Stimulation by glucose of cyclic AMP accumulation in mouse pancreatic islets is mediated by protein kinase C

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The mechanism of glucose-stimulated cyclic AMP accumulation in mouse pancreatic islets was studied. In the presence of 3-isobutyl-1-methylxanthine, both glucose and the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA), an activator of protein kinase C, enhanced cyclic AMP formation 2.5-fold during 60 min of incubation. Both TPA-stimulated and glucose-stimulated cyclic AMP accumulations were abolished by the omission of extracellular Ca^{2+} . The Ca^{2+} ionophore A23187 did not affect cyclic AMP accumulation itself, but affected the time course of TPA-induced cyclic AMP accumulation, the effect of A23187 + TPA mimicking the time course for glucose-induced cyclic AMP accumulation. A 24 h exposure to TPA, which depletes islets of protein kinase C, abolished the effects of both TPA and glucose on cyclic AMP production. Both TPA-induced and glucose-induced cyclic AMP productions were inhibited by anti-glucagon antibody, and after pretreatment with this antibody glucose stimulation was dependent on addition of glucagon. Pretreatment of islets with TPA for 10 min potentiated glucagon stimulation and impaired somatostatin inhibition of adenylate cyclase activity in a particulate fraction of islets. Carbamoylcholine, which is supposed to activate protein kinase C in islets, likewise stimulated cyclic AMP accumulation in islets. These observations suggest that glucose stimulates islet adenylate cyclase by activation of protein kinase C, and thereby potentiates the effect of endogenous glucagon on adenylate cyclase.

INTRODUCTION

Cyclic AMP has been shown to have a marked potentiating effect on the ability of glucose to stimulate insulin release from islets. In addition, intracellular concentrations of cyclic AMP can be modestly increased by stimulatory concentrations of glucose (Charles *et al.*, 1973; Grill & Cerasi, 1974; Capito & Hedeskov, 1974). However, the mechanism by which glucose affects the cyclic AMP content in islets is not known.

Some evidence suggests that the raised intracellular Ca^{2+} concentration in response to glucose is responsible for stimulation of the adenylate cyclase. Thus, as previously demonstrated in rat and *ob/ob*-mouse islets, the effect of glucose in increasing cyclic AMP depends on the presence of extracellular Ca^{2+} (Charles *et al.*, 1975; Hellman, 1976). It has been reported that Ca^{2+} -calmodulin stimulates rat islet adenylate cyclase activity (Valverde *et al.*, 1979; Sharp *et al.*, 1980). However, since Ca^{2+} -calmodulin fails to affect mouse islet adenylate cyclase (Thams *et al.*, 1982), Ca^{2+} -calmodulin activation of adenylate cyclase does not seem to be a plausible mechanism for glucose stimulation of adenylate cyclase.

It has been demonstrated that isolated β -cells exhibit a markedly lower cyclic AMP formation than do β -cells in intact islets. Glucose alone does not influence cyclic AMP concentrations in purified β -cells, but enhances glucagon-induced cyclic AMP formation in these cells, suggesting that this lack of glucose stimulation is caused by the separation of β -cells from glucagon-containing α -cells (Schuit & Pipeleers, 1985). Similarly, somatostatin produced in islet D-cells, which inhibits cyclic AMP

accumulation in islets (Bent-Hansen *et al.*, 1979), may be involved in paracrine regulation of β -cell function.

Several lines of evidence have implicated protein kinase C in regulation of insulin secretion. Protein kinase C-dependent protein phosphorylation has been identified in pancreatic islets (Lord & Ashcroft, 1984; Thams *et al.*, 1984), and glucose has been found to induce protein kinase C-mediated protein phosphorylation in neonatal islets (Dunlop & Larkins, 1986). Furthermore, the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) has been found to stimulate insulin secretion from both rat and mouse islets (Virji *et al.*, 1978; Malaisse *et al.*, 1980; Thams *et al.*, 1986).

