Hormonal Regulation of Pancreatic Islet Adenyl Cyclase†

W. J. Thompson,* D. G. Johnson, and R. H. Williams

ABSTRACT: Adenyl cyclase activity of rat pancreatic islet membrane was increased by secretin, pancreozymin, and isoproterenol, while ACTH, glucagon, growth hormone, and insulin had no effect. Both secretin and isoproterenol activations were enhanced by prostaglandin E_1 (PGE_1) and GTP. Isoproterenol activation was additive with PGE_1, as was that of secretin with PGE_1, but only in the presence of GTP. Secretin activation in the presence of PGE_1 and GTP was equivalent to NaF stimulation. Kinetic analysis indicated that secretin and GTP increased the maximum velocity of the adenyl cyclase and tended to decrease the apparent affinity of the enzyme for ATP. Glucagon activation of islet membrane adenyl cyclase was dependent upon prior treatment of the membrane preparation with EGTA and the use of inhibitors of proteolytic enzymes during the collagenase digestion phase of islet preparation. These results suggest that hormonal regulation of insulin secretion may be affected by PGE_1 and guanine nucleotide modulation of the adenyl cyclase activation process.

Secretion of insulin from pancreatic β cells is increased by several hormones, many of which are known to activate adenyl cyclase. Some of these hormones, such as glucagon, also increase cAMP[1] levels in pancreatic islets (Turtle and Kipnis, 1967; Miller et al., 1972), apparently by activating adenyl cyclase (Davis and Lazarus, 1972; Howell et al., 1973; Kuo et al., 1973). This evidence has led to the concept that cAMP is an important regulator of insulin secretion (Turtle and Kipnis, 1967; Sussman and Vaughan, 1967; Montague and Cook, 1971; Atkins and Matty, 1971).

Pancreatic islet adenyl cyclase is of particular interest because of the large number of hormones reported to increase enzymatic activity (Miller et al., 1972; Davis and Lazarus, 1972; Kuo et al., 1973). Previous studies in our laboratory demonstrated that stimulation of pancreatic islet adenyl cyclase by prostaglandins was dependent on the presence of GTP (Johnson et al., 1974). Kuo et al. (1973) reported that GTP also augmented the activation of pancreatic islet adenyl cyclase by glucagon. Thus, it appears that GTP might play an important modulatory role in the activation of pancreatic islet adenyl cyclase by hormones, much as it does in several other adenyl cyclase systems (Rodbell et al., 1971, Krishna et al., 1972; Wolff and Cook, 1973). This report extends our studies testing the modulation of pancreatic islet adenyl cyclase activation by hormones, PGE_1 and GTP.

Experimental Section

Materials. Male Wistar rats weighing 300–400 g (Simonson Laboratories) were used throughout these studies. Chemicals were 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); isoproterenol, creatine phosphokinase, phosphocreatine, ATP, GTP, cAMP, soybean trypsin inhibitor, type 1-S (Sigma); neutral aluminum oxide, activity I (E. Merck, Darmstadt, Germany); manganese dioxide (Matheson Coleman and Bell); enzyme-grade sucrose (Mann); bovine serum albumin (fraction V) (Nutritional Biochemical Corp.); glucagon and insulin (Eli Lilly); ACTH (Parke Davis); and growth hormone (NIH-GH-B16 bovine). The prostaglandins were kindly supplied by Dr. John Pike, Upjohn Co., Kalamazoo, Michigan. Prostaglandins were dissolved in a minimal amount of ethanol, and 10^{-2} M stock solutions made with nitrogen-gassed Krebs-Ringer bicarbonate (Umbreit et al., 1945) were stored at −20 °C. Porcine secretion and pancreozymin were obtained from Dr. Jorpes, Karolinska Institutet, Stockholm, Sweden.

