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Translocation of protein kinase C in rat islets of Langerhans

Effects of a phorbol ester, carbachol and glucose

Shanta J. Persaud, Peter M. Jones, David Sugden and Simon L. Howell

Biomedical Sciences Division, King's College London, Kensington, London W8 7AH, England

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In unstimulated rat islets (2 mM glucose), most of the ion-exchange purified protein kinase C (PKC) activity was associated with the cytosolic fraction. Both carbachol and phorbol myristate acetate caused a significant translocation of PKC activity from cytosolic to membrane fractions, but under the same conditions, glucose (20 mM) did not cause such a redistribution of PKC activity. PMA-induced translocation of PKC to the membrane fraction was also observed in electrically permeabilised islets, in which recovery of the enzyme activity was enhanced by buffering the intracellular Ca^{2+} concentration to 50 nM and supplying the permeabilised islets with protease inhibitors.

Protein kinase C; Translocation; Glucose; Carbachol; Phorbol ester; (Islets of Langerhans)

1. INTRODUCTION

Protein kinase C (PKC) is a closely related family of Ca^{2+}- and phospholipid-dependent kinases which have been identified in a wide range of tissues [1]. At physiological concentrations of Ca^{2+}, PKC can be activated by increases in the intracellular availability of diacylglycerols (DAG), or by tumour-promoting phorbol esters, such as phorbol myristate acetate (PMA), which can substitute for DAG [1,2].

PKC has been identified and characterised in islets of Langerhans and insulin-secreting tumour cells [3,4], and DAG analogues [5] or phorbol esters [6,7] have been shown to stimulate insulin secretion in vitro. However, the precise physiological role of PKC in the control of insulin secretion in response to nutrient secretagogues (e.g. glucose) or receptor-mediated secretagogues (e.g. cholinergic muscarinic agonists) is still uncertain.

Correspondence address: S.J. Persaud, Biomedical Sciences Division, King's College London, Kensington, London W8 7AH, England

The activation of PKC is thought to involve translocation of the enzyme from the cytosol to membranes, where it associates with phosphatidylserine forming a catalytically active complex [8]. We have therefore determined the effects of glucose, PMA and the muscarinic agonist carbachol (CCh) on the distribution of PKC activity between cytosolic and membrane fractions prepared from isolated rat islets of Langerhans.

2. MATERIALS AND METHODS

2.1. *Materials*

Collagenase (type XI), bovine serum albumin (BSA, fraction V), leupeptin, phenylmethylsulphonyl fluoride (PMSF), PMA, CCh, Nonidet P-40, diolein, phosphatidylserine, histone type IIIs and adenosine 5′-triphosphate (ATP) were obtained from Sigma (England). Tissue culture medium was from Gibco (Europe). [γ-^{32}P]ATP (3000 Ci/mmol) was from Amersham International (England). All other reagents were of analytical grade from BDH (England).

2.2. *Islet isolation and permeabilisation*

Islets of Langerhans were isolated from fed Wistar rats of either sex (150–200 g) by collagenase digestion of the pancreas [9] and maintained in a bicarbonate-buffered (pH 7.4) physiological salt solution (Gey-Gey buffer [10]) supplemented

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with 2 mM glucose, 2 mM $CaCl_2$ and 0.5 mg/ml BSA. To determine the effects of CCh on the intracellular distribution of PKC, islets were first cultured for 20 h at 37°C in RPMI 1640 in a humidified atmosphere of 5% CO_2/95% O_2 to facilitate receptor regeneration. In some experiments insulin secretion into the supernatant (5 islets/0.5 ml) was measured by radioimmunoassay [11] and in others, islets were electrically permeabilised as in [7,12]. For permeabilisation, islets were thoroughly washed in a K^+-glutamate-based Ca^{2+}/EGTA buffer (permeation buffer, pH 6.6) with $CaCl_2$ added to give a free Ca^{2+} concentration of 50 nM, then permeabilised by 5 exposures (200 μs) to an electric field of 3.4 kV/cm. A modified permeation buffer, containing 50 μg/ml leupeptin and 1 mM PMSF, was used for incubation of permeabilised islets.