It has been reported that treatment of intact human platelets and cyc⁻ S49 lymphoma cells with TPA impairs inhibition of adenylate cyclase by hormonal factors and potentiates hormonal stimulation of adenylate cyclase (Jakobs *et al.*, 1985a; Katada *et al.*, 1985), owing to a protein kinase C-mediated phosphorylation of the α -subunit of the inhibitory coupling protein N_i of adenylate cyclase (Katada *et al.*, 1985). Likewise, TPA treatment has been shown to potentiate hormonal stimulation through the stimulatory N_s protein of adenylate cyclase in several other tissues (Bell *et al.*, 1985; Sibley *et al.*, 1986; Olinas & Onali, 1986; Johnson *et al.*, 1986).

In seeking an explanation for the ability of glucose to increase cyclic AMP in pancreatic islets, the present study aimed to investigate the effect of protein kinase C on regulation of adenylate cyclase. The results demonstrate that protein kinase C stimulates cyclic AMP accumulation through a potentiation of glucagon stimulation and an impairment of somatostatin inhibition of

Abbreviations used: TPA, 12-*O*-tetradecanoylphorbol 13-acetate; 4 α -PDD, 4 α -phorbol 12,13-didecanoate; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol; H-7, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine; IBMX, 3-isobutyl-1-methylxanthine.

adenylate cyclase. Both glucose-stimulated and TPA-stimulated cyclic AMP accumulations are dependent on endogenously released glucagon, suggesting that glucose-stimulated cyclic AMP accumulation is caused by protein kinase C activation, modifying the paracrine effects of glucagon and probably of somatostatin.

EXPERIMENTAL

Materials

Crude bacterial collagenase, cyclic AMP and GTP (dilithium salt) were obtained from Boehringer, Mannheim, Germany. ATP (disodium salt; A2383), TPA, 4 α -PDD, OAG, H-7, Ca²⁺ ionophore A23187, carbamoylcholine chloride and somatostatin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Cyclic [³H]AMP (sp. radioactivity 48 Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Glucagon and pancreatic-glucagon-specific anti-glucagon antibody (K5563) were obtained from Novo Industries A/S, Bagsvaerd, Denmark. All other chemicals were of analytical grade.

Preparation of islets

Islets were prepared by collagenase digestion of the pancreases of male albino mice (approx. 26 g body wt.) fed *ad libitum* on a standard laboratory diet. When specified, islets were kept in tissue culture for 24 h in TCM199 medium, supplemented with 10% (v/v) newborn-calf serum (Gibco), 20 mM-Hepes, 5 mM-NaHCO₃, 100 units of penicillin/ml and 100 μ g of streptomycin/ml.

Adenylate cyclase assay

Groups of 250 islets were collected in 1 ml of Krebs-Henseleit (1932) buffer, pH 7.40, supplemented with 2 mg of human serum albumin/ml and 3.3 mM-glucose. After preincubation for 30 min at 37 °C, 800 μ l of the medium was replaced by 800 μ l of the same medium also containing test agents as indicated in the Tables and Figures, and the islets were incubated for 10 min at 37 °C. The islets were then collected in 250 μ l of 25 mM-Tes (pH 7.60)/5 mM-MgCl₂/1 mM-EGTA for the preparation of a 27000 g particulate fraction (Thams *et al.*, 1982). Adenylate cyclase activity was measured without Ca²⁺ as described previously (Thams *et al.*, 1982). Protein was measured with bovine serum albumin as standard as described by Groves *et al.* (1968).

Islet cyclic AMP accumulation

Islet cyclic AMP accumulation was measured in batch-type incubations. Batches of 20 medium-sized islets were transferred to test tubes containing 100 μ l of Krebs-Henseleit buffer with 2 mg of human serum albumin/ml and 3.3 mM-glucose. After preincubation for 30 min at 37 °C, 80 μ l of the medium was replaced by 80 μ l of the same medium also containing 0.1 mM-IBMX and test agents as indicated in the Tables and Figures, and the islets were incubated for 10–60 min; 0.5 mM-EGTA was included in Ca²⁺-free medium. When added, EGTA, H-7 and anti-glucagon antibody were present during both the preincubation and the incubation period. Anti-glucagon antibody (K5563) was dialysed against 25 mM-Tes buffer, pH 7.40, and diluted 1:8 in Krebs-Henseleit buffer with 2 mg of human serum albumin/ml and 3.3 mM-glucose

before use (final dilution 1:1600, binding capacity approx. 0.1 pmol of glucagon/100 μ l in the radioimmunoassay by Novo). Islet metabolism was arrested by addition of 10 μ l of 1 M-HCl. The test tubes were cooled on ice, and the contents sonicated (10 s, 40 W). The precipitates were removed by centrifugation at 6000 g for 5 min, and the pH of the supernatant was adjusted to 5.5 with 1 M-NaOH and 0.25 M-potassium phosphate buffer, pH 5.5. After neutralization the supernatants were stored at –20 °C until assay for cyclic AMP.