Adenyl Cyclase Assay. Adenyl cyclase activity was measured by a modification of the method of Ramachandran (1971), as previously described by Johnson et al., 1974. 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 0.01 ml. Assay incubations were for 15 min at 30 °C. [^{32}P]cAMP formed was then isolated, using manganese dioxide followed by neutral alumina–lead sulfate chromatography. Fresh solutions of ATP and prostaglandins were prepared for each experiment.

Isolation of Pancreatic Islets. Pancreatic islets were prepared by the collagenase technique of Lacy and Kostianovsky (1967), using discontinuous Ficoll gradients (Lindall et al., 1969) according to the previously described modification by Johnson et al. (1973). Unless otherwise noted, 200 islets (4 rats) from the Ficoll gradient layer enriched in islet tissue were microdissected, sized, suspended in 1.2 ml of 0.32 M sucrose (4 °C), and homogenized by hand using 6–8 strokes in a Duall-type glass homogenizer fitted with a

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Abbreviations used: PGE_1, prostaglandin E_1; cAMP, cyclic 3′,5′-adenosine monophosphate; ACTH, adrenocorticotropic hormone; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

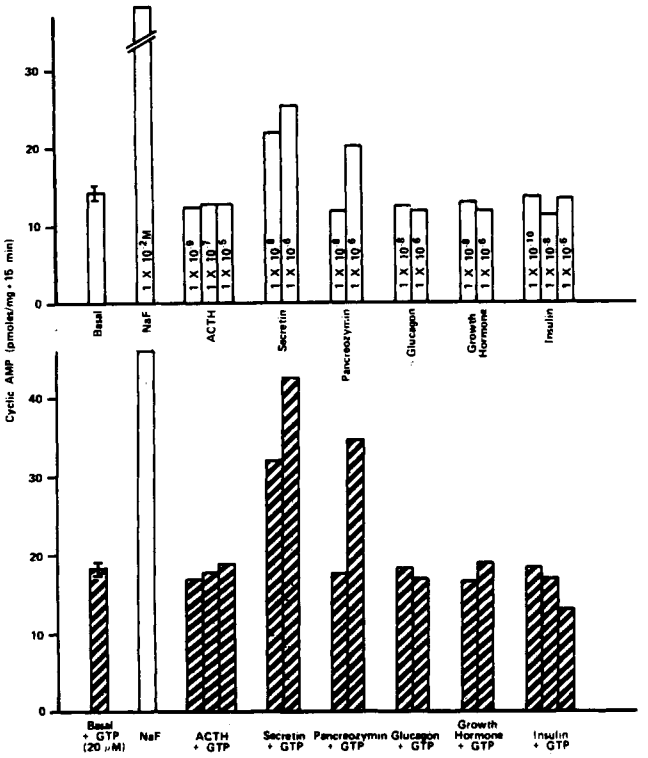

FIGURE 1: Effect of hormones on rat islet membrane adenyl cyclase in the presence and absence of GTP. Rat pancreatic islets were isolated and their membranes prepared as in Methods. The upper panel represents adenyl cyclase activities with various hormones included in the assay at the concentrations indicated. The lower panel was the identical experiment except GTP (20 μM) was included in the incubation mixture. ATP substrate concentration was 20 μM; incubation time was 15 min at 30 °C; and each assay contained 37 μg of islet protein/assay. Single values are given at each hormone concentration; the basal and fluoride values are quadruplicate means with standard error. All determinations were with the same membrane preparation.

