Comparison of effects of phorbol esters and glucose on protein kinase C activation and insulin secretion in pancreatic islets

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The tumour-promoting phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induces insulin secretion from isolated pancreatic islets, and this suggests a potential role for protein kinase C in the regulation of stimulus–secretion coupling in islets. In the present study, the hypothesis that the insulinotropic effect of TPA is mediated by activation of protein kinase C in pancreatic islets has been examined. TPA induced a gradual translocation of protein kinase C from the cytosol to a membrane-associated state which correlated with the gradual onset of insulin secretion. The pharmacologically inactive phorbol ester 4 α -phorbol 12,13-didecanoate did not mimic this effect. TPA also induced a rapid time-dependent decline of total protein kinase C activity in islets and the appearance of a Ca²⁺- and phospholipid-independent protein kinase activity. Insulin secretion induced by TPA was completely suppressed (IC₅₀ ~ 10 nM) by staurosporine, a potent protein kinase C inhibitor. Staurosporine also inhibited islet cytosolic protein kinase C activity at similar concentrations (IC₅₀ ~ 2 nM). In addition, staurosporine partially (~ 60%) inhibited glucose-induced insulin secretion at concentrations (IC₅₀ ~ 10 nM) similar to those required to inhibit TPA-induced insulin secretion, suggesting that staurosporine may act at a step common to both mechanisms, possibly the activation of protein kinase C. However, stimulatory concentrations of glucose did not induce down-regulation of translocation of protein kinase C, and the inhibition of glucose-induced insulin release by staurosporine was incomplete. Significant questions therefore remain unresolved as to the possible involvement of protein kinase C in glucose-induced insulin secretion.

INTRODUCTION

Protein kinase C is a Ca²⁺- and phospholipid-dependent protein kinase which is thought to participate in a wide variety of cellular activation processes (Kikkawa & Nishizuka, 1986; Nishizuka, 1986). The role of protein kinase C in physiologically induced insulin secretion is, however, at present unresolved (for reviews, see Prentki & Matschinsky, 1987; Metz, 1988). A number of studies have established that pancreatic islets contain protein kinase C activity (Tanigawa *et al.*, 1982; Lord & Ashcroft, 1984; Hubinont *et al.*, 1984) and a number of possible endogenous substrates have been suggested (Thams *et al.*, 1984; Brocklehurst & Hutton, 1984; Lord & Ashcroft, 1984; Dunlop & Larkins, 1986). Recently, two independent studies have demonstrated that 1,2-diacyl-*sn*-glycerol, an endogenous activator of protein kinase C, accumulates in glucose-stimulated islets by virtue, probably, of synthesis *de novo* from glucose (Peter-Riesch *et al.*, 1988; Wolf *et al.*, 1989). Since the exposure of isolated islets to synthetic cell-permeable diacylglycerol (i.e. 1-oleoyl-2-acetyl-glycerol) stimulates insulin secretion (Malaisse *et al.*, 1985; Wolf *et al.*, 1989), it is attractive to postulate that secretagogue-in-

duced activation of protein kinase C promotes insulin secretion.

Tumour-promoting phorbol esters (e.g. 12-*O*-tetradecanoylphorbol 13-acetate, TPA), are thought to be relatively specific activators of protein kinase C (Castagna *et al.*, 1982). These compounds bind at the diacylglycerol site on protein kinase C and thereby promote activation of the enzyme (Sharkey *et al.*, 1984). TPA has been reported to induce insulin secretion from isolated islets (Virji *et al.*, 1978; Malaisse *et al.*, 1980; Hii *et al.*, 1986; Bozem *et al.*, 1987) and insulinoma cells (Hutton *et al.*, 1984; Wollheim *et al.*, 1988), and to activate islet protein kinase C *in vitro* (Hubinont *et al.*, 1984). The observation that TPA induces a slowly evolving but sustained secretory response from islets has been taken to imply that protein kinase C activation regulates the second phase of secretagogue-induced insulin secretion (Zawalich *et al.*, 1983). TPA has also been demonstrated to inhibit ATP-sensitive K⁺ channels in insulinoma cells and thereby to promote depolarization (Wollheim *et al.*, 1988). The secretory response of permeabilized islets to Ca²⁺ is also enhanced by TPA (Jones *et al.*, 1985).