Cyclic AMP assay

Cyclic AMP was measured as described by Geisler *et al.* (1977), except that the assay medium was 0.25 M-potassium phosphate, pH 5.50, containing 20 mM-nitrilotriacetic acid, 6 mM-2-mercaptoethanol and 0.02% human serum albumin.

Miscellaneous

TPA was added in a small volume of dimethyl sulphoxide, final concn. 0.01–0.02% (v/v). At these low concentrations, dimethyl sulphoxide did not affect cyclic AMP accumulation or adenylate cyclase activity.

Results are given as means $\pm$ S.E.M. for *n* experiments. Statistical evaluation of the data was made by Student's paired *t* test; NS, not significant (*P* > 0.05).

RESULTS

Effect of TPA on cyclic AMP accumulation

TPA (1.6 μ M) stimulated cyclic AMP accumulation from 0.20 to 0.50 pmol/20 islets during a 60 min incubation (Table 1). In comparison, glucose (16.7 mM) enhanced cyclic AMP to 0.48 pmol/20 islets. Removal of Ca²⁺ from the incubation medium and inclusion of EGTA (0.5 mM) slightly inhibited cyclic AMP accumulation at 3.3 mM-glucose and totally abolished TPA (1.6 μ M)-stimulated and glucose (16.7 mM)-stimulated cyclic AMP accumulation (Table 1). The Ca²⁺ ionophore A23187 (2.5 μ M) did not have any effect on cyclic AMP production, either alone or in combination with TPA (1.6 μ M) determined after 60 min (Table 1).

TPA, A23187+TPA and glucose all caused a rapid increase in cyclic AMP in islets (Fig. 1); the time course for TPA showed a progressive increase in cyclic AMP during 60 min of incubation. In comparison, cyclic AMP accumulation in the presence of A23187+TPA or glucose reached a plateau after 10 min of incubation, followed by a second increase between 30 and 60 min, suggesting that A23187+TPA mimic the effect of glucose, which is known to stimulate both Ca²⁺ fluxes and phosphatidylinositol hydrolysis in islets (Prentki & Wollheim, 1984; Best *et al.*, 1984).

TPA caused a dose-dependent stimulation of cyclic AMP accumulation (Table 2). A significant stimulation was observed at 0.16 μ M-TPA, which caused maximum stimulation of cyclic AMP production. The effect of TPA was mimicked by the synthetic diacylglycerol OAG, which previously has been shown to stimulate protein kinase C and insulin secretion from islets (Malaisse *et al.*, 1985). The inactive phorbol ester 4 α -PDD did not affect cyclic AMP production in islets (Table 2).

The isoquinolinesulphonamide H-7 (50 μ M), an inhibitor of protein kinase C and cyclic AMP-dependent protein kinase (Hidaka *et al.*, 1984), failed to inhibit both TPA- and glucose-induced cyclic AMP production

Table 1. Effect of TPA on cyclic AMP accumulation

Islet cyclic AMP accumulation was measured after 60 min of incubation in the presence of IBMX (0.1 mM) as described in the Experimental section. Results are means $\pm$ S.E.M. for the numbers of experiments given in parentheses. The significance of the differences from the paired control values was assessed by using a paired *t* test: NS, not significant ($P > 0.05$).