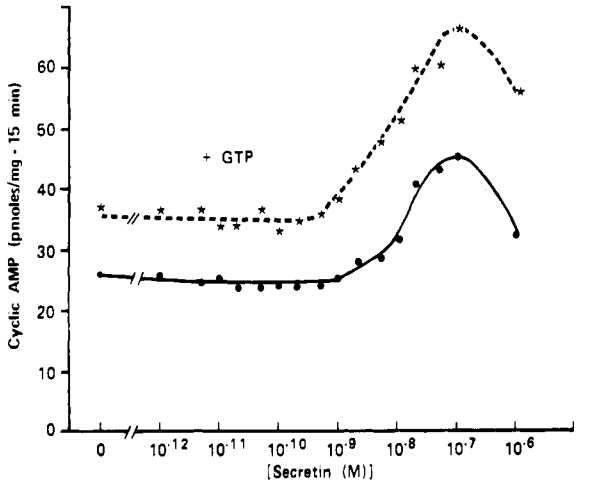

FIGURE 2: Secretin stimulation of rat islet membrane adenyl cyclase in the presence and absence of GTP (20 μM). All preparations, assay conditions, and data expression are identical with those of Figure 1.

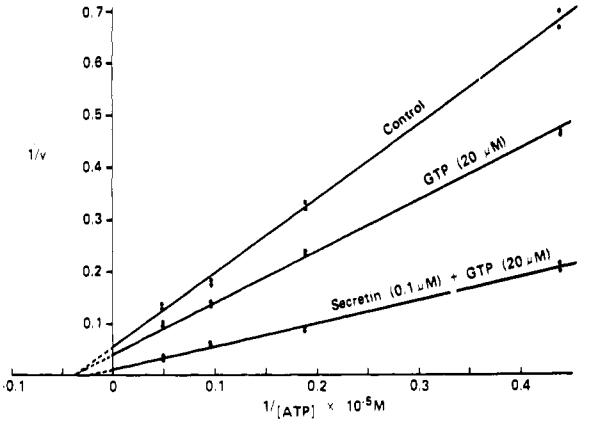

FIGURE 3: Kinetic plots of secretin and GTP activation of islet membrane adenyl cyclase activity. Islets and membranes were prepared as in Methods. Incubation time was 15 min at 30 °C. Data are plotted according to Lineweaver-Burk (1934) and statistically analyzed according to Cleland (1967).

Teflon plunger. The islet cell membranes used for these studies were prepared by transferring the homogenate with a cold Pasteur pipet to a Nalgene test tube (15 × 100 mm), centrifuging (4 °C) at 13 000g for 10 min, and resuspending the pellet in fresh 0.32 M sucrose with a cold, smooth glass rod. A homogeneous membrane preparation was achieved by gentle rehomogenization with 1-2 strokes of a Teflon plunger in the original vessel.

Results

Effects of Hormones on Rat Islet Membrane Adenyl Cyclase in the Presence and Absence of GTP. ACTH, secretin, pancreozymin, glucagon, growth hormone, and insulin were tested as activators of rat islet membrane adenyl cyclase activity (Figure 1, panel a). Secretin activated membrane adenyl cyclase activity at 0.01 μM as well as 1 μM, while pancreozymin only activated this enzyme at 1 μM, in agreement with previous reports (Howell et al., 1973; Davis and Lazarus, 1972; Kuo et al., 1973). None of the other hormones tested had any detectable effect on this enzyme preparation.

Since GTP had been shown previously to affect PGE_1 activation of islet adenyl cyclase (Johnson et al., 1974), hormonal activation of islet adenyl cyclase also was tested in the presence of GTP (Figure 1, panel b). GTP (20 μM), which alone had a slight effect on basal enzyme activity, enhanced both secretin and pancreozymin activations. GTP had no effect on the other hormones tested. Activation of adenyl cyclase with secretin plus GTP was nearly equivalent to NaF (10 mM) activation. NaF activation was unaffected by GTP in this system. Concentration-activity curves indicated that 0.1 μM secretin caused maximum stimulation (Figure 2). Maximal GTP activation was obtained with 20 μM. The K_a for secretin (8-10 nM) was not affected by GTP.

Lineweaver-Burk analysis of kinetic data indicated that the activation of membrane adenyl cyclase by secretin (0.1 μM) plus GTP (20 μM) involved the same kinetic parameters as previously shown for PGE_1 plus GTP activation (Figure 3). Maximum velocity was increased (from 13.8 ± 1.3 to 52 ± 4.5 picomol/5 islets, for 15 min), but the apparent K_m for ATP increased (from 168 ± 27 μM to 201 ± 29 μM).