Such effects of TPA on insulin-secreting cells are presumed to be mediated by activation of protein kinase

Abbreviations used: TPA, 12-*O*-tetradecanoylphorbol 13-acetate; 4 α -PDD, 4 α -phorbol 12,13-didecanoate; PMSF, phenylmethanesulphonyl fluoride; BSA, bovine serum albumin.

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C. The present study was undertaken to directly examine this hypothesis. In a variety of tissues, the activation of cellular responses by phorbol esters or hormones is accompanied by the translocation of protein kinase C from the cytosol to a membrane-associated state (Niedel & Blackshear, 1986; Blackshear, 1988). We have assessed the ability of TPA to induce such a translocation of protein kinase C in islets. We have also examined the influence of the potent protein kinase C inhibitor staurosporine (Tamaoki *et al.*, 1986) on the insulinotropic effects of TPA. The ability of the insulin secretagogue D-glucose to induce islet protein kinase C translocation and the influence of protein kinase C inhibitors on glucose-induced insulin secretion have also been examined.

MATERIALS AND METHODS

Male Sprague-Dawley rats (160–180 g) were purchased from Sasco (O'Fallon, MO, U.S.A.) and maintained on Rodent Chow 5001 (Ralston Purina, St. Louis, MO, U.S.A.) fed *ad libitum* for at least 7 days prior to use. Collagenase (CLS-IV) was obtained from Cooper Biochemical (Freehold, NJ, U.S.A.) or Boehringer-Mannheim (Indianapolis, IN, U.S.A.). Tissue culture medium (CMRL-1066), penicillin, streptomycin, Hanks' buffer, heat-inactivated fetal calf serum and L-glutamine were obtained from Gibco (Grand Island, NY, U.S.A.). Pentex bovine serum albumin (BSA) was obtained from Miles Laboratories (Ekhart, IN, U.S.A.). Phosphatidylserine (from bovine brain) was obtained from Avanti Polar Lipids (Pelham, AL, U.S.A.). Leupeptin, phenylmethanesulphonyl fluoride (PMSF), TPA, 4 α -phorbol 12,13-didecanoate (4 α -PDD) and 1,2-diolein were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). D-Glucose was purchased from the National Bureau of Standards (Washington, DC, U.S.A.). [γ - 32 P]ATP was purchased from Amersham Corporation (Arlington Heights, IL, U.S.A.). Staurosporine was purchased from Kamiya Biomedical Company (Thousand Oaks, CA, U.S.A.). K252a was generously given by Dr. Hiroshi Kase (Tokyo Research Laboratories, Tokyo, Japan).

Isolation and culture of islets

Pancreatic islets were isolated aseptically from rats by methods previously described (McDaniel *et al.*, 1983). Islets were cultured overnight at 24 °C in tissue culture medium CMRL-1066 containing 5.5 mM-glucose, 1% L-glutamine, 10% (w/v) heat-activated fetal bovine serum, 0.5% penicillin and 0.5% streptomycin under an atmosphere of air/CO₂ (19:1).

Incubation of islets

For translocation studies, isolated islets were washed three times in Krebs-Ringer/Hepes buffer (25 mM-Hepes (pH 7.4)/115 mM-NaCl/24 mM-NaHCO₃/5 mM-KCl/2.5 mM-CaCl₂/1 mM-MgCl₂) containing 3 mM-glucose and 0.1% BSA (basal medium), counted into siliconized borosilicate tubes (300–400/tube) and preincubated for 30 min at 37 °C in basal medium (300 μ l) under an atmosphere of air/CO₂ (19:1). The medium was then removed from each tube and replaced with basal medium supplemented with agonist (28 mM-glucose or 100 nM-TPA) prewarmed to 37 °C. The tubes were then incubated with shaking for 1, 2, 5, 10 or 30 min at 37 °C. At the end of this period, the medium was removed, and in some experiments it was analysed for

insulin content. The remaining islets were washed with ice-cold homogenization medium [25 mM-Tes/NaOH (pH 7.4)/1 mM-EGTA/0.5 mM-dithiothreitol/1 mM-PMSF/100 μ g of leupeptin/ml]. After rapid centrifugation, the medium was removed and replaced with homogenization medium (130 μ l), immediately sonicated (5 $\times$ 1 s, setting 2, Branson Cell Disruptor; Danburg, CT, U.S.A.), and placed on ice. Samples (125 μ l) were then centrifuged (Beckman airfuge for 10 min, 179–207 kPa). The supernatant (cytosolic fraction) was placed in fresh tubes, and the pellet (particulate fraction) was washed and then resuspended (using sonication) in homogenization medium (125 μ l). Triton X-100 was added to cytosolic and particulate fractions (final concentration 0.1%), and the samples were incubated on ice for 45–60 min prior to assay.