2.3. *Extraction and partial purification of PKC*

Groups of 300–450 islets were incubated for 5 min, unless otherwise indicated, at 37°C in 200 μl of appropriate buffer (Gey-Gey buffer supplemented with 500 μM CCh, 500 nM PMA or 20 mM glucose for intact islets; modified permeation buffer supplemented with 500 nM PMA for permeabilised islets). Following incubation, islets were pelleted by a brief centrifugation (9000 × *g*, 15 s), the supernatant was discarded, and 200 μl ice-cold buffer A (20 mM Tris-HCl, 2 mM EDTA, 0.5 mM EGTA, 50 μg/ml leupeptin, 1 mM PMSF and 0.1% mercaptoethanol, pH 7.5) were added to the pellet. Islets were sonicated (MSE Soniprobe, 3 × 15 s, 4 μm) and membranes were separated from the cytosol by centrifugation at 30000 × *g* for 15 min at 4°C. The resulting membrane pellets were dispersed by sonication (2 × 15 s, 4 μm) in 200 μl buffer A and enzyme activity was solubilised by incubation (30 min, 4°C) in the presence of 1% Nonidet P-40. Membrane and supernatant fractions were applied to DEAE-cellulose DE52 columns (0.2 ml, pre-equilibrated with 1 ml buffer A) and the columns were washed with 3 ml buffer A. The enzyme was eluted from the columns using 300 μl buffer A supplemented with 120 mM NaCl.

2.4. *Protein kinase C assay*

PKC activity in eluate samples was assayed by measuring incorporation of ^{32}P from [γ-^{32}]ATP into histone type IIIs. Aliquots of the column eluates were incubated at 30°C in the presence of 11.1 mM magnesium acetate, 1.3 mM $CaCl_2$, 96 μg/ml phosphatidylserine, 6.4 μg/ml diolein, 1.11 mg/ml histone and 111 μM ATP, in a final volume of 45 μl. Reactions were started by the addition of [γ-^{32}P]ATP (final spec. act. 0.3 Ci/mmol), and terminated with 1 ml of cold 10% trichloroacetic acid containing 10 mM sodium pyrophosphate and 1 mM ATP. 500 μg BSA were added as a carrier and the precipitated protein was collected onto Whatman GF/C filters using a Millipore 1225 filtration manifold, then washed four times with 5 ml of 5% trichloroacetic acid. ^{32}P incorporation was estimated by liquid scintillation counting of the filters (Beckman LS 7500). All samples were corrected for ^{32}P binding to the filter in the absence of added enzyme activity (<0.2% total radiolabel). Each eluate sample was assayed in triplicate in the absence and presence of phosphatidylserine and diolein, and the difference in ^{32}P incorporation between these conditions was taken to reflect PKC activity in the sample. Differences between treatments were assessed by Student's paired or unpaired *t*-tests, as appropriate.

3. RESULTS

In unstimulated islets (2 mM glucose) most of the PKC activity was associated with the cytosolic fraction (95.6 ± 2% of total activity, mean ± SE, n = 5), with little detectable activity in the membrane fraction (4.4 ± 2% of total activity). Progress curves in assays of PKC activity in both cytosolic and membrane fractions were linear with time for incubation periods of up to 10 min, as shown in fig.1a. PKC activity was also dependent on enzyme concentration, showing a linear increase in activity with increasing volume of column eluate added to the incubation mixture, over the range 2.5–10 μl (fig.1b). For subsequent assays a sample volume of 10 μl column eluate and an incubation time of 10 min were used.