Effect of GTP on Basal Adenyl Cyclase Activity. As noted above, GTP alone produced a small activation of adenyl cyclase activity. This effect was variable with different islet preparations and was apparent in approximately 70% of our membrane preparations. Lineweaver-Burk plots (Figure 3) showed that, when this activation was seen, GTP increased the maximum velocity of the enzyme (from 13.8 ± 1.3 to 19.0 ± 0.9 picomol/5 islets, for 15 min), with no

Table I: Effect of GTP and Glucagon on Basal Pancreatic Islet Membrane Adenyl Cyclase Activity.[a]

Collagenase Digestion Additions	Homogenization Media: Sucrose (0.32 M)	Homogenization Media: $NaHCO_3$ (1 mM)	Homogenization Media: EGTA (5 mM)	Assay Additions	Adenyl Cyclase Activity (pmol/5 islets, 15 min)
Benzamidine	+	−	−	None	0.33 ± 0.05
Benzamidine	+	−	−	Glucagon (1 μM)	0.35 ± 0.01
STI	−	+	+	None	0.06 ± 0.01
STI	−	+	+	GTP (20 μM)	0.22 ± 0.04
STI	−	+	+	Glucagon (1 μM)	0.12 ± 0.01
STI	−	+	+	Glucagon (1 μM) + GTP (20 μM)	0.27 ± 0.03
STI	−	+	+	NaF (10 mM)	0.30 ± 0.05
STI	+	−	+	None	0.17 ± 0.02
STI	+	−	+	GTP (20 μM)	0.42 ± 0.03
STI	+	−	+	Glucagon (1 μM)	0.27 ± 0.05
STI	+	−	+	Glucagon (1 μM) + GTP (20 μM)	0.47 ± 0.04
STI	+	−	+	NaF (10 mM)	0.53 ± 0.06

[a] Adenyl cyclase activities were measured as in Methods. Homogenization procedures were as described in Methods and Results. Collagenase concentrations used were 2 mg/ml. Soybean trypsin inhibitor (STI) (20 mg/ml) or benzamidine (5 mM) was used by mixing with the collagenase solution before pancreas infusion, incubation, mincing, and dispersal. ATP substrate concentration was 20 μM; incubation time 15 min at 30 °C; and the data are expressed as the mean (±SEM) of triplicate determinations. Additional values with GTP have been omitted from this table for the sake of simplicity but will be furnished to the interested reader by writing directly to the authors.

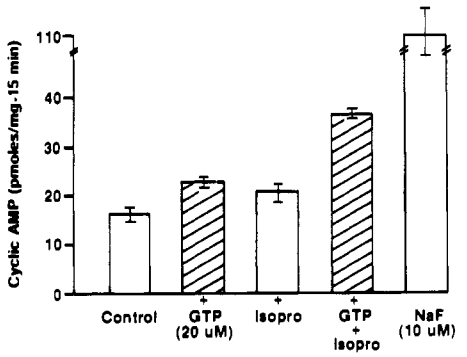

FIGURE 4: Effect of isoproterenol (isopro) on rat islet membrane adenyl cyclase activity. All preparations and assay conditions are as in Figure 2, except that 34 μg of islet protein was used in each assay. Values are mean of quadruplicate determination with SEM indicated.

effect on the apparent K_m for ATP.

Experiments were then designed to control the effect of GTP on basal activity. The GTP effect was entirely dependent on the level of basal activity which, in turn, depended on the treatment of the membranes. When the proteolytic enzyme inhibitors, soybean trypsin inhibitor (type 1) or benzamidine, were included in the collagenase digestion, the basal activities of the membrane adenyl cyclases subsequently prepared were three times the level obtained by the usual procedure (Table I). Since islets isolated in the presence of the inhibitors contained larger amounts of contaminant protein from the isolation procedure, the results were expressed in terms of islets used to prepare the membranes rather than milligrams of protein.

