Effects of Ca^{2+} and a phorbol ester on insulin secretion from islets of Langerhans permeabilised by high-voltage discharge

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Isolated rat islets of Langerhans permeabilised by high-voltage discharge secreted insulin in response to elevations in Ca^{2+} over the range 100 nM to 10 μM Ca^{2+}. The phorbol ester, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), had no effects on insulin secretion in the absence of Ca^{2+}. In the presence of Ca^{2+} concentrations of > 10 nM, TPA produced dose-related shifts in the Ca^{2+}-activation curve to lower Ca^{2+} concentrations, together with marked increases in the maximum secretory response to Ca^{2+}. These results suggest that, in islets, the activation of protein kinase C is important in modulating both the sensitivity of the exocytotic mechanism to intracellular Ca^{2+}, and the magnitude of the insulin secretory response.

Islets of Langerhans *High-voltage discharge* *Cytosolic Ca^{2+}* *Exocytosis* *Phorbol ester*

1. INTRODUCTION

The secretion of insulin from pancreatic B cells may be regulated in part by the hydrolysis of the plasma membrane phospholipids, phosphatidylinositol 4,5-bisphosphate, to produce *myo*-inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) [1,2].

IP3 can mobilise intracellular Ca^{2+} in islets [3] and insulin-secreting tumour cells [4] and may therefore be involved in the control of cytosolic Ca^{2+}, which is thought to be of prime importance in regulating insulin secretion [5]. DAG may also play an important role in the regulation of insulin secretion by activating the phospholipid- and Ca^{2+}-dependent protein kinase C (PK-C), an enzyme which has been identified in islets [6] and insulin-secreting cell lines [7]. Tumour-promoting phorbol esters, such as TPA, which mimic the DAG activation of PK-C [8] are potent insulin secretagogues [7,9–11], suggesting a physiological role for PK-C in insulin secretion.

Studies of TPA-stimulated secretion in intact tissues offer little direct insight into the relationships between cytosolic Ca^{2+}, PK-C activation and hormone release. We have therefore used islets permeabilised by high-voltage discharge [12] to investigate the roles of intracellular Ca^{2+} and PK-C activation by TPA in the regulation of insulin secretion.

2. MATERIALS AND METHODS

Islets of Langerhans were isolated from rat pancreata by collagenase digestion as described [13], and incubated for 60 min at 37°C in a bicarbonate-buffered physiological salt solution [14] containing 2 mM glucose and 2 mM $CaCl_2$. The islets were then washed 5 times at 4°C with a glutamate-based Ca^{2+}/EGTA buffer (permeation buffer), containing 140 mM K-glutamate, 15 mM Hepes, 7 mM $MgSO_4$, 5 mM adenosine 5′-triphosphate (ATP, Sigma), 5 mM glucose, 1 mM EGTA, 0.5 mg/ml bovine serum albumin (fraction V, Sigma), pH 6.6, with $CaCl_2$ added to produce a Ca^{2+} concentration of 10 nM. Ca^{2+} concentrations in the

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permeation buffer were calculated using the dissociation constants of Portzehl et al. [15], and confirmed by direct measurements with a Ca^{2+} electrode using ionophore 1001 (Fluka), as described in [16].

Groups of 50–100 islets were resuspended in permeation buffer (2°C) of various Ca^{2+} concentrations (10 nM to 10 μM) and permeabilised by 5 exposures to an electric field of 3.4 kV/cm. Permeabilised islets were washed twice with permeation buffer at 2°C and groups of 10 islets transferred by micropipette to incubation vials containing 1.0 ml permeation buffer of various Ca^{2+} concentrations and the appropriate concentration of TPA dissolved in dimethyl sulphoxide (DMSO). Controls (no TPA) contained DMSO alone. Islets were incubated for 30 min at 37°C, the incubation vials centrifuged at 9000 × g for 30 s, and insulin measured in the supernatant by radioimmunoassay [12]. Differences between treatments were assessed by analysis of variance or Student's unpaired t-test, as appropriate.

3. RESULTS

Incubation of permeabilised islets in buffers containing increasing concentrations of Ca^{2+} (10^{-8}–10^{-4} M) produced dose-related increases in insulin secretion over the range 100 nM to 10 μM Ca^{2+} (fig.1), with 50% of the maximum response at approx. 1.0 μM Ca^{2+}.