The tumour-promoting phorbol ester, PMA, produced a redistribution of PKC activity in islets. Incubation of intact islets at 37°C in the presence of 500 nM PMA and 2 mM glucose caused a time-dependent decrease in cytosolic PKC activity with a maximal effect observed at 2 min, as shown in fig.2. Further exposure to PMA (up to 5 min) did not produce any further decrease in PKC activity associated with the cytosol (fig.2). The decrease in cytosolic PKC activity was accompanied by increased activity in the membrane fraction. Table 1 (rows A) shows the increase in membrane-associated PKC activity concomitant with a decrease in cytosolic activity in islets exposed to 500 nM PMA for 5 min. The total amount of cellular PKC activity (cytosol plus membrane) was reduced following PMA-induced translocation of PKC activity. In four experiments in which islets were exposed to 500 nM PMA for 5 min, total PKC activity recovered was 77.5 ± 4% of controls (no PMA). Since activated PKC is a substrate for a Ca^{2+}-activated protease [2], this reduction in enzyme activity may reflect proteolytic cleavage of PKC which had become activated and membrane-associated by the presence of PMA. We therefore measured the effects of PMA treatment on PKC distribution in electrically permeabilised islets, in which the protease inhibitors leupeptin and PMSF were introduced directly into the intracellular compartment, and cytosolic Ca^{2+} was fixed at 50 nM by chelating buffers. As in intact islets, treatment of electrically permeabilised islets with 500 nM PMA for 5 min caused a significant reduction in

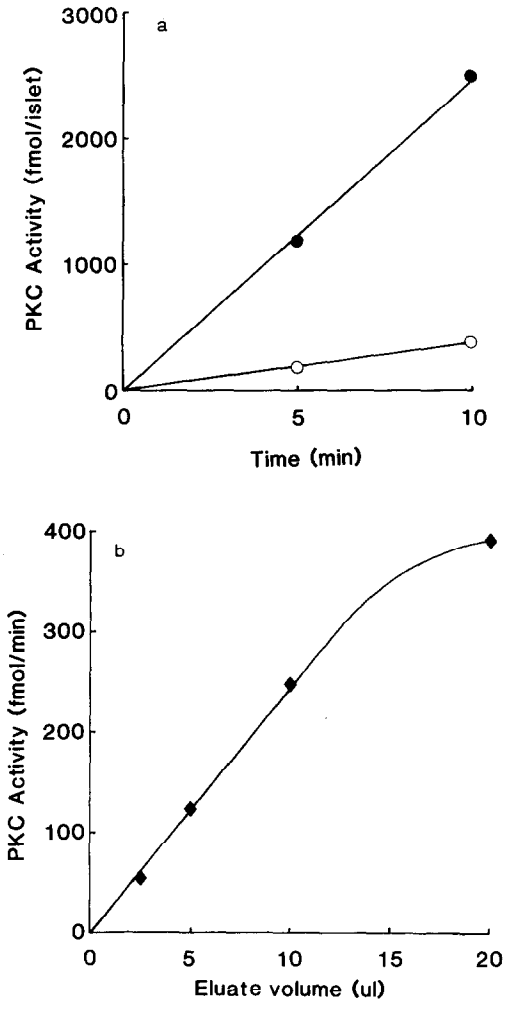

Fig.1. PKC activity in cytosol and membranes of unstimulated islets. (a) Ca^{2+}- and phospholipid-dependent PKC activity measured in cytosolic (•) and membrane (○) fractions prepared from unstimulated intact islets was linear with time up to incubation periods of 10 min. (b) Cytosolic PKC activity was concentration-dependent, showing a linear increase in activity over the range 2.5–10 μl of column eluate added per incubation.

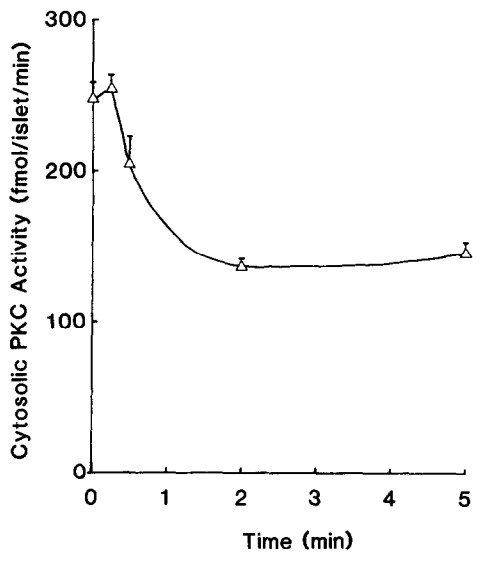

Fig.2. Time course of disappearance of PKC from cytosol in response to PMA. Incubation of intact islets in the presence of 500 nM PMA (2 mM glucose) produced a time-dependent decrease in cytosolic PKC activity.

cytosolic PKC activity with a concomitant increase in the activity associated with the membrane fraction (table 1, rows B). In three such experiments the total PKC activity (cytosol plus membrane) recovered was 90.3 ± 4% of control.