If islets that had been prepared by collagenase digestion in the presence of trypsin inhibitor were homogenized and the membrane preparation was washed once in 0.32 M sucrose containing 5 mM EGTA, the elevated basal adenyl cyclase activity was reduced to the activity seen with the original procedure (Table I). These membranes were resuspended in 0.32 M sucrose containing 1 mM EGTA for measurement of activity.

Further, if islets prepared by collagenase plus trypsin inhibitor were homogenized in 1 mM sodium bicarbonate plus 5 mM EGTA instead of sucrose-EGTA and the membrane preparation was subsequently resuspended in 0.32 M sucrose plus 1 mM EGTA, according to Goldfine et al. (1972), the basal adenyl cyclase activity was further depressed (Table I). If GTP (20 μM) alone was added to these membrane preparations, the activation by this nucleotide was 80% that seen with NaF (10 mM). Neither GTP-stimulated adenyl cyclase nor this basal activity was affected by KCl (5 mM).

Effect of Glucagon on Adenyl Cyclase Activity. Adenyl cyclase activity of membranes from islets prepared by the classical collagenase digestion technique and homogenized in 0.32 M sucrose was not activated by 0.01 or 1 μM glucagon in the presence or absence of GTP (20 μM) using 0.05 or 0.5 mM ATP substrate concentrations (Figure 1). Similarly, it was not possible to demonstrate adenyl cyclase activation by glucagon using pancreatic islets isolated from mice. Activation of rat islet membrane adenyl cyclase by glucagon (1 μM) was seen when the pancreas was digested with collagenase in the presence of an inhibitor of proteolytic enzymes and the islets were homogenized in either 0.32 M sucrose or 1 mM sodium bicarbonate containing 5 mM EGTA (Table I). The increases in activity were comparable to those reported in other rat islet studies (Howell et al., 1973; Kuo et al., 1973; and Davis and Lazarus, 1972). Glucagon did not increase the activation seen with GTP alone. The glucagon stimulation was not seen by homogenizing in sucrose-EGTA alone with no protease inhibitor in the collagenase digestion step.

Activation of Islet Adenyl Cyclase by Catecholamines. Isoproterenol stimulated islet membrane adenyl cyclase activity (Figure 4). These results are in agreement with previous reports (Atkins and Matty, 1971; Kuo et al., 1973; and Miller et al., 1972). The effect of isoproterenol alone was

Table II: Effect of Adrenergic Blockers on Isoproterenol Stimulated Rat Islet Membrane Adenyl Cyclase.[a]

	Adenyl Cyclase Activity (% Control)
Control + None	100 ± 8
Control + Propranolol (20 μM)	93 ± 4
Control + Phentolamine (20 μM)	99 ± 5
Control + Isoproterenol (2 μM)	127 ± 5
Control + Isoproterenol + Propranolol (20 μM)	106 ± 5
Control + Isoproterenol + Phentolamine (20 μM)	129 ± 5
Control + Isoproterenol + Propranolol + Phentolamine	97 ± 5
Control + GTP (20 μM)	153 ± 7
Control + GTP + Propranolol (20 μM)	158 ± 3
Control + GTP + Phentolamine (20 μM)	155 ± 4
Control + GTP + Isoproterenol (2 μM)	239 ± 8
Control + GTP + Isoproterenol + Propranolol	170 ± 3
Control + GTP + Isoproterenol + Phentolamine	230 ± 14
Control + Isoproterenol + Propranolol + Phentolamine	158 ± 2

[a] Adenyl cyclase activities were determined as described in Methods. ATP substrate concentration was 20 μM; incubation time was 15 min at 30 °C; and each assay contained 37 μg of protein per assay. Control activity was 15.2 picomol of cAMP formed/mg, 15 min. Assays were performed in triplicate and values are given as the mean (±SEM).