Assay of protein kinase C activity

Protein kinase C activity in cytosol (10 μ l) and particulate (10–30 μ l) fractions was assayed by incorporation of [32 P]P_i from [γ - 32 P]ATP into histone III-S at 30 °C. The total assay volume was 100 μ l, and the assay medium contained 100 mM-Tes/NaOH (pH 7.4), 5 mM-MgCl₂, 1 mM-EGTA, 400 μ g of histone III-S/ml, with or without 1.3 mM-Ca²⁺ (free Ca²⁺ concentration $\sim$ 300 μ M), with or without 125 μ g of phosphatidylserine/ml, and with or without 1,2-diolein (when added, 2.4 μ g/ml). After a preincubation period of 2 min, the reaction was initiated by the addition of 10 μ l of [γ - 32 P]-ATP (final concentration 20 μ M, $\sim$ 1.4 μ Ci/tube). After 30 s, 50 μ l of stop buffer (186 mM-Tris/HCl (pH 6.7)/9 mM-SDS/6 mM-2-mercaptoethanol/15% glycerol) was added and protein was denatured by boiling for 2 min. Phosphorylated histone bands (120 μ l) were separated from endogenous proteins by SDS/polyacrylamide-gel electrophoresis (PAGE) in 12% gels. After staining with Coomassie Blue [0.1% Coomassie Blue R-250 in 10% (v/v) acetic acid], the three histone bands of highest molecular mass (accounting for $\sim$ 95% of histone radioactivity) were excised from the gel and treated with 1.0 ml of Protosol. The 32 P content was determined by liquid scintillation spectrometry.

Protein kinase C activity was calculated after the subtraction of [32 P]P_i incorporation into histone in the absence of Ca²⁺, phosphatidylserine or diacylglycerol (basal activity). The inclusion of these cofactors resulted in a 2–3-fold and 7–8-fold stimulation of 'basal' histone phosphorylation in the particulate and cytosol fractions derived from islets respectively. Protein kinase C-catalysed incorporation of [32 P]P_i into histone III-S was found to be linear with time and with protein concentration over the course of the assay period.

Insulin secretion

Isolated islets were randomly counted (20/tube) into 10 mm $\times$ 75 mm siliconized borosilicate tubes and preincubated for 30 min at 37 °C with shaking in basal medium containing no BSA (200 μ l) with or without staurosporine or K252a under an atmosphere of air/CO₂ (19:1). The medium was then removed and replaced with fresh basal medium (no BSA; 200 μ l) supplemented with 16.5 mM-glucose with or without inhibitors. The incubation was continued for 30 min before an equal volume of basal medium containing 0.2% BSA was added. The supernatant was then withdrawn and the insulin content was determined by radioimmunoassay.

RESULTS

Translocation of protein kinase C to a membrane-associated state

TPA (100 nM) was found to induce insulin secretion in the presence of a basal concentration (3 mM) of glucose (Fig. 1*a*). This insulinotropic effect of TPA was not mimicked by a pharmacologically inactive analogue of TPA (4 α -PDD). The possibility that TPA-induced insulin secretion might be associated with translocation of protein kinase C was addressed by determining the activity of the enzyme in cytosolic and membrane fractions from TPA-stimulated islets. Protein kinase C activity was assessed as Ca²⁺- and phospholipid-dependent histone phosphorylating activity. In resting islets incubated in basal glucose medium (Krebs-Ringer/Hepes buffer containing 3 mM-glucose and 0.1 % BSA), approx. 90–95 % of the total cellular protein kinase C activity was recovered in the cytosol fraction after the cells had been disrupted in buffer containing EGTA. As illustrated in Fig. 1*b*, TPA (100 nM) induced a 5-fold increase in the proportion of the total cellular protein kinase C activity associated with the membrane fraction over the time period 0–30 min. This was accompanied by a loss of protein kinase C activity from the cytosol fraction (results not shown). It was noted that only 20–30 % of protein kinase C activity lost from the cytosol appeared in the particulate fractions, indicating that proteolytic degradation of membrane-associated protein kinase C had occurred (see below). Protein kinase C translocation was not induced by the pharmacologically inactive phorbol ester 4 α -PDD. Comparison of TPA-induced translocation of protein kinase C and TPA-induced insulin secretion revealed a striking temporal correlation between the two parameters, suggesting that translocation may represent a crucial step in the cellular effects of TPA.