Line	Added agents				Cyclic AMP (pmol/ 20 islets)	<i>P</i>
	Glucose (mM)	Ca ²⁺ (mM)	TPA (μ M)	A23187 (μ M)		
1	3.3	2.5	—	—	0.20 $\pm$ 0.02 (22)	
2	3.3	—	—	—	0.16 $\pm$ 0.03 (3)	Versus 1: < 0.005
3	3.3	2.5	1.6	—	0.50 $\pm$ 0.07 (12)	Versus 1: < 0.001
4	3.3	—	1.6	—	0.17 $\pm$ 0.04 (3)	Versus 2: NS
5	3.3	2.5	—	2.5	0.17 $\pm$ 0.01 (4)	Versus 1: NS
6	3.3	2.5	1.6	2.5	0.50 $\pm$ 0.04 (5)	Versus 1: < 0.01; Versus 3: NS
7	16.7	2.5	—	—	0.48 $\pm$ 0.07 (16)	Versus 1: < 0.001
8	16.7	—	—	—	0.18 $\pm$ 0.02 (3)	Versus 2: NS

Fig. 1. Time courses of cyclic AMP accumulation by mouse islets

Islet cyclic AMP accumulation was determined in the presence of 3.3 mM-glucose and 0.1 mM-IBMX as described in the Experimental section, with the following additions: (a) without (○) and with (●) 1.6 μ M-TPA; (b) with 2.5 μ M-A23187 (○) or with 2.5 μ M-A23187 + 1.6 μ M-TPA (●); (c) without (○) or with (●) 16.7 mM-glucose. Results are means $\pm$ S.E.M. ($n = 3-4$). The significance of the differences from controls in the absence of TPA (1.6 μ M) or glucose (16.7 mM) was assessed by using a paired *t* test: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

Table 2. Specificity of TPA effect on cyclic AMP accumulation

Islet cyclic AMP accumulation was measured after 60 min of incubation in the presence of glucose (3.3 mM) and IBMX (0.1 mM) as described in the Experimental section. Results are means $\pm$ S.E.M. ($n = 3$). Statistical evaluation of the data was made by a paired *t* test: NS, not significant ($P > 0.05$).

Added agents			Cyclic AMP (pmol/ 20 islets)	<i>P</i> (versus control)
TPA (μ M)	4 α -PDD (μ M)	OAG (mM)		
—	—	—	0.22 $\pm$ 0.06	
0.0016	—	—	0.25 $\pm$ 0.02	NS
0.016	—	—	0.29 $\pm$ 0.06	NS
0.16	—	—	0.38 $\pm$ 0.04	< 0.005
1.6	—	—	0.37 $\pm$ 0.03	< 0.005
—	1.6	—	0.21 $\pm$ 0.01	NS
—	—	0.25	0.35 $\pm$ 0.02	< 0.01

(results not shown). H-7 competes with ATP for protein kinase C and may be a poor inhibitor of the kinase at the millimolar concentrations of ATP prevailing in islet cells (Hidaka *et al.*, 1984).

To substantiate further a role for protein kinase C in glucose-stimulated cyclic AMP accumulation, islets were pretreated by 24 h exposure to 0.16 μ M-TPA, which has been shown to deplete islets of total cellular protein kinase C activity (Hii *et al.*, 1987). Islets were cultured for 24 h in TCM 199 medium containing either 4 α -PDD (0.16 μ M) or TPA (0.16 μ M). In 4 α -PDD-treated islets, TPA (0.16 μ M) and glucose (16.7 mM) stimulated cyclic AMP production from 0.31 $\pm$ 0.04 (3) at 3.3 mM-glucose to 0.56 $\pm$ 0.03 (3) ($P < 0.05$) and 0.54 $\pm$ 0.02 (3) ($P < 0.05$) pmol/60 min per 20 islets respectively. In contrast, TPA-treated islets failed to respond to both TPA and glucose, cyclic AMP production amounting to 0.36 $\pm$ 0.02 (3) at 3.3 mM-glucose and to 0.38 $\pm$ 0.01 (3) and 0.38 $\pm$ 0.09 (3) pmol/60 min per 20 islets in the presence of TPA (0.16 μ M) and glucose (16.7 mM) respectively.