small and variable from preparation to preparation, with maximal activation at 2 μM. However, GTP (20 μM) greatly enhanced the activation by this catecholamine. The effect of isoproterenol was blocked by the β-adrenergic blocker, propranolol, but not by the α-adrenergic blocker, phentolamine (Table II). Propranolol also blocked the stimulation of isoproterenol in the presence or absence of GTP. Dopamine (20 μM) and acetylcholine had no effect on islet membrane adenyl cyclase activity.

Hormonal Activation of Islet Membrane Adenyl Cyclase in the Presence or Absence of GTP. Since the activations of islet adenyl cyclase by gastrointestinal hormones, catecholamines, and prostaglandins all appeared to be affected by GTP, we tested these agents in combination with and without GTP (Table III). All agents were studied using maximally stimulating concentrations. Each effector used alone was enhanced by GTP, as previously shown. No two of the agents tested were additive with each other in the absence of GTP. However, in the presence of GTP, isoproterenol was additive with PGE_1 and secretin was additive with PGE_1. Secretin activation was not additive to that of isoproterenol in the presence of GTP. The effects of secretin with PGE_1 and GTP were equivalent or greater than NaF stimulation.

Table III: Effect of Isoproterenol, Secretin, PGE_1, and GTP Alone and in Combination on Rat Islet Membrane Adenyl Cyclase Activity.[a]

Additive	% Activity	Increase Above Additive Alone
None	100 ± 8	
GTP (20 μM)	122 ± 5	
Isoproterenol (20 μM)	107 ± 8	
Secretin (0.125 μM)	169 ± 11	
PGE_1 (20 μM)	97 ± 6	
NaF (10 mM)	295 ± 10	
GTP + PGE_1	217 ± 13	Yes
GTP + Isoproterenol	164 ± 6	Yes
GTP + Secretin	215 ± 14	Yes
PGE_1 + Isoproterenol	123 ± 13	No
PGE_1 + Secretin	179 ± 16	No
Secretin + Isoproterenol	177 ± 11	No
GTP + PGE_1 + Isoproterenol	258 ± 3	Yes
GTP + Isoproterenol + Secretin	288 ± 17	No
GTP + PGE_1 + Secretin	317 ± 6	Yes

[a] Adenyl cyclase activity was measured and the enzyme prepared as in Methods. ATP substrate concentration was 40 μM; incubation time was 15 min at 30 °C; and each assay contained 44 μg of protein. Control activity was 35.7 picomol of cAMP formed/mg; 15 min. Assays were performed in triplicate and values are given as the mean (±SEM).

Discussion

Elevated cAMP levels in isolated rat islets produced by prostaglandin activation of adenyl cyclase are associated with an increase in glucose-induced insulin secretion (Johnson et al., 1973). Our previous studies of pancreatic islet adenyl cyclase suggested that GTP may be important in regulating the activation of islet adenyl cyclase. The adenyl cyclase studies presented here suggest that GTP can also affect the activation of islet adenyl cyclase by gastrointestinal hormones and catecholamines. Enhancement of the secretin activation of islet adenyl cyclase by GTP produced apparent changes in the kinetic parameters very similar to those seen with PGE_1 and GTP. The maximum velocity of the enzyme was increased, but there was a decrease in its apparent affinity for substrate. The mechanism of these apparent kinetic changes is unknown.