Suppression of TPA-induced insulin secretion by staurosporine

The role of protein kinase C in the secretory response to TPA was further examined by determining the

Fig. 2. Inhibition of islet cytosol protein kinase C activity by staurosporine

Cytosol was prepared from islets (approx. 1500, obtained from five rats) essentially by the method of McDaniel *et al.* (1983). Islets were suspended in 500 μ l of homogenization media (see the Materials and methods section) and disrupted with 13 strokes of a Teflon/glass homogenizer while standing on ice. The homogenate was centrifuged in a Beckman SW50.1 rotor at 135000 *g* for 60 min at 4 °C. The supernatant was assayed for protein kinase C in the absence and presence of staurosporine (0–1000 nM).

influence of staurosporine, a potent inhibitor of protein kinase C, on TPA-induced insulin secretion. As illustrated in Fig. 2, staurosporine inhibited protein kinase C activity in islet cytosol in a concentration-dependent manner, with an IC₅₀ value (concn. of inhibitor causing 50 % inhibition) of approx. 2 nM. This is similar to the IC₅₀ value of this compound for inhibition of protein kinase C activity in a variety of other tissues (Kiyoto *et al.*, 1987). Complete inhibition (95–100 %) of islet cytosolic protein kinase C activity was achieved at

Fig. 1. Correlation of TPA-induced insulin secretion (a) with TPA-induced translocation of protein kinase C (b)

Islets (300–400/tube) were incubated in basal medium (□) or in basal medium containing TPA (100 nM) (●) or the inactive phorbol ester 4 α -PDD (100 nM) (○). At the indicated times, the medium was removed and assayed for insulin content (a). The remaining islets were washed, sonicated and fractionated, and protein kinase C activity associated with particulate and cytosol fractions was determined (b).

Fig. 3. Inhibition of TPA-induced insulin secretion by staurosporine

Islets (20/tube) were preincubated for 30 min at 37 °C in basal medium (3 mM-glucose, no BSA) in the absence or presence of staurosporine (0–1000 nM). Subsequently, islets were stimulated with TPA (100 nM) (●) in the presence of various concentrations of staurosporine (0–1000 nM). After 30 min, the medium was removed from the islets and assayed for insulin content.

staurosporine concentrations above 100 nM. Staurosporine also inhibited TPA-induced insulin secretion in a concentration-dependent manner (Fig. 3). Half-maximal inhibition of insulin secretion was achieved at a staurosporine concentration of about 10 nM. Complete inhibition was achieved at concentrations above 100 nM.

The staurosporine concentrations required to inhibit TPA-induced insulin secretion are therefore similar to those required to inhibit islet cytosolic protein kinase C activity *in vitro* (compare Fig. 3 with Fig. 2).

TPA-induced down-regulation of protein kinase C

Although TPA initially activates protein kinase C, numerous studies have demonstrated that prolonged stimulation of many cell types with TPA induces a time-dependent decrement in the total cellular protein kinase C activity (Kikkawa & Nishizuka, 1986; Nield & Blackshear, 1986). During the course of the experiments described above on TPA-induced translocation of protein kinase C, it was observed that TPA also induced an apparent disappearance (down-regulation) of total islet protein kinase C activity with time. Under basal conditions (i.e. Krebs–Ringer/Hepes buffer containing 3 mM-glucose and 0.1% BSA), total measurable islet protein kinase C activity decreased by 35% after 30 min in the absence of any stimulus (Fig. 4a). Stimulation of islets with TPA (100 nM) greatly accelerated the spontaneous loss of protein kinase C activity. During 30 min of incubation with TPA, more than 65% of the total islet protein kinase C activity was lost. This TPA-induced decline in islet protein kinase C activity was accompanied by the appearance of a Ca^{2+} - and phospholipid-independent protein kinase activity (Fig. 4b). This latter effect was not observed in experiments in which cytosol and particulate fractions were separated (i.e. Figs. 1 and 6), indicating that this proteolytically derived species may be unstable during centrifugation.