Fig. 2. Effect of anti-glucagon antibody on TPA-induced and glucose-induced cyclic AMP accumulation

Islet cyclic AMP accumulation was measured as described in the Experimental section without (□) or with (▨) anti-glucagon antibody and further additions as indicated. The antibody was present during both the preincubation and the incubation period. Results are means $\pm$ S.E.M. ($n = 3-4$). The significance of the differences from controls in the absence of the antibody was assessed by using a paired t test: * $P < 0.025$; ** $P < 0.02$; *** $P < 0.005$; **** $P < 0.001$.

Effect of anti-glucagon antibody on cyclic AMP accumulation

Anti-glucagon antibody slightly but significantly inhibited cyclic AMP accumulation at 3.3 mM-glucose (Fig. 2). Furthermore, the antibody abolished the effects of glucose (16.7 mM) and TPA (1.6 μ M) on cyclic AMP. The effect of antibody was not reversed by concomitant addition of glucagon (10 μ M). Similar inhibitory effects were not produced by antibody-free serum (results not shown).

To investigate the reversibility of the inhibition by antibody, islets were preincubated with glucagon antibody for 30 min, washed in antibody-free medium, and then examined for glucose (16.7 mM)-stimulated and glucagon (10 μ M)-stimulated cyclic AMP accumulation (Fig. 3). Both these cyclic AMP accumulations were severely impaired by pretreatment with antibody. The reason for this partially non-reversible inhibition of glucagon stimulation is not known. However, when added in combination glucagon (10 μ M)+glucose (16.7 mM) caused a significant increase in cyclic AMP after pretreatment with anti-glucagon antibody, which suggests that glucose stimulation of cyclic AMP formation is dependent on glucagon, as previously suggested by Schuit & Pipeleers (1985).

Effect of TPA on adenylate cyclase activity

Pretreatment of intact islets with TPA (1.6 μ M) for 10 min had no effect on basal adenylate cyclase activity,

Fig. 3. Reversibility of anti-glucagon-antibody inhibition of cyclic AMP accumulation

Islet cyclic AMP accumulation was measured as described in the Experimental section. Islets were preincubated in the absence (a) or presence (b) of anti-glucagon antibody for 30 min, washed in antibody-free Krebs–Henseleit buffer supplemented with 2 mg of human serum albumin/ml and 3.3 mM-glucose, and then examined for glucose-stimulated and glucagon-stimulated cyclic AMP accumulation. Results are means $\pm$ S.E.M. ($n = 3$). The significance of the differences from controls in the absence of glucose and glucagon was assessed by using a paired t test: * $P < 0.02$.

whereas both GTP (10 μ M)-stimulated and GTP (10 μ M)+NaCl (100 mM)-stimulated activities were enhanced by 10 ± 2 (3)% ($P < 0.02$) and 12 ± 3 (3)% ($P < 0.025$) (Table 3).

Glucagon stimulation of adenylate cyclase was dependent on GTP. Pretreatment with TPA enhanced glucagon stimulation from 14% to 29% at 10 μ M-glucagon (Table 3), suggesting that TPA potentiates N_s -mediated stimulation of islet adenylate cyclase. The potentiation of glucagon-stimulated adenylate cyclase activity was accompanied by an impairment of somatostatin inhibition of the enzyme; thus somatostatin inhibition was decreased from 16% to 6% at 0.6 μ M and from 31% to 15% at 6 μ M-somatostatin (Table 3). The somatostatin inhibition was also dependent on GTP, this effect being greatly enhanced in the presence of NaCl (results not shown), suggesting that NaCl may decrease N_s -mediated enzyme inhibition exerted by the sole addition of GTP (Jakobs *et al.*, 1985b).

Table 3. Effect of TPA on adenylate cyclase activity

The effect of TPA on adenylate cyclase activity was assessed after pretreatment of islets without (a) or with (b) 1.6 μM -TPA for 10 min as described in the Experimental section. Results are means $\pm$ S.E.M. ($n = 3$). Glucagon stimulation and somatostatin inhibition are presented as mean percentages ($\pm$ S.E.M.) of the activity in the presence of GTP (10 μM) and GTP (10 μM) + NaCl (100 mM) respectively. The significance of the differences from control (a) was assessed by using a paired t test: NS, not significant ($P > 0.05$).