In the adenyl cyclase system of pancreatic islets, the kinetic parameters, fluoride activation, and dose-response curves with secretin and GTP all differ from the results reported for GTP regulation of the stimulation of adenyl cyclase in liver membranes by glucagon (Rodbell et al., 1974; Rodbell et al., 1971). Those much more detailed studies led to the conclusion that GTP was perhaps an obligatory intermediate in the glucagon binding and activation mechanism. The regulation of pancreatic islet adenyl cyclase by GTP appears to involve a different mechanism. Our findings indicate that PGE_1, as well as GTP, can regulate hormonal stimulation of islet adenyl cyclase. PGE_1 was additive to isoproterenol or secretin activation only in the presence of

GTP. None of these hormones were additive with each other unless GTP was present. Therefore, it appears that GTP may act as an allosteric modulator to couple the hormone receptor–prostaglandin complex to a common activation site. Evidence that prostaglandins might be important in the mechanism of hormonal activation is indicated by the fact that the activations by secretin and isoproterenol were not additive in the presence of GTP alone. The data regarding additivity obtained with prostaglandins and maximally stimulating concentrations of hormones suggest that different hormones do not each act on different or multiple adenyl cyclases.

Failure of glucagon to activate adenyl cyclase of islet homogenates or membrane fractions was a puzzling finding. Glucagon is a well recognized insulin secretagogue. It elevates cAMP in isolated islets (Turtle and Kipnis, 1967) and has no effect on islet cAMP phosphodiesterase (personal observations). This insensitivity of islet membranes to glucagon has been reported previously (Kuo et al., 1973). These investigators attributed this effect to pancreatic proteolysis, because prior administration of pilocarpine allowed preparation of glucagon-sensitive crude islet homogenates, even after collagenase digestion of the pancreas.

Since glucagon receptors are known to be sensitive to trypsin (Rodbell et al., 1970), we investigated causative factors in the preparation of the islets that might release trypsin or other proteolytic enzymes and destroy the glucagon receptors. We discovered that inclusion of proteolytic enzyme inhibitors in the pancreas digestion step had a profound effect on the basal adenyl cyclase activity, but did not affect glucagon insensitivity. Glucagon only activated our islet membrane preparations when the membranes were first washed with EGTA, regardless of whether the homogenization medium was sodium bicarbonate or sucrose. EGTA had been reported previously to enhance glucagon activation (Kuo et al., 1973) and calcium is an inhibitor of basal adenyl cyclase activity (Davis and Lazarus, 1972). We conclude from these findings that bound calcium can inhibit glucagon activation of islet adenyl cyclase, as proposed for other systems (Hepp et al., 1970).

The effect of GTP alone on membrane adenyl cyclase activity was also related to bound calcium. GTP was nearly as effective as sodium fluoride in activating an EGTA-washed islet membrane preparation. These results suggest the hypothesis that hormones may affect an interaction of calcium and GTP on an adenyl cyclase regulatory site to promote cAMP synthesis and insulin secretion.

Glucose activation of pancreatic islet adenyl cyclase has not been demonstrated in vitro or in vivo (Davis and Lazarus, 1972; Howell et al., 1973; Kuo et al., 1973). Glucose effects on cAMP content of pancreatic islets are controversial; some studies are positive (Grill and Cerasi, 1974; Charles et al., 1973), most are negative (e.g., Montague and Cook, 1971; Cooper et al., 1973), and one was positive, but indicated that increased cAMP was unrelated to insulin release (Hellman et al., 1974). Isolated islets have been demonstrated to have altered glucose sensitivity related to pronase treatment (Orci et al., 1973), neuraminidase treatment and membrane sialic acid content (Hahn et al., 1974), sulfhydryl oxidation (Hellman et al., 1973; Wizeman et al., 1973), and calcium (Malaisse-Lagae and Malaisse, 1971; Herman et al., 1973). Our studies and others indicate that adenyl cyclase activation by hormones is also sensitive to many factors. It would appear that a great deal of caution is necessary in interpreting in vitro studies of glucose effects on adenyl cyclase. Much more detailed and rigorous biochemical studies will be required before either accepting or discarding this mechanism of regulating insulin secretion.