A cholinergic muscarinic agonist had similar effects to PMA on the distribution of PKC activity in intact islets. Incubation of cultured (20 h, 37°C) intact islets for 5 min at 37°C with 500 μM CCh at

Table 1

Effects of PMA, CCh and glucose on redistribution of PKC activity

	PKC activity (fmol/islet per min)	
	Cytosol	Membrane
(A) 2 mM glucose	275 ± 19	undetectable
2 mM glucose + 500 nM PMA	140 ± 10[b]	43 ± 7[b]
(B) 50 nM Ca^{2+}	145 ± 5	11 ± 9
50 nM Ca^{2+} + 500 nM PMA	80 ± 8[b]	47 ± 4[a]
(C) 2 mM glucose	225 ± 21	14 ± 6
2 mM glucose + 500 μM CCh	159 ± 14[a]	46 ± 5[b]
(D) 2 mM glucose	320 ± 27	undetectable
20 mM glucose	300 ± 11	undetectable

500 nM PMA caused a translocation of PKC activity from the cytosol to membranes in both intact (A) and electrically permeabilised (B) islets. 500 μM CCh had similar effects to PMA in intact islets (C), but under the same conditions 20 mM glucose was unable to cause a redistribution of PKC activity (D). Data are expressed as means ± SE of 3–4 observations from single experiments (A–D), each representative of results obtained in 3–5 separate experiments. Levels of significance by unpaired *t*-tests: [a] $p < 0.05$, [b] $p < 0.01$ vs appropriate PKC activity of unstimulated islets

a substimulatory glucose concentration (2 mM) produced a significant increase in membrane-associated PKC activity together with a reduction in enzyme activity in the cytosolic fraction, as shown in table 1 (rows C).

In contrast, exposure of intact islets to 20 mM glucose for 5 min at 37°C did not cause a detectable decrease in cytosolic PKC activity, or an increase in membrane-associated activity, compared to controls incubated in the presence of 2 mM glucose (table 1, rows D). In parallel experiments under the same conditions, 20 mM glucose caused a significant stimulation of insulin secretion from intact islets during a 5 min incubation at 37°C (2 mM glucose, 28.3 ± 8 pg/islet per 5 min; 20 mM glucose, 94.8 ± 16, $p < 0.01$, $n = 5$).

4. DISCUSSION

Here, we have attempted to evaluate the involvement of PKC in receptor-mediated and glucose-induced insulin secretion by measuring the translocation of PKC from cytosol to membranes in isolated rat islets of Langerhans. In common with many other cell types [13–16], exposing intact islets to a phorbol ester produced a detectable shift of PKC activity to the membrane fraction. Despite the relatively high concentrations of phorbol esters used in our experiments, the extent of PKC translocation in islets was less than that reported in other tissues [13,14], including a rat insulinoma cell line [16]. This appears to be a real difference between tissues, since we were able to show considerably more translocation of PKC activity in parallel experiments using rat anterior pituitary tissue (46% of total in membranes in pituitary vs 24% in islets; Persaud and Jones, unpublished). The PMA-induced translocation of PKC activity in electrically permeabilised islets incubated in a sub-stimulatory concentration of Ca^{2+} is in accordance with previous reports that phorbol esters, by activating PKC, stimulate insulin secretion from permeabilised islets [7,17]. Furthermore, maintaining intracellular Ca^{2+} at a sub-stimulatory concentration and introducing protease inhibitors into the permeabilised cells enhanced the recovery of PKC activity from permeabilised islets compared to intact islets, perhaps suggesting that membrane-associated PKC is susceptible to proteolytic degradation by a Ca^{2+}-activated protease in islets, as has previously been reported in other tissues [2].

