Protein kinase C activators modulate differentiated thyroid function in vitro

Jody Ginsberg and Patricia G. Murray

Department of Medicine, University of Alberta, Edmonton, Alberta T6G 2G3, Canada

Received 30 July 1986

Exposure of porcine thyroid cells to the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) leads to inhibition of differentiated thyroid function. We investigated whether this effect is mediated via protein kinase C activation. TPA, phorbol 12,13-didecanoate and phorbol 12,13-dibutyrate inhibited TSH-stimulated iodine organification in porcine thyroid cells by 98, 96 and 45%, respectively. Non-tumour promoting phorbol esters had no effect. The diacylglycerol analogue, *sn*-1,2-dioctanoylglycerol had similar but quantitatively less activity than TPA. Dibutyryl cAMP could not reverse any inhibition noted. Under conditions that caused significant inhibition of differentiated function, TPA caused translocation of thyroidal protein kinase C from the cytosol to its membrane-bound form. These data provide evidence that the mechanism of phorbol action on thyroid function in vitro includes activation of protein kinase C.

Protein kinase C *Phorbol ester* *Diacylglycerol* *Thyroid function*

1. INTRODUCTION

Tumour-promoting phorbol esters, e.g. 12-*O*-tetradecanoylphorbol 13-acetate (TPA), activate protein kinase C by causing its translocation from the cytosol to an active membrane-bound form [1,2]. This is because of their structural similarity to the endogenous activator, diacylglycerol which is produced following turnover of membrane inositol phosphates [3]. However, TPA has many effects [4], not all of which appear mediated by protein kinase C activation [5–7]. Exposure of thyroid cells to TPA leads to inhibition of differentiated thyroid function [8–10]. The present studies provide evidence that such effects are, at least in part, mediated by protein kinase C.

2. MATERIALS AND METHODS

2.1. *Chemicals*

TPA, phorbol 12,13-dibutyrate (PDBu), phorbol 12,13-didecanoate, 4α-phorbol 12,13-didecanoate, 4β-phorbol, H1 histone type IIIS and dibutyryl cAMP [$(Bu)_2$ cAMP] were obtained from Sigma. *sn*-1,2-Dioctanoylglycerol (DOG) was purchased from Avanti Polar Lipids. [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from New England Nuclear. DEAE cellulose (DE52) was from Whatman. Bovine TSH (Thytropar) was supplied by Armour Pharmaceuticals.

2.2. *Methods*

2.2.1. Iodine organification

TSH-stimulated iodine organification in cultured porcine thyroid cells was based on the method of Plannels et al. [11] as modified by Ginsberg et al. [12]. Briefly, isolated porcine thyroid cells were incubated in serum-free medium with a test substance for 40 min at 37°C prior to washing and plating in Falcon 3047 multiwell plates (10^6 cells per well in 1 ml MEM containing 10% newborn calf serum). The cells were incubated with TSH (10^3 μU) or $(Bu)_2$ cAMP

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

(1 mM) in quadruplicate for 18 h. $Na^{125}