In summary, our studies indicate that guanine nucleotides may play an important role in regulating insulin secretion, particularly in response to hormones. Hormone receptor units may be influenced by PGE_1 and GTP modulation of cyclase activation. The gastrointestinal hormones appear to be much more potent activators of pancreatic islet adenyl cyclase than glucagon or catecholamines.

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Determination of Molar Ratios of Vesicular Stomatitis Virus Induced RNA Species in BHK_{21} Cells†

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ABSTRACT: A modified procedure for analysis of RNA in denaturing formamide–polyacrylamide slab gels containing 6 M urea is described. Using this technique, in conjunction with fluorographic analysis, we determined molecular weights and molar ratios of the various vesicular stomatitis virus (VSV) induced RNAs in BHK_{21} cells. A comparison of the molar ratios of virus-specific mRNAs and their putative protein products in these cells suggests that there is little, if any, translational control of viral gene expression during acute VSV infection.

A technique for the electrophoresis of RNA in formamide–acrylamide gels has previously been described in detail (Pinder et al., 1974). This technique worked well for tube gel analysis of RNA, but attempts to apply it to slab gel electrophoresis followed by fluorographic analysis of tritiated RNA (Bonner and Laskey, 1974) met substantial difficulties in this laboratory. The modifications described here facilitate the handling of low percentage acrylamide gels containing formamide and allow quantitative fluorographic analysis (Laskey and Mills, 1975) of tritiated RNAs of high molecular weight. The method is applied to an analysis of virus-induced RNA species in vesicular stomatitis virus (VSV[1]) infected cells. VSV is an enveloped negative strand RNA virus with an associated transcriptase. RNA synthesized in VSV-infected cells in the presence of actinomycin D is predominantly virus-specific "plus" (messenger) strand (for review, see Wagner, 1975). Recently, electrophoretic separation of VSV-specific RNAs was accomplished under denaturing conditions (Rose and Knipe, 1975; Grubmann et al., 1975). In vitro translation of these RNAs allowed identification of the coding capacity of four of the five VSV-specific mRNAs (Knipe et al., 1975). The remaining RNA species (28S) is thought to code for the large (L) VSV structural protein (Morrison et al., 1974). In infected cells all five VSV-specific proteins are synthesized throughout the infectious cycle, in about the same relative proportion (Mudd and Summers, 1970), but the molar amounts of the individual proteins differ greatly from one another, suggesting a rather stringent regulation of VSV protein synthesis. The data presented in this paper suggest that this regulation occurs at the level of transcription rather than translation.

Materials and Methods

Materials. 99% formamide was purchased from Mallinckrodt. Acrylamide was from BDH. *N,N'*-Methylenebisacrylamide and *N,N,N',N'*-tetramethylethylenediamine (Temed) were from Eastman. Other chemicals were reagent grade.

Deionization of Formamide–Urea. Urea was dissolved in formamide to a final concentration of 6 M. The solution was then mixed with mixed-bed, ion-exchange resin (Amberlite coarse MB-1, 8 g/100 ml) and stirred for 30 min at room temperature during which the conductivity of the solution dropped to below 5 μmho. The formamide–urea was filtered and stored in the dark at 5 °C. The solution could be stored for up to 10 days before conductivity rose to undesirable levels.

Preparation of RNA Gels. Gels were 2.8% (w/v) polyacrylamide with 2.38% acrylamide and 0.4% *N,N'*-methylenebisacrylamide. In 30 ml of the deionized formamide–urea solution was dissolved 0.715 g of acrylamide in 0.126 g of *N,N'*-methylenebisacrylamide. To this solution was then added 0.11 g of barbital and 0.072 ml of Temed. The apparent pH was adjusted to 9.0 using a standard glass elec-

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[1] Abbreviations used: VSV, vesicular stomatitis virus; Temed, *N,N,N',N'*-tetramethylethylenediamine; L VSV structural protein, large VSV structural protein; G protein, glycosylated protein; N protein, nucleocapsid protein; NS protein, minor protein; M protein, matrix protein.