Glucose-induced insulin secretion and protein kinase C

In view of the ability of staurosporine to inhibit TPA-induced insulin secretion, it was of interest to examine the effect of this inhibitor on glucose-induced insulin

Fig. 4. TPA-induced down-regulation of protein kinase C activity and formation of Ca^{2+} - and phospholipid-independent protein kinase activity in intact islets

Islets (300–400/tube) were incubated at 37 °C for the indicated times in basal medium (□) or in basal medium supplemented with TPA (100 nM) (●) or glucose (28 mM) (■). Islets were disrupted by sonication, and Triton X-100 was added to a final concentration of 0.1%. After 45–60 min, incorporation of [32 P]P_i into histone III-S was determined in the absence and presence of Ca^{2+} and phosphatidylserine. (a) Total protein kinase C activity, (b) protein kinase activity in the absence of Ca^{2+} and phosphatidylserine (basal activity).

Fig. 5. Inhibition of glucose-induced insulin secretion by staurosporine and K252a

Islets (20/tube) were preincubated for 30 min at 37 °C in the absence or presence of staurosporine (0–10 μM) or K252a (0–100 μM). Subsequently, islets were challenged with stimulatory concentrations of glucose (16.5 mM) in the presence of staurosporine (0–10 μM) (●) or K252a (0–100 μM) (○). After 30 min, the medium was removed and assayed for insulin content.

secretion. Staurosporine suppressed glucose (16.5 mM)-induced insulin secretion in a concentration-dependent manner (Fig. 5). Moreover, the concentrations of staurosporine required to inhibit glucose-induced insulin secretion were essentially identical to those required to inhibit TPA-induced insulin secretion (Fig. 5, cf. Fig. 3). Half-maximal inhibition of insulin secretion was achieved

Fig. 6. Effects of stimulatory concentrations of glucose on translocation of protein kinase C

Islets (300–400/tube) were incubated in the presence of basal medium (□), basal medium supplemented with 100 nM-TPA (●), or basal medium supplemented with 28 mM-glucose (■). At the indicated times, islets were sedimented, washed and disrupted by sonication in media containing 0.1 μM-Ca²⁺. After fractionation, the particulate and cytosol fractions were assayed for protein kinase C activity as described in the Materials and methods section.

at staurosporine concentrations of approx. 10 nM in both cases. Although staurosporine completely suppressed TPA-induced insulin secretion, glucose-induced secretion was inhibited by only 60% by maximally effective concentrations of staurosporine. Glucose-induced insulin secretion is therefore mediated in part by staurosporine-insensitive mechanisms. Glucose-induced insulin secretion was also markedly inhibited (IC₅₀ ~ 2 μM) by another protein kinase C inhibitor, K252a (Kase *et al.*, 1986). Basal insulin secretion was not affected by staurosporine (100 nM) or K252a (100 nM) (results not shown). As illustrated in Fig. 6, however, stimulation of islets with glucose (28 mM) did not induce the translocation of protein kinase C to a membrane-associated state under conditions in which TPA induced a marked effect. Furthermore, glucose at a concentration which maximally stimulates insulin secretion was not observed to accelerate the spontaneous decline in total islet protein kinase C activity (Fig. 4a) or to induce the appearance of Ca²⁺- and phospholipid-independent protein kinase C activity (Fig. 4b). Thus glucose (28 mM) did not mimic the effects of TPA on translocation or down-regulation of protein kinase C activity.

DISCUSSION

There is considerable evidence to indicate that the physiological regulation of protein kinase C is modulated by its interaction with cellular membranes, and agonist-induced translocation of protein kinase C from the cytosol to a membrane-associated state is often observed in stimulated cells (Niedel & Blackshear, 1986; Thomas *et al.*, 1987). In the present study, we have demonstrated that TPA induces translocation of protein kinase C activity from cytosol to membrane fractions of isolated pancreatic islets. The gradual induction of protein kinase C translocation in response to TPA was closely correlated in time with TPA-induced insulin secretion. These observations indicate that TPA activates protein kinase C in islets and suggest that this event could play a role in the insulinotropic action of TPA. Additional observations supporting this suggestion are that TPA induces a rapid down-regulation of total islet protein kinase C activity and that the protein kinase C inhibitor staurosporine suppresses TPA-induced insulin secretion.