In the absence of Ca^{2+} (and the presence of 1 mM EGTA) TPA had no significant effects on insulin secretion, the secretory response to 0, 50 and 500 nM TPA being (mean ± SE, n = 4) 128 ± 22, 131 ± 23 and 165 ± 28 pg/islet per h, respectively. However, in the presence of even very low levels of Ca^{2+}, TPA had marked effects on insulin secretion by the permeabilised islets. At Ca^{2+} concentrations of 10 nM, 100 nM and 10 μM, TPA (50 nM to 5 μM) produced dose-related increases in insulin secretion (fig.2). Interestingly, TPA promoted insulin secretion above the maximum (10 μM) Ca^{2+}-activated response. At concentrations of 50 nM, 500 nM and 5 μM TPA produced increases of 14 ± 5, 82 ± 20 and 124 ± 16%, respectively, over the insulin release evoked by 10 μM Ca^{2+} alone.

Both the Ca^{2+}-induced and TPA-stimulated insulin secretion were temperature dependent. Fig.3 shows the results of an experiment in which permeabilised islets were exposed to 10 nM or 10 μM Ca^{2+} in the presence or absence of 0.5 μM TPA at either 2 or 37°C. The 3-fold increase in insulin release in response to elevated Ca^{2+} (fig.3, lower panel) was totally abolished by incubation at 2°C (fig.3, upper panel), as was the stimulatory effect of TPA at both 10 nM and 10 μM Ca^{2+} (fig.3).

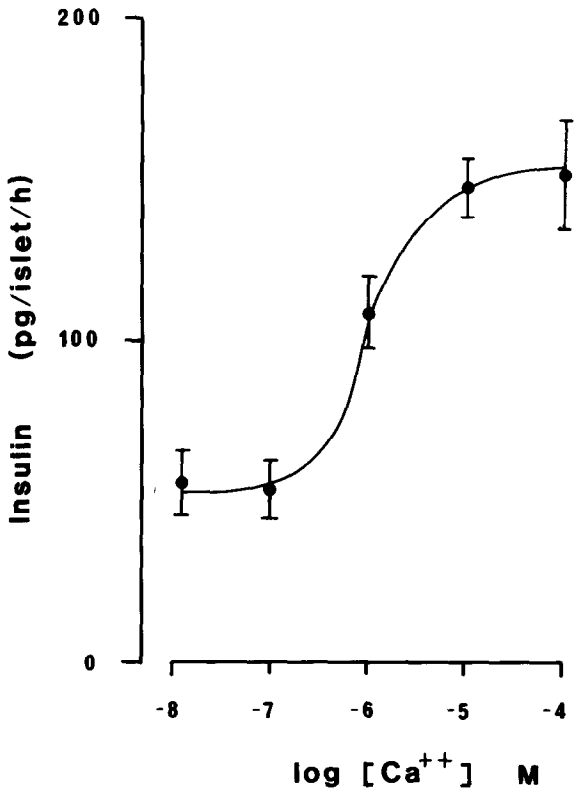

Fig.1. Ca^{2+}-dependent exocytosis in permeabilised rat islets. Isolated islets were permeabilised at 2°C and incubated at 37°C in Ca^{2+}/EGTA buffers of various free Ca^{2+} concentrations. Increasing Ca^{2+} produced dose-related increases in insulin secretion. Points show means ± SE for 8 or 9 observations.

The secretory responses to 10 μM Ca^{2+} alone and to TPA in the presence of 10 μM Ca^{2+} were both dependent on the presence of MgATP (fig.4). Omission of ATP from the permeation buffer caused a reduction of 78 ± 13% (± SE, n = 4) in the response to 10 μM Ca^{2+}, and of 64 ± 7% in the response to 10 μM Ca^{2+} plus 0.5 μM TPA.