Added agents				Activity (pmol of cyclic AMP/h per μg of protein)			Activity (%)		
GTP (μM)	NaCl (mM)	Glucagon (μM)	Somatostatin (μM)	Control (a)	TPA (b)	P	Control (a)	TPA (b)	P
–	–	–	–	1.56 ± 0.08	1.67 ± 0.02	NS			
10	–	–	–	3.07 ± 0.08	3.39 ± 0.02	< 0.02	100 ± 2	100 ± 1	
10	–	1	–				110 ± 3	115 ± 1	NS
10	–	10	–				114 ± 2	129 ± 5	< 0.02
10	100	–	–	3.84 ± 0.11	4.32 ± 0.13	< 0.025	100 ± 3	100 ± 3	
10	100	–	0.6				84 ± 2	94 ± 2	< 0.001
10	100	–	6				69 ± 2	85 ± 3	< 0.005

Effects of carbamoylcholine on cyclic AMP accumulation

Carbamoylcholine, which potentiates glucose-stimulated insulin secretion, has previously been demonstrated to stimulate Ca^{2+} fluxes and phosphatidylinositol hydrolysis (Gagerman *et al.*, 1980; Best, 1986; Morgan *et al.*, 1987) in pancreatic islets, suggestive of an activation of protein kinase C. Carbamoylcholine likewise stimulated cyclic AMP accumulation in mouse islets. In the presence of glucose (3.3 mM) and IBMX (0.1 mM), cyclic AMP accumulation was enhanced from 0.12 ± 0.01 (3) pmol/60 min per 20 islets in the absence of carbamoylcholine to 0.19 ± 0.01 (3) ($P < 0.001$), 0.29 ± 0.01 (3) ($P < 0.005$) and 0.30 ± 0.01 (3) pmol/60 min per 20 islets ($P < 0.001$) in the presence of 1 μM -, 10 μM - and 100 μM -carbamoylcholine respectively. Thus half-maximum stimulation was observed at approx. 2 μM and maximum stimulation at 10 μM -carbamoylcholine. The time course for carbamoylcholine-stimulated cyclic AMP accumulation showed a maximum after 10 min of incubation (results not shown).

DISCUSSION

The results demonstrate that protein kinase C may play a role in glucose-stimulated cyclic AMP accumulation in mouse islets. Thus both TPA and glucose stimulated cyclic AMP accumulation in a fashion which was dependent on extracellular Ca^{2+} and abolished after long-term exposure to TPA. Furthermore, the results with adenylate cyclase demonstrate that protein kinase C potentiates glucagon stimulation and impairs somatostatin inhibition of adenylate cyclase, suggesting that glucose in stimulation of cyclic AMP in β -cells modifies paracrine effects of glucagon and somatostatin released from α -cells and D-cells respectively. In accordance, both TPA-stimulated and glucose-stimulated cyclic AMP accumulation appeared to be dependent on endogenous released glucagon, confirming previous results with isolated single β -cells, which demonstrated that glucose-induced cyclic AMP production is dependent on glucagon (Schuit & Pipeleers, 1985).

In accordance with previous results in human platelets and cyc[−] lymphoma cells (Jakobs *et al.*, 1985a; Katada *et al.*, 1985), the stimulatory effect of protein kinase C on adenylate cyclase in islets may be due to phosphorylation of the α_1 subunit of the regulatory GTP-binding component N_i of adenylate cyclase. In agreement with this, glucose has been found to stimulate phosphorylation of a M_r -40000 protein in a particulate fraction of neonatal-rat islets (Dunlop & Larkins, 1986), which may be identical with the α_1 subunit of N_i (Katada *et al.*, 1985). However, additional stimulatory effects of protein kinase C should probably not be excluded. Thus TPA has been found to enhance N_s stimulation in frog erythrocytes without any significant effects on N_i inhibition of adenylate cyclase (Sibley *et al.*, 1986).

At variance with previous observations with rat pancreatic islets (Karl *et al.*, 1975), A23187 itself failed to stimulate cyclic AMP formation in mouse islets, probably reflecting the absence of Ca^{2+} -calmodulin-stimulated adenylate cyclase activity in mouse islets (Thams *et al.*, 1982).

