Biochem. J. (1986) 233, 287–289 (Printed in Great Britain)

Enhancement of glucagon secretion from isolated rat islets of Langerhans by phorbol 12-myristate 13-acetate

Charles S. T. HII, Jane STUTCHFIELD and Simon L. HOWELL*
Department of Physiology and MRC Secretory Mechanisms Group, King's College London (KQC), University of London, Campden Hill Road, London W8 7AH, U.K.

The phorbol ester 4β-phorbol 12-myristate 13-acetate (PMA), at concentrations of 0.1 μM and above, stimulated secretion of glucagon and of insulin from isolated rat islets of Langerhans incubated in the presence of 5.5 mM-glucose. Stimulation of secretion of both hormones by 1 μM-PMA persisted in the absence of external Ca^{2+}, and could be abolished by incubating the islets at 4 °C. These findings suggest a role of protein kinase C in the α-cell (and β-cell) secretory mechanism.

INTRODUCTION

The phorbol ester PMA, structurally resembling diacylglycerol, has been shown by Castagna *et al.* (1982) to activate protein kinase C, a Ca^{2+}–phospholipid-dependent protein kinase, *in vitro*. This enzyme, distributed widely in different tissues (reviewed by Nishizuka, 1984), including the islets of Langerhans (Tanigawa *et al.*, 1982; Thams *et al.*, 1984; Harrison *et al.*, 1984), is thought to play a crucial role in signal transduction for a variety of biologically active substrates which activate cellular functions.

PMA has stimulatory effects on various cell types, such as platelets and chromaffin cells (reviewed by Nishizuka, 1984). In the β-cells of islets of Langerhans (Yamamoto *et al.*, 1982; Zawalich *et al.*, 1983) and insulinoma cells (Hutton *et al.*, 1984) insulin secretion is also stimulated by PMA. We now extend the list of cell types that can be stimulated by this ester to include the α-cells of isolated rat islets of Langerhans, by reporting the effect of PMA on glucagon secretion from these islets.

EXPERIMENTAL

Media

Islet isolation and static secretion experiments were carried out in a bicarbonate-buffered medium (Gey & Gey, 1936) equilibrated to pH 7.4 with O_2/CO_2 (19:1). The medium contained either 5.5 mM- or 11 mM-glucose, 0.5 mg of bovine serum albumin/ml and 1 mM-$CaCl_2$ (unless stated otherwise). PMA was dissolved in dimethyl sulphoxide, and all controls contained a similar volume of the solvent alone. The maximum concentration of the solvent presented to the islets never exceeded 1% (v/v), which did not affect hormone release.

Islet isolation

Islets were isolated from the body and tail of the pancreas, known to contain a high proportion of α-cells (Trimble & Renold, 1981), of male Wistar rats (150–200 g) by a collagenase digestion technique (Howell & Taylor, 1968). After isolation, the islets were preincubated for 1 h at 37 °C.

Islet incubations

At the end of 1 h, the islets were washed three times by repeated centrifugation (200 *g* for 30 s). Groups of ten islets were incubated for 30 min at either 37 °C or 4 °C. The islets were then centrifuged (9000 *g*) for 45 s to sediment any cells that might have become detached. Samples of the supernatant medium were then taken for hormone radioimmunoassays.

Hormone radioimmunoassays

Glucagon was assayed by using rabbit anti-(bovine glucagon) serum (obtained from Guildhay Antisera, University of Surrey), purified pig/bovine glucagon standards (Novo, Bagsvaerd, Denmark) and pig/bovine glucagon [^{125}I]iodinated in our laboratory by the method of Jorgensen & Larsen (1972). Insulin was assayed by using guinea-pig anti-(rat insulin) serum (given by Dr. W. Montague, University of Leicester), purified rat insulin standards (Novo, Denmark) and ^{125}I-labelled insulin prepared by a chloramine-T method (Hunter & Greenwood, 1962). In both assays, the antibody-bound fraction was separated from the unbound fraction by precipitation in 12% (w/v) polyethylene glycol 6000 (Desbuquois & Aurbach, 1977).

Statistical analysis

Values were expressed as means ± S.E.M. Differences were analysed by Student's *t* test and were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Islets isolated from the body and tail of the rat pancreas contained on average 4.7 ng of glucagon/islet. This value compares favourably with those reported by Trimble & Renold (1981). When challenged with arginine, glucagon secretion was stimulated (control, 776 ± 92 pg/30 min per 10 islets; +5 mM-L-arginine hydrochloride, 1190 ± 162 pg/30 min per 10 islets; $P < 0.03$, $n = 15$).

Secretion of glucagon as well as that of insulin were stimulated by PMA at concentrations of 0.1 μM and

Abbreviation used: PMA, 4β-phorbol 12-myristrate 13-acetate.

* To whom reprint requests should be addressed.

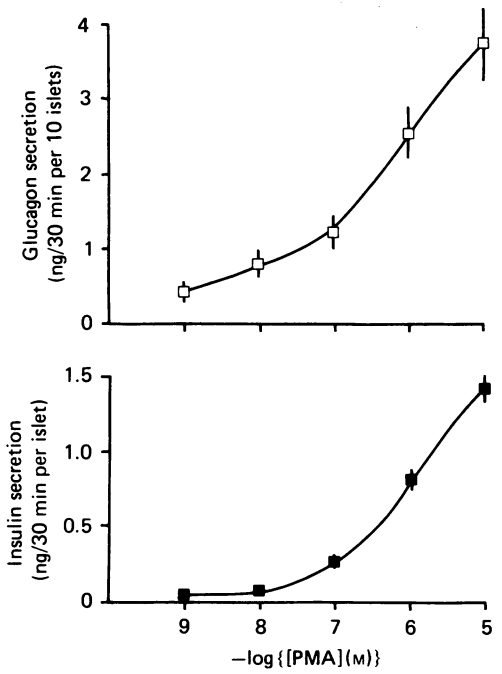

Fig. 1. Effects of PMA on secretion of glucagon and insulin

Isolated islets were incubated in the presence of 5.5 mM-glucose as described in the Experimental section. Points and vertical lines are means ± S.E.M. for 16–20 observations from four experiments.

above (Fig. 1); maximal secretory rates were not reached at the highest PMA dose tested. Dimethyl sulphoxide, at concentrations over the range, did not affect secretion of either glucagon or insulin (results not shown). The α-cells responded differently from the β-cells to changes in external Ca^{2+} concentrations (Fig. 2). In the absence of 1 μM-PMA, complete removal of Ca^{2+} almost doubled the rate of glucagon secretion (0 mM-Ca^{2+} versus 0.25 mM-, 0.5 mM- or 1 mM-Ca^{2+}, $P < 0.05$); insulin secretion was unaffected. Whereas glucagon secretion was not significantly enhanced on raising the Ca^{2+} concentration in the medium from 1 to 10 mM ($P = 0.059$), *a 4–5-fold* enhancement in insulin secretion was apparent on raising the external Ca^{2+} concentration from 0 to 10 mM ($P < 0.00001$), with a threshold of 2 mM. In the presence of 1 μM-PMA, glucagon secretion remained relatively constant over a Ca^{2+} concentration range of 0–2 mM, but secretion was increased by 10 mM-Ca^{2+} compared with that observed at either 0.5 mM- or 1 mM-Ca^{2+} ($P < 0.02$); the rate of insulin secretion, on the other hand, rose sharply in the presence of 1 μM-PMA, reaching a maximum at around 1 mM-Ca^{2+} (Fig. 2). At all the Ca^{2+} concentrations tested, greater amounts of both hormones were secreted in the presence than in the absence of 1 μM-PMA. Stimulation of secretion of both hormones by 1 μM-PMA could be abolished by incubating the islets at 4 °C (Table 1). Under this condition, glucagon secretion, but not the basal insulin secretion from control islets seen in the absence of 1 μM-PMA, was significantly impaired ($P < 0.01$).

These results show that secretion of glucagon, like that of insulin (Yamamoto *et al.*, 1982; Zawalich *et al.*, 1983), can be stimulated by PMA. The PMA dose–response curves (Fig. 1) are closely similar, suggesting that PMA probably activated a set of similar secretory mechanisms in the α and β cells. The PMA dose–response curves obtained in this study are strikingly similar to those reported for rat insulinoma cells by Hutton *et al.* (1984).Those workers found the concentration of PMA sufficient to stimulate insulin release to be in the same order of magnitude as that reported here. Similar results have been observed by other workers using either normal (Yamamoto *et al.*, 1982) or electrically permeabilized (Jones *et al.*, 1985) islets. However, these values are considerably higher than those reported for some other cell types, such as the chromaffin cells (Knight & Baker, 1983; Brocklehurst & Pollard, 1985). This discrepancy could be due to real differences between the various cell types and/or the technical procedures used in tissue preparation.

Ca^{2+} omission has been reported either to stimulate or to inhibit glucagon secretion (reviewed by Leclercq-Meyer & Malaisse, 1983). In the present study glucagon secretion in the absence of PMA was enhanced in an

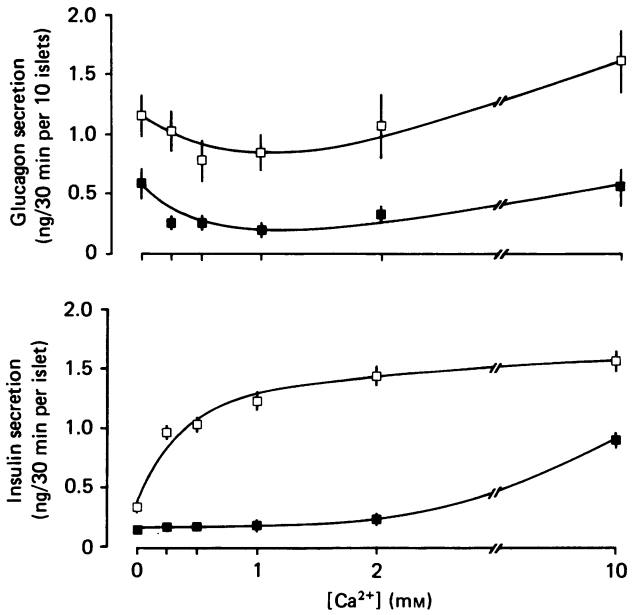

Fig. 2. Dependence of secretion of glucagon and insulin on external Ca^{2+}

Isolated islets were incubated in the presence of 11 mM-glucose. Ca^{2+}-depleted medium was obtained by omission of $CaCl_2$ from and the addition of 0.5 mM-EGTA to the medium (constituted in distilled deionized water). Ca^{2+} measurements in this medium with a Ca^{2+} electrode showed nanomolar values. When the medium contained 10 mM-Ca^{2+}, the NaCl concentration was lowered to maintain iso-osmolality, and the total PO_4^{2-} concentration was lowered to 0.1 mM. In Fig. 1 and Table 1, glucagon radioimmunoassay was carried out by using a phosphate buffer (0.04 M, pH 7.4) for standards and label dilution and heat-inactivated horse serum for antibody dilution. In Fig. 2, glucagon radioimmunoassay was performed with a borate buffer (0.133 M, pH 8.0) for all dilutions. This gave a lower degree of non-specific binding (3–4%) and a higher degree of specific binding (22–27%), and this might account for the different secretory rates seen here compared with Fig. 1 and Table 1. Points (■, control; □, +1 μM-PMA) and vertical lines are means ± S.E.M. for 16–20 observations from four experiments.

Table 1. Temperature-dependence of PMA-stimulated secretion of hormones

Islets were incubated in the presence of 5.5 mM-glucose at either 4 °C or 37 °C. Values are means ± S.E.M. for 16–20 observations from four experiments. Significance of difference: $*P < 0.1$ versus 4 °C; $**P < 0.001$ versus control at 37 °C.

	Glucagon (ng/30 min per 10 islets)		Insulin (ng/30 min per islet)	
Temp. (°C)	Control	+1 μM-PMA	Control	+1 μM-PMA
4	0.58 ± 0.07	0.75 ± 0.08	0.09 ± 0.01	0.11 ± 0.01
37	0.99 ± 0.12	3.02 ± 0.53**	0.10 ± 0.01	0.83 ± 0.05**

EGTA-containing Ca^{2+}-depleted medium. This phenomenon, abolished by cooling the islets to 4 °C, also occurred in an EGTA-free Ca^{2+}-depleted medium (C. S. T. Hii & S. L. Howell, unpublished work). On the other hand, an enhancement of glucagon secretion in the presence of 10 mM-Ca^{2+} has been observed by Wollheim *et al.* (1977) in monolayer cultures of neonatal pancreas. A similar effect, though not statistically significant, was observed in the control islets (Fig. 2).

It has been reported that very low intracellular Ca^{2+} concentrations are required for PMA-induced cell activation. In either electrically permeabilized (Jones *et al.*, 1985) or digitonin-permeabilized islets (Tamagawa *et al.*, 1985), insulin secretion could be stimulated by PMA at a Ca^{2+} concentration as low as 10 nM. The ability of PMA to stimulate secretion of both glucagon and insulin in an EGTA-containing Ca^{2+}-depleted medium is not inconsistent with these findings, although Malaisse *et al.* (1983) found insulin secretion to be stimulated by PMA only in EGTA-free Ca^{2+}-depleted medium.

It is unlikely that the effects of PMA observed in this study could be attributed to non-specific leakages, since the stimulation of secretion of both glucagon and insulin by 1 μM-PMA could be totally abolished by lowering the ambient temperature (Table 1). Given that phosphorylation of endogenous insulinoma granule membrane proteins only occurred at PMA concentrations greater than 10 nM (Hutton *et al.*, 1984), that protein kinase C activation appears to be the most likely mechanism of action for PMA (Niedal *et al.*, 1983), and that protein kinase C is present in the islets of Langerhans (Tanigawa *et al.*, 1982; Thams *et al.*, 1984; Harrison *et al.*, 1984), a role of protein kinase C in the α-cell secretory mechanism is strongly suggested. Highly purified α-cell populations will be required to analyse its role in detail.

Financial assistance towards the cost of this study from the British Diabetic Association and the M.R.C. via the Secretory Mechanisms Group is gratefully acknowledged.

Received 30 August 1985/22 October 1985; accepted 25 October 1985

REFERENCES

Brocklehurst, K. W. & Pollard, H. B. (1985) FEBS Lett. **183**, 107–110
Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) J. Biol. Chem. **257**, 7847–7851
Desbuquois, G. & Aurbach, G. D. (1977) J. Clin. Endocrinol. Metab. **33**, 732–738
Gey, G. O. & Gey, M. L. (1936) Am. J. Cancer **27**, 45–76
Harrison, D. E., Ashcroft, S. J. H., Christie, M. R. & Lord, J. M. (1984) Experientia **40**, 1075–1084
Howell, S. L. & Taylor, K. W. (1968) Biochem. J. **108**, 17–24
Hunter, W. M. & Greenwood, F. C. (1962) Nature (London) **194**, 495–496
Hutton, J. C., Pershavaria, M. & Brocklehurst, K. W. (1984) Biochem. J. **224**, 483–490
Jones, P. M., Stutchfield, J. & Howell, S. G. (1985) FEBS Lett., in the press
Jorgensen, K. H. & Larsen, U. D. (1972) Horm. Metab. Res. **4**, 223–224
Knight, D. E. & Baker, P. F. (1983) FEBS Lett. **160**, 98–100
Leclercq-Meyer, V. & Malaisse, W. J. (1983) Glucagon II: Handb. Exp. Pharmacol. **66/II**, 58–74
Malaisse, W. J., Lebrun, P., Herchuelz, A., Sener, A. & Malaisse-Lagae, F. (1983) Endocrinology (Baltimore) **113**, 1870–1877
Niedal, J. E., Kuhn, L. J. & Vandenbark, G. R. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 36–40
Nishizuka, Y. (1984) Nature (London) **308**, 693–698
Tamagawa, T., Niki, H. & Niki, A. (1985) FEBS Lett. **183**, 430–432
Tanigawa, K., Kuzuya, H., Imura, H., Taniguchi, H., Baba, S., Takai, Y. & Nishizuka, Y. (1982) FEBS Lett. **138**, 183–186
Thams, P., Capito, K. & Hedeskov, C. J. (1984) Biochem. J. **221**, 247–253
Trimble, E. & Renold, A. E. (1981) Am. J. Physiol. **240**, E422–E427
Wollheim, C. B., Blondel, B., Renold, A. E. & Sharp, G. W. (1977) Endocrinology (Baltimore) **101**, 911–919
Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, K. & Kato, R. (1982) Biochem. Biophys. Res. Commun. **105**, 759–765
Zawalich, W., Brown, C. & Rasmussen, H. (1983) Biochem. Biophys. Res. Commun. **117**, 448–455