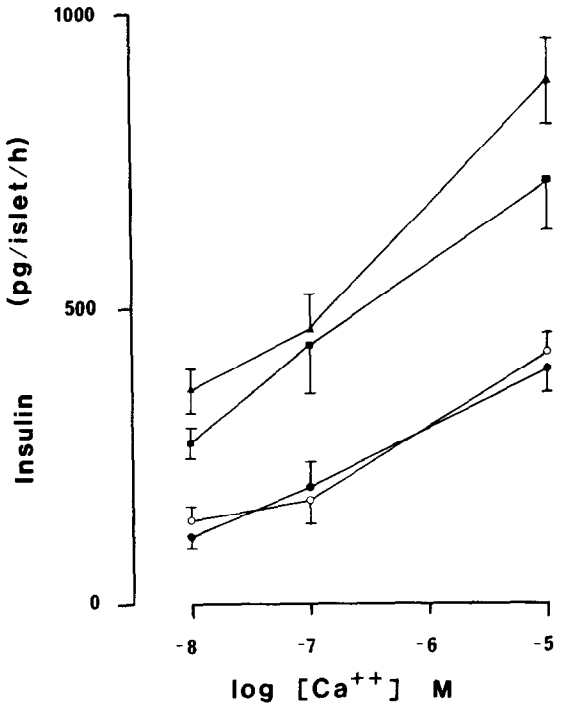

Fig.2. Stimulation of Ca^{2+}-dependent insulin secretion by TPA. The figure shows insulin secretion by permeabilised islets incubated in various concentrations of TPA [control, no TPA (●), 50 nM (○), 0.5 μM (■), 5.0 μM (▲)] at different levels of Ca^{2+}. Note that 0.5 and 5.0 μM TPA significantly increased the secretory response at all Ca^{2+} concentrations ($p < 0.05$), and stimulated secretion by approx. 100% over the maximum Ca^{2+}-activated response (10 μM Ca^{2+}). Points show means ± SE, $n = 4$ or 5.

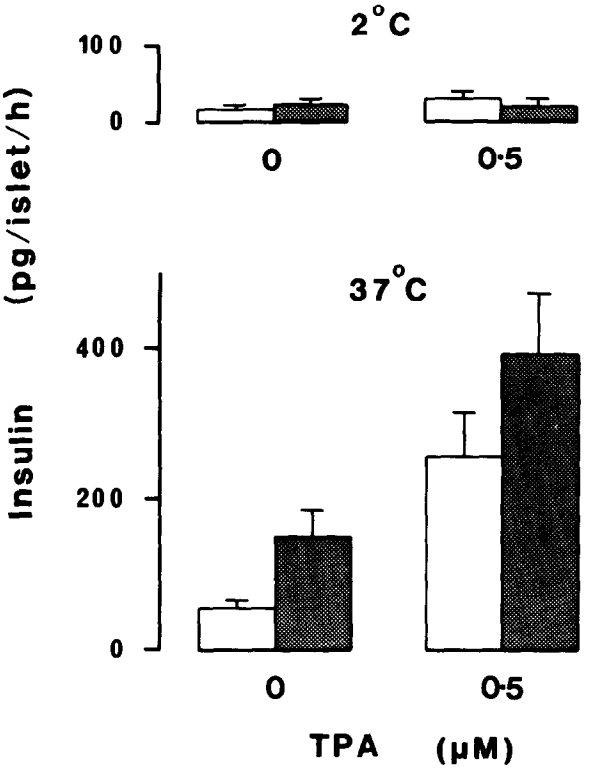

Fig.3. Temperature dependence of Ca^{2+}-induced and TPA-stimulated insulin secretion. Permeabilised islets were incubated in buffers containing 10 nM (open bars) or 10 μM (hatched bars) Ca^{2+}, in the presence or absence of 0.5 μM TPA. Islets incubated at 2°C (upper panel) showed no significant secretory response to either Ca^{2+} or to TPA. Incubation at 37°C (lower panel) produced a 3-fold increase in insulin secretion in response to 10 μM Ca^{2+} ($p < 0.05$), and stimulation of secretion by TPA at both 10 nM ($p < 0.05$) and 10 μM ($p < 0.01$) Ca^{2+}. Bars show means ± SE, $n = 4$.

4. DISCUSSION

Exposure to a high-intensity electric field induces the formation of stable pores in the plasma membrane of a variety of cell types [12,17–19], allowing direct access to the interior of the cell. Although digitonin-permeabilised islets have recently been used to study Ca^{2+} handling [3,20] and insulin secretion [20,21], electrical permeabilisation is perhaps more appropriate when studying membrane associated events such as exocytosis, since the membrane disruption produced by high-voltage discharge is confined to the locality of the pores and there is no generalised chemical alteration of the plasma membrane.

There is considerable evidence that elevated cytosolic Ca^{2+} is an important initiator of insulin secretion [5], and in the present studies increasing the concentration of free Ca^{2+} produced dose-related increases in insulin secretion from permeabilised islets. Although there was some inter-experimental variation in the absolute levels of insulin secretion (e.g. see figs 3 and 4), the magnitude of the response to Ca^{2+} was remarkably consistent between experiments, with a maximum Ca^{2+}-dependent stimulation of about 3-fold over basal secretion.

