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Phorbol esters have a dual action through protein kinase C in regulation of proliferation of FRTL-5 cells

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In quiescent rat thyroid (FRTL-5) cells, phorbol-12-myristate-13-acetate (PMA) inhibited DNA synthesis induced by a combination of insulin and thyrotropin (TSH) or dibutyryl cyclic AMP (Bt_2cAMP). This inhibitory effect of PMA was observed even when PMA was added after addition of these growth factors, and was maximal when PMA was added 2–4 h after the growth factors (late in the G_1-phase of the cell cycle). On the other hand, PMA alone induced DNA synthesis and also enhanced that induced by Bt_2cAMP or insulin in these quiescent cells. 1-Oleoyl-2-acetylglycerol mimicked these effects of PMA, but 4α-phorbol-12,13-didecanoate had no effect. These data demonstrate that in FRTL-5 cells protein kinase C has a stimulatory effect on the G_0 to G_1 transition and an inhibitory effect on the G_1 to S transition in the cell cycle.

Cell proliferation; Cell cycle; Phorbol ester; Protein kinase C; (FRTL-5 cell)

1. INTRODUCTION

It is of great interest to study the control mechanisms of proliferation in FRTL-5 cells as an in vitro model of goitrous thyroid diseases. In human, rat and dog thyroid cells, TSH is a potent growth factor [1–3]. However, recently in human and rat thyroid, TSH was found to stimulate not only cAMP synthesis but also PIP_2 hydrolysis with the generation of IP_3 and DAG [4–6].

Protein kinase C, an enzyme that is activated by DAG or phorbol esters in the presence of Ca^{2+} and phospholipid, plays important roles in transmembrane signaling [7].

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Abbreviations: PMA, phorbol-12-myristate-13-acetate; TSH, thyrotropin; Bt_2cAMP, dibutyryl cyclic AMP; OAG, 1-oleoyl-2-acetylglycerol; 4α-PDD, 4α-phorbol-12,13-didecanoate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PDGF, platelet-derived growth factor

Although phorbol esters have been known for many years to stimulate cell proliferation in various cell types, some examples of their growth inhibition have been reported [8–11].

In this paper, we show that protein kinase C activated by phorbol esters can exert both stimulatory and inhibitory effects that may be related to different phases in the cell cycle.

2. MATERIALS AND METHODS

2.1. *Materials*

The following materials were used: [*methyl*-^{3}H]thymidine (DuPont); PMA and 4α-PDD (LC Services Co.); OAG (Serdary Res. Lab.); TSH, Bt_2cAMP, insulin and 3-isobutyl-1-methylxanthine (Sigma); cAMP RIA kits (Yamasu Shoyu Co.). Other materials and chemicals were obtained from commercial sources.

2.2. *Cell culture and assay for DNA synthesis*

FRTL-5 cells were seeded into 24-well costar trays (approx. 2×10^5 cells/well) in Coon's modified Ham F-12 medium containing 5% calf serum, TSH (10^2 μU/ml), transferrin (5 μg/ml), insulin (10 μg/ml), somatostatin (10 ng/ml), cortisone (10 nM) and the tripeptide glycyl-L-histidyl-L-lysine acetate (10 ng/ml) as described previously [12], and were in-

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cubated at 37°C in a humidified atmosphere (95% air, 5% CO_2) for at least one week. The culture medium was changed twice weekly.

For measurement of DNA synthesis, the cells were washed twice with PBS(−) and then incubated in RPMI-1640 containing 0.1% BSA and 0.1% DMSO for 48 h. They were then incubated for 48 h in RPMI containing 0.1% BSA, 0.1% DMSO, transferrin (5 μg/ml) and [*methyl*-^{3}H]thymidine (0.5 μCi/well) and with or without one or more of the following compounds: insulin (10 μg/ml), TSH (10^2 μU/ml), Bt_2cAMP (0.1 mM) and PMA (10^{-7} M). PMA also was added to the wells at different times before and after addition of other compounds.

The reaction was stopped by addition of 10% trichloroacetic acid and the radioactivity in acid-insoluble materials was counted in a liquid scintillation spectrometer.

3. RESULTS

Addition of PMA to quiescent FRTL-5 cells slightly enhanced DNA synthesis induced by insulin, but markedly inhibited that induced by a combination of TSH and insulin (fig.1). Addition of PMA at different times before and after addition of the growth factors showed that it inhibited DNA synthesis even when added after TSH and insulin, its effect being maximal when it was added 2–4 h after their addition (late in the G_1 phase of the cell cycle).

Most effects of TSH on the thyroid are mediated through activation of the adenylate cyclase-cyclic AMP system. We investigated whether adenylate cyclase in FRTL-5 cells was modulated by pretreatment of the cells with PMA for various periods. Results showed that PMA pretreatment inhibited the TSH-induced cAMP response, inhibition being maximal with a pretreatment period of 4–6 h (fig.2).