It has been demonstrated that TPA inhibits cyclic AMP phosphodiesterase activity in rat hepatocytes (Irvine *et al.*, 1986). The possible effect of TPA on phosphodiesterase in islets was not investigated in the present study. However, it seems unlikely that inhibition of phosphodiesterase has made a major contribution to the present findings, since all experiments were performed in the presence of IBMX (0.1 mM), which causes a strong inhibition of phosphodiesterase in islets (Christie & Ashcroft, 1984). Furthermore, the ability of glucose to enhance cyclic AMP must rely on activation of adenylate cyclase, since glucose stimulation of cyclic AMP persists even in the presence of 1 mM-IBMX (results not shown), which causes a total abolition of phosphodiesterase in islets (Christie & Ashcroft, 1984).

Both glucose and carbamoylcholine have been shown to stimulate Ca^{2+} fluxes and phosphatidylinositol hydrolysis in islets (Gagerman *et al.*, 1980; Best, 1986), indicative of activation of protein kinase C. In agreement, carbamoylcholine possessed a capacity for stimulation of cyclic AMP accumulation. Like the effects of carbamoylcholine on phosphatidylinositol hydrolysis (Best &

Malaisse, 1983) and on Ca^{2+} fluxes (Morgan *et al.*, 1987), this effect is observable in the micromolar range. Previous studies have shown that cholinergic stimulation does not stimulate, or only slightly stimulates, cyclic AMP accumulation in islets (Gagerman *et al.*, 1978; Trus *et al.*, 1979). The reason for this discrepancy is not known, but in one of the studies glucose also failed to stimulate cyclic AMP accumulation (Trus *et al.*, 1979).

The physiological significance of glucose-potentiated glucagon stimulation of adenylate cyclase remains to be established. Glucose inhibits glucagon secretion from the perfused pancreas (Gerich *et al.*, 1974), and it is possible that isolated islets release glucagon as a result of the isolation procedure. Consequently, the effect of glucose *in vivo* may be to sensitize β -cells to non-islet hormonal factors such as gut glucagon, cholecystokinin and secretion, which all may be expected to activate adenylate cyclase in islets (Kuo *et al.*, 1973).

In conclusion, the present findings have clearly demonstrated that protein kinase C potentiates glucagon stimulation and impairs somatostatin inhibition of adenylate cyclase in mouse islets. The involvement of protein kinase C in glucose-stimulated cyclic AMP formation is substantiated by the findings that both TPA-induced and glucose-induced cyclic AMP accumulations are dependent on Ca^{2+} and glucagon, and are abolished after long-term exposure to TPA. In addition, carbamoylcholine, which activates protein kinase C, also stimulates cyclic AMP accumulation.