The threshold of about 100 nM Ca^{2+} for insulin secretion from permeabilised islets is in good

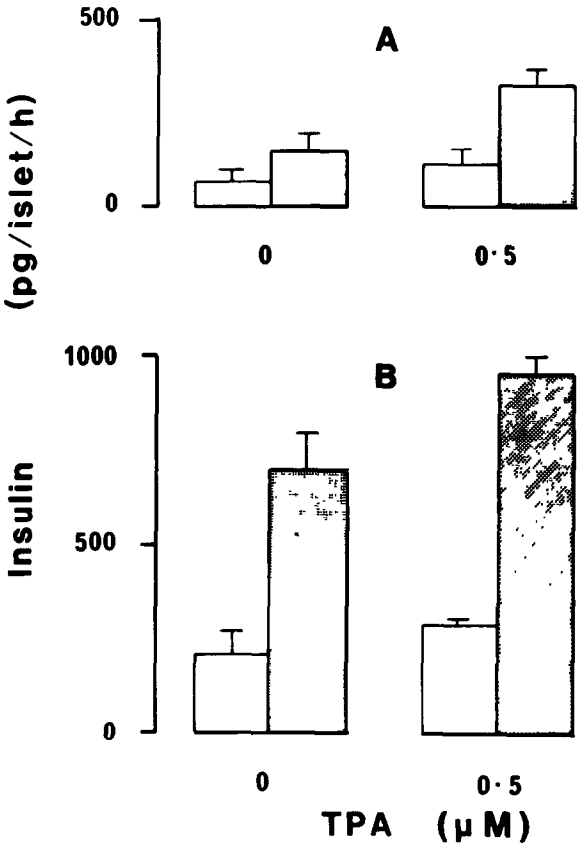

Fig.4. ATP dependence of insulin secretion in permeabilised islets. Permeabilised islets were incubated in buffers containing 10 nM (open bars) or 10 μM (hatched bars) Ca^{2+}, in the presence or absence of 0.5 μM TPA. (A) In the absence of MgATP permeabilised islets showed no significant increase in insulin secretion in response to Ca^{2+}, and the stimulatory effects of 0.5 μM TPA were significantly reduced ($p < 0.01$). (B) In the presence of 5 mM MgATP, 10 μM Ca^{2+} produced a 3.5-fold increase in secretion ($p < 0.01$) and this Ca^{2+}-dependent secretion was further stimulated by TPA. Points show means ± SE, $n = 4$ or 5.

agreement with estimates of basal cytosolic Ca^{2+} in unstimulated insulin-secreting cells, whether measured by fluorescent probes [22,23], or by a Ca^{2+} microelectrode in suspensions of digitonin-permeabilised cells [4]. The maximum secretory responses to Ca^{2+} in this work, and in others using digitonin-permeabilised islets [20,22], were seen at Ca^{2+} levels of 1–10 μM, somewhat higher than those measured in stimulated intact tumour cells.

TPA had marked effects on the Ca^{2+}-dependent exocytosis in permeabilised islets, with concentrations above 50 nM producing significant increases in insulin release. TPA may have direct effects on ion fluxes across the B cell plasma membrane [24,25]. While these effects might prove to be a significant component of the response to TPA in whole cells, they are unlikely to be important in permeabilised islets where the extracellular and intracellular medium are contiguous. Since TPA readily binds to membrane phospholipids [25], some of its effects could be ascribed to a non-physiological disruption of the secretory granule or plasma membranes. However, the temperature sensitivity and ATP dependence of the exocytotic response to TPA suggest more than a simple solvent effect on membranes. Furthermore, in the absence of Ca^{2+}, TPA had no stimulatory effect on insulin secretion, as might be expected if the response was due to activation of a Ca^{2+}-dependent kinase.

In this work one effect of TPA on insulin secretion was a shifting of the Ca^{2+}-activation curve to the left, thus inducing exocytosis at lower levels of intracellular Ca^{2+}. Similar findings have been reported in a number of other secretory tissues [17–19,26], although the effective doses of TPA vary between tissues. The concentrations of TPA which stimulate secretion from electrically permeabilised islets are similar to those which enhance insulin release from digitonin-treated islets [21] and promote secretion [7,9–11] and affect the membrane potential [25] in intact islets. Electrically permeabilised chromaffin cells [17], platelets [18] and acinar cells [19] respond to levels of TPA which were without effect in our experiments (i.e. <50 nM), although in normal [27] and digitonin-permeabilised [26] chromaffin cells higher levels of TPA are required.

TPA also produced considerable increases in the maximum secretory response to Ca^{2+} by permeabilised islets. A similar, though somewhat smaller effect has been reported in permeabilised acinar cells [19], but TPA does not increase the maximum Ca^{2+}-dependent exocytosis in either permeabilised chromaffin cells [17] or platelets [18]. This may indicate that PK-C activation is quantitatively more important in secretion from B cells than from some other tissues.

Finally, these studies in permeabilised islets raise

the possibility that the physiological activation of PK-C by DAG could stimulate insulin secretion without the need for elevations in cytosolic Ca^{2+} above the reported basal levels of 100–200 nM [4,22,23]. Whether a physiological release of insulin can occur in intact islets without any increase in cytosolic Ca^{2+} remains to be seen.

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