted at presumably saturating levels of hormone, as were the effects on glucagon activation of liver membrane adenylyl cyclase by GTP (Rodbell *et al.*, 1971c). However, PGE_1 and peptide hormones, such as glucagon, may act by entirely different mechanisms. Evidence has been presented that TSH and PGE_1 act at separate sites (Wolff and Cook, 1973).

Most modifiers of enzyme activity exert negative effects, but positive modifiers have been described that generally affect only one kinetic parameter dependent on the state of the enzyme (Atkinson, 1966; Gerhart, 1970). Some enzyme systems in the Embden–Meyerhoff pathway are modulated by single metabolic intermediates affecting the affinity of one enzyme and the maximum velocity of the reverse enzyme (Atkinson, 1966). The exact mechanisms by which a GTP-induced decrease in the binding affinity of a hormone activator could result in a concomitant decrease in the affinity of adenylyl cyclase for substrate ATP are still unknown. It is interesting in this regard that oxytocin stimulation of adenylyl cyclase activity of toad bladder increased both the affinity for ATP and the maximum velocity of that enzyme system (Bockaert *et al.*, 1972). In studying membrane preparations or homogenates the accessibility of ATP substrate to adenylyl cyclase may also be an important factor in determining reaction kinetics or the possible allosteric action of GTP on kinetic parameters.

Cyclic AMP appears to play an important role in β cell secretion of insulin. Most evidence suggests that cAMP modulates insulin secretion rather than directly mediating the exocytotic process. Even in the presence of high intracellular cAMP, insulin secretion appears to require a stimulating concentration of glucose. PGE_1-induced elevation of cAMP does not release insulin from pancreatic islets incubated in medium containing glucose, 30 mg/100 ml (Johnson *et al.*, 1973). However, increasing the cAMP concentration, either by activating adenylyl cyclase with prostaglandins or by inhibiting phosphodiesterase with theophylline, augments the rate of insulin secretion. GTP exerts a large effect on the response of pancreatic adenylyl cyclase to prostaglandins

in vitro. Further studies are in progress to delineate whether the metabolism of guanyl nucleotides is modified *in vivo* by agents such as glucose as a mechanism for regulating insulin secretion.

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