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REFERENCES

- Bell, J. D., Buxton, I. L. O. & Brunton, L. L. (1985) *J. Biol. Chem.* **260**, 2625–2628
- Bent-Hansen, L., Capito, K. & Hedeskov, C. J. (1979) *Biochim. Biophys. Acta* **585**, 240–249
- Best, L. (1986) *Biochem. J.* **238**, 773–779
- Best, L. & Malaisse, W. J. (1983) *Biochim. Biophys. Acta* **750**, 157–163
- Best, L., Dunlop, M. & Malaisse, W. J. (1984) *Experientia* **40**, 1085–1091
- Capito, K. & Hedeskov, C. J. (1974) *Biochem. J.* **142**, 653–658
- Charles, M. A., Fanska, R., Schmid, F. G., Forsham, P. H. & Grodsky, G. M. (1973) *Science* **179**, 569–571
- Charles, M. A., Lawecki, J., Pictet, R. & Grodsky, G. M. (1975) *J. Biol. Chem.* **250**, 6134–6140
- Christie, M. R. & Ashcroft, S. J. H. (1984) *Biochem. J.* **218**, 87–99
- Dunlop, M. E. & Larkins, R. G. (1986) *Arch. Biochem. Biophys.* **248**, 562–569
- Gagerman, E., Idahl, L.-A., Meissner, H. P. & Täljedal, I.-B. (1978) *Am. J. Physiol.* **235**, E493–E500
- Gagerman, E., Sehlin, J. & Täljedal, I.-B. (1980) *J. Physiol. (London)* **300**, 505–513
- Geisler, A., Klysner, R., Thams, P. & Christensen, S. (1977) *Acta Pharmacol. Toxicol.* **40**, 356–368
- Gerich, J., Charles, M. & Grodsky, G. (1974) *J. Clin. Invest.* **54**, 833–841
- Grill, V. & Cerasi, E. (1974) *J. Biol. Chem.* **249**, 4196–4201
- Groves, W. E., Davis, F. C. & Milner, B. H. (1968) *Anal. Biochem.* **22**, 195–210
- Hellman, B. (1976) *Experientia* **32**, 155–157
- Hidaka, H., Inagaki, M., Kawamoto, S. & Sasaki, Y. (1984) *Biochemistry* **23**, 5036–5041
- Hii, C. S. T., Jones, P. M., Persaud, S. J. & Howell, S. L. (1987) *Biochem. J.* **246**, 489–493
- Irvine, F., Pyne, N. J. & Houslay, M. D. (1986) *FEBS Lett.* **208**, 455–459
- Jakobs, K. H., Bauer, S. & Watanabe, Y. (1985a) *Eur. J. Biochem.* **151**, 425–430
- Jakobs, K. H., Aktories, K., Minuth, M. & Schultz, G. (1985b) *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **19**, 137–150
- Johnson, J. A., Goda, T. J. & Clark, R. B. (1986) *J. Cyclic Nucleotide Protein Phosphorylation Res.* **11**, 199–215
- Karl, R. C., Zawulich, W. S., Ferrendelli, J. A. & Matschinsky, F. M. (1975) *J. Biol. Chem.* **250**, 4575–4579
- Katada, T., Gilman, A. G., Watanabe, Y., Bauer, S. & Jakobs, K. H. (1985) *Eur. J. Biochem.* **151**, 431–437
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Kuo, W.-N., Hodgins, D. S. & Kuo, J. F. (1973) *J. Biol. Chem.* **248**, 2705–2711
- Lord, J. M. & Ashcroft, S. J. H. (1984) *Biochem. J.* **219**, 547–551
- Malaisse, W. J., Sener, A., Herchuelz, A., Carpinelli, A. R., Poloczek, P., Winand, J. & Castagna, M. (1980) *Cancer Res.* **40**, 3827–3831
- Malaisse, W. J., Dunlop, M. E., Mathias, P. C., Malaisse-Lagae, F. & Sener, A. (1985) *Eur. J. Biochem.* **149**, 23–27
- Morgan, N. G., Rumford, G. M. & Montague, W. (1987) *Biochem. J.* **244**, 669–674
- Olianas, M. C. & Onali, P. (1986) *J. Neurochem.* **47**, 890–897
- Prentki, M. & Wollheim, C. B. (1984) *Experientia* **40**, 1052–1060
- Schuit, F. C. & Pipeleers, D. G. (1985) *Endocrinology (Baltimore)* **117**, 834–840
- Sharp, G. W. G., Wiedenkel, D. E., Kaelin, D., Siegel, E. G. & Wollheim, C. B. (1980) *Diabetes* **29**, 74–77
- Sibley, D. R., Jeffs, R. A., Daniel, K., Nambi, P. & Lefkowitz, R. J. (1986) *Arch. Biochem. Biophys.* **244**, 373–381
- Thams, P., Capito, K. & Hedeskov, C. J. (1982) *Biochem. J.* **206**, 97–102
- Thams, P., Capito, K. & Hedeskov, C. J. (1984) *Biochem. J.* **221**, 247–253
- Thams, P., Capito, K. & Hedeskov, C. J. (1986) *Biochem. J.* **237**, 131–138
- Trus, M. D., Hintz, C. S., Weinstein, J. B., Williams, A. D., Pagliara, A. S. & Matschinsky, F. M. (1979) *J. Biol. Chem.* **254**, 3921–3929
- Valverde, I., Vandermeers, A., Anjaneyulu, R. & Malaisse, W. J. (1979) *Science* **206**, 225–227
- Virji, M. A. G., Steffes, M. W. & Estensen, R. D. (1978) *Endocrinology (Baltimore)* **102**, 706–711