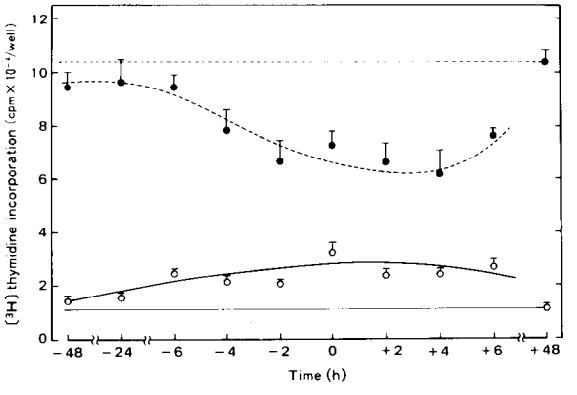

Fig.1. Stimulation and inhibition of DNA synthesis by PMA in FRTL-5 cells. PMA (10^{-7} M) was added to the cells at the indicated times relative to the addition of insulin only or with TSH. +2 h represents addition of PMA 2 h after the growth factors and so +48 h represents no addition of PMA. (○) DNA synthesis in the presence of insulin (10 μg/ml); (●) DNA synthesis in the presence of TSH (10^2 μU/ml) and insulin (10 μg/ml). Points and bars indicate means ± SD for triplicate determinations.

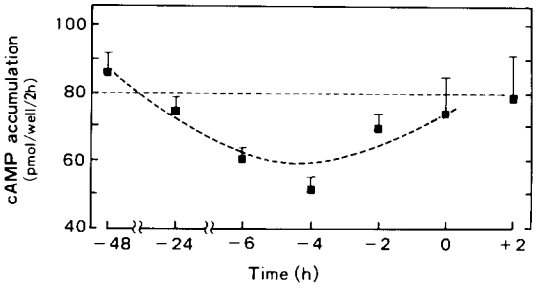

Fig.2. Effect of PMA pretreatment on TSH-induced cAMP accumulation in FRTL-5 cells during 2 h incubation. PMA (10^{-7} M) was added to the wells at the indicated times relative to the addition of TSH (10^2 μU/ml). −2 h represents 2 h preincubation with PMA and so +2 h represents no addition of PMA. Points and bars indicate means ± SD for triplicate determinations.

We next examined whether the inhibitory effect of PMA on DNA synthesis resulted from inhibition of TSH-induced cAMP accumulation. PMA inhibited DNA synthesis induced by a combination of Bt_2cAMP and insulin, and the inhibition was greatest when PMA was added after addition of the growth factors (fig.3).

PMA alone induced DNA synthesis and also did not inhibit that induced by Bt_2cAMP (table 1).

OAG mimicked these dual effects of PMA on DNA synthesis, but 4α-PDD was ineffective (not shown). Moreover, in cells in which the level of

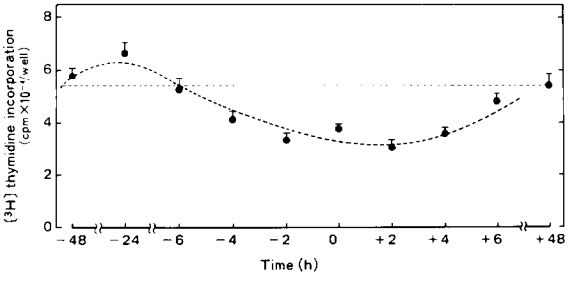

Fig.3. Inhibition by PMA of DNA synthesis induced by Bt_2cAMP (0.1 mM) plus insulin (10 μg/ml). Times of addition of PMA are represented as described for fig.1. Points and bars indicate means ± SD for triplicate determinations.

Table 1

Effect of PMA on DNA synthesis

Additions	[^{3}H]Thymidine incorporation (cpm × 10^{-4} /well)	
	Without PMA	With PMA (10^{-7} M)
None	0.96 ± 0.07	2.01 ± 0.18
Bt_2cAMP (0.1 mM)	3.58 ± 0.13	4.13 ± 0.41
Insulin (10 μg/ml)	1.09 ± 0.07	3.27 ± 0.37
Bt_2cAMP + insulin	5.10 ± 0.07	4.26 ± 0.13

DNA synthesis was assayed in the presence of the indicated additions. Values are means ± SD for triplicate determinations

protein kinase C was reduced by 48 h pretreatment with 10^{-6} M PMA, PMA showed no stimulatory or inhibitory effect on DNA synthesis induced by one or more growth factors (table 2).

4. DISCUSSION

In this work we found that PMA had both stimulatory and inhibitory effects on DNA synthesis in FRTL-5 cells depending on the presence of other growth factors. It is known that PMA and OAG activate protein kinase C, whereas 4α-PDD does not. Since these effects of PMA on DNA synthesis were mimicked by OAG but not by 4α-PDD, and were abolished by down regulation of protein kinase C, we conclude that they are mediated through the activation of protein kinase C.