It has been known for some time that the response of intact cells to active tumour-promoting phorbol esters involves a time-dependent decrease in total cellular protein kinase C content, as assessed by a variety of methods including loss of protein kinase C enzymic activity, loss of specific phorbol ester binding activity or loss of immunoreactive protein kinase C (Hovis *et al.*, 1986; Blackshear, 1988). The prolonged incubation of islets with TPA for 20–24 h has also recently been shown to result in the loss of protein kinase C enzymic activity (Hii *et al.*, 1987). The present study indicates that the TPA-induced decline in total islet protein kinase C activity occurs relatively rapidly. The mechanism underlying phorbol ester-induced down-regulation of protein kinase C is not fully understood but reflects, in part, proteolytic degradation of the enzyme in the membrane-associated (i.e. activated) state. This process appears to involve the action of a Ca²⁺-dependent protease (Inoue *et al.*, 1977; Tapley & Murray, 1985; Melloni *et al.*, 1986; Blackshear, 1988) and to result in dissociation of the catalytic domain of protein kinase C (molecular mass 50 kDa,

often referred to as protein kinase M, PKM) from the lipid-binding (regulatory) domain (Lee & Bell, 1986). The free catalytic domain retains protein kinase activity but is no longer dependent on Ca^{2+} or phospholipid. Whether this 'constitutively active' form of the enzyme participates in cellular activation processes is an unresolved question. Our observations that TPA induces the formation of a Ca^{2+} - and phospholipid-independent protein kinase activity nonetheless reflects TPA-induced activation of islet protein kinase C.

TPA-induced insulin secretion could also be completely suppressed by the protein kinase C inhibitor staurosporine. That this effect of staurosporine is attributable to inhibition of protein kinase C in islets is indicated by the similar concentration-dependence of inhibition of insulin secretion and of inhibition of islet cytosolic protein kinase C activity *in vitro*. The sum of the observations with TPA therefore strongly supports the hypothesis that the insulinotropic action of TPA is mediated by activation of islet protein kinase C.

The predominant physiological regulator of insulin secretion is D-glucose. The role of protein kinase C in glucose-induced insulin secretion is at present unresolved. In the present study, the protein kinase C inhibitor staurosporine was observed to profoundly inhibit insulin secretion induced by glucose in a manner very similar to the inhibition of TPA-induced insulin secretion. The observation that the concentration-dependence of inhibition of glucose-induced insulin secretion by staurosporine is virtually identical to that for inhibition of TPA-induced insulin secretion by staurosporine suggests that this inhibitor acts at a step shared by both secretory processes. Activation of protein kinase C could be this step. The possibility that staurosporine inhibits enzymes other than protein kinase C which participate in insulin secretion cannot, however, be excluded. A role for protein kinase C in insulin secretion is further supported by the observation that a second inhibitor of protein kinase C, K252a (Kase *et al.*, 1986), also suppressed glucose-induced insulin secretion with an appropriate IC_{50} value ($2\text{ }\mu\text{M}$). However, it was noted that while staurosporine totally prevents TPA-induced insulin secretion, glucose-induced insulin secretion was only partially ($\sim 60\%$) inhibited at maximally effective concentrations. These data suggest that protein kinase C may not be a fundamental component of the secretory processes but rather may serve as a modulator of these processes.

In contrast with TPA, glucose was not observed to induce the translocation of islet protein kinase C to a membrane-associated state, as assessed by protein kinase C activity measurements in membrane and cytosol fractions. Glucose (28 mM) also did not mimic TPA in inducing down-regulation of protein kinase C. The possibility exists, however, that stimulation of islets with glucose results in an activation of protein kinase C that cannot be detected with our present assay for translocation of enzyme activity. For example, it is possible that translocation may occur in intact islets in response to glucose but that this effect cannot be preserved during disruption and fractionation procedures owing to the transience of physiological signals which induce translocation, such as diacylglycerol accumulation or elevation of the intracellular Ca^{2+} concentration (May *et al.*, 1985; Dougherty & Niedel, 1986; Hughes & Ashcroft, 1988; Terbush *et al.*, 1988). Alternatively, glucose may induce

the translocation of an isoenzyme of protein kinase C which is distinct from the isoenzyme translocated by TPA (Nishizuka, 1988) or for which histone III-S is a poor substrate (Cochet *et al.*, 1986; Ramsdell *et al.*, 1986; Cooper *et al.*, 1987). However, it is yet to be determined whether islets contain several isoenzymic forms of protein kinase C. Consequently, a full assessment of the involvement of protein kinase C in glucose-induced insulin secretion requires the development of additional techniques capable of addressing these questions.

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