The cholinergic muscarinic agonist, CCh, promoted translocation of PKC from cytosol to membranes in intact islets, as has also been recently reported in studies using rat insulinoma cells [16] and, in a preliminary report, a hamster cell line [18]. These observations concur with the known effects of cholinergic agonists to promote DAG formation in islet membranes [19], and intact islets [20], and suggest that the DAG thus formed is involved in the activation and translocation of PKC.

There have been several demonstrations that glucose stimulates the breakdown of inositol phospholipids in pancreatic islets with the subsequent generation of inositol phosphates and, presumably, DAG [21,22]. Furthermore, there is evidence that glucose stimulates the de novo synthesis of DAG in islets [20]. Despite this, we were unable to detect an effect of glucose on PKC translocation under conditions in which both PMA and CCh produced significant changes in the distribution of the enzyme, and in which glucose stimulated insulin secretion. It is possible that glucose activates only a minority subspecies of PKC within B cells and that translocation of this minority species is not detectable in the present assay which measures the total activity of all PKC subspecies. Alternatively, glucose may activate, either by DAG or by some other activator [1], an isoform of PKC which does not translocate to membranes upon activation. However, the most likely explanation for our results is that the DAG generated in response to glucose does not activate PKC, perhaps because of its cellular localisation, and that the translocation of PKC is not obligatory for glucose-induced insulin secretion.

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REFERENCES

[1] Nishizuka, Y. (1988) Nature 334, 661–665.
[2] Nishizuka, Y. (1984) Nature 308, 693–698.
[3] Tanigawa, K., Kuzuya, H., Imura, H., Taniguchi, H., Baba, S., Takai, Y. and Nishizuka, Y. (1982) FEBS Lett. 138, 183–186.

[4] Lord, J.M. and Ashcroft, S.J.H. (1984) Biochem. J. 219, 547–551.
[5] Malaisse, W.J., Dunlop, M.E., Mathias, P.C.F., Malaisse-Lagae, F. and Sener, A. (1985) Eur. J. Biochem. 149, 23–27.
[6] Hubinont, C.J., Best, L., Sener, A. and Malaisse, W.J. (1984) FEBS Lett. 170, 247–253.
[7] Jones, P.M., Stutchfield, J. and Howell, S.L. (1985) FEBS Lett. 191, 102–106.
[8] Blumberg, P.M. (1988) Cancer Res. 48, 1–8.
[9] Bjaaland, T., Jones, P.M. and Howell, S.L. (1988) J. Mol. Endocrinol. 1, 171–178.
[10] Gey, G.O. and Gey, M.K. (1936) Am. J. Cancer 27, 45–76.
[11] Jones, P.M., Salmon, D.M.W. and Howell, S.L. (1988) Biochem. J. 254, 397–403.
[12] Yaseen, M.A., Pedley, K.C. and Howell, S.L. (1982) Biochem. J. 206, 81–87.
[13] Wooten, M.W. and Wrenn, R.W. (1984) FEBS Lett. 171, 183–186.
[14] Zatz, M., Mahan, L.C. and Reisine, T. (1987) J. Neurochem. 48, 106–110.
[15] Sugden, D., Vanacek, V., Klein, D.C., Thomas, T.P. and Anderson, W.B. (1985) Nature 314, 359–361.
[16] Yamatani, T., Chiba, T., Kadowaki, S., Hishikawa, R., Yamaguchi, A., Inui, T., Fujita, T. and Kawazu, S. (1988) Endocrinology 122, 2826–2832.
[17] Stutchfield, J., Jones, P.M. and Howell, S.L. (1986) Biochem. Biophys. Res. Commun. 136, 1001–1006.
[18] Regazzi, R. and Wollheim, C.B. (1988) Diabetologia 31, 534A.
[19] Dunlop, M.E. and Malaisse, W.J. (1986) Arch. Biochem. Biophys. 244, 421–429.
[20] Peter-Riesch, B., Fathi, M., Schlegel, W. and Wollheim, C.B. (1988) J. Clin. Invest. 81, 1154–1161.
[21] Best, L., Dunlop, M. and Malaisse, W.J. (1984) Experientia 40, 1085–1090.
[22] Morgan, N.G., Rumford, G.M. and Montague, W. (1985) Biochem. J. 228, 713–718.