Pretreatment of the cells with PMA decreased TSH-induced cAMP accumulation. In the late G_1 phase of the cell cycle PMA also inhibited DNA synthesis induced by a combination of TSH and insulin. However, since PMA also inhibited DNA synthesis induced by a combination of Bt_2cAMP and insulin in the late G_1 phase, it probably has another mechanism for the inhibition of DNA synthesis besides that of inhibiting the TSH-induced cAMP response.

Table 2

Effect of down-regulation of protein kinase C on DNA synthesis

Additions	[^{3}H]Thymidine incorporation (cpm × 10^{-4}/well)	
	Without PMA	With PMA (10^{-7} M)
Insulin (10 μg/ml)	1.16 ± 0.09	1.15 ± 0.01
Insulin + TSH (10^2 μU/ml)	7.58 ± 0.14	7.36 ± 0.02
Insulin + Bt_2cAMP (0.1 mM)	5.07 ± 0.23	4.81 ± 0.20

Cells were depleted of protein kinase C by pretreatment with 10^{-6} M PMA for 48 h. Then their DNA synthesis was assayed in the presence of the indicated additions. Values are means ± SD for triplicate determinations

There are reports of growth inhibition by phorbol esters [8–11]. In rat vascular smooth muscle, PMA was recently shown to inhibit thrombin-induced inositol trisphosphate release and DNA synthesis induced by thrombin at the G_1 to S transition, but not DNA synthesis induced by platelet-derived growth factor (PDGF) [10]. Moreover, rabbit aortic smooth muscle cells are sensitive to both the stimulatory and the inhibitory actions of protein kinase C, depending on the presence of the other growth factors in the regulation of proliferation [11].

In FRTL-5 cells, PMA alone induced DNA synthesis and also enhanced that induced by insulin or Bt_2cAMP. Conceivably PMA stimulates DNA synthesis at the G_0 to G_1 transition in the cell cycle, since it inhibits the G_1 to S transition in FRTL-5 cells. Thus our results demonstrate that protein kinase C has dual effects on two well-defined transition points in the cell cycle, namely the G_0 to G_1 transition and the G_1 to S transition in FRTL-5 cells.

Inhibition of growth is important in the development of normal tissues and in tumor formation [13]. Therefore, the dual action of protein kinase C at two transition points of FRTL-5 cells is very important for the fine regulation of the complex system involved in cell proliferation.

Protein kinase C has both positive and negative actions that depend on the function of the target substrate protein, in which it phosphorylates the seryl and threonyl residues [7]. The protein product of the ras proto-oncogene is known to be required for initiation of the S phase of the cell cycle in response to stimulation by serum [14]. In addition, the product of the Harvery ras oncogene, p21, is a substrate for protein kinase C in vitro [15]. The inhibitory action of protein kinase C at the G_1 to S transition in the cell cycle may possibly be due to its phosphorylation of the ras proto-oncogene product.

But the mechanisms by which protein kinase C modulates cell proliferation positively and negatively remain to be clarified.

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REFERENCES

[1] Roger, P.P. and Dumont, J.E. (1987) Biochem. Biophys. Res. Commun. 149, 707–711.
[2] Dere, W.H. and Rapoport, B. (1986) Mol. Cell. Endocrinol. 44, 195–199.
[3] Roger, P.O., Servais, P. and Dumont, J.E. (1987) Exp. Cell Res. 172, 282–292.
[4] Laurent, E., Mockel, J., Van Sande, J., Graff, I. and Dumont, J.E. (1987) Mol. Cell. Endocrinol. 52, 273–278.
[5] Philp, N.J. and Grollmann, E.F. (1986) FEBS Lett. 202, 193–196.
[6] Field, J.B., Ealey, P.A., Marshall, N.J. and Cockcroft, S. (1987) Biochem. J. 247, 519–524.
[7] Nishizuka, Y. (1986) Science 233, 305–312.
[8] Doctrow, S.R. and Folkman, J. (1987) J. Cell. Biol. 104, 678–687.
[9] Leach, K.L., Powers, E.A., Mayo, J.K., Abraham, I., Burnett, B.A. and Groppi, V.E. (1987) J. Cell. Physiol. 132, 463–472.
[10] Huang, C.-L. and Ives, H.E. (1987) Nature 329, 849–850.
[11] Kariya, K., Fukumoto, Y., Tsuda, T., Kawahara, Y., Fukuzaki, H., Yamamoto, T. and Takai, Y. (1987) FEBS Lett. 217, 69–73.
[12] Ambesi-Impiombato, F.S., Picone, R. and Coon, H.G. (1980) Proc. Natl. Acad. Sci. USA 77, 3455–3459.
[13] Marx, J.L. (1986) Science 232, 1093–1095.
[14] Mulcahy, L.S., Smith, M.R. and Stacey, D.W. (1985) Nature 313, 241–243.
[15] Jeng, A.Y., Srivastava, S.K., Local, J.C. and Blumberg, P.M. (1987) Biochem. Biophys. Res. Commun. 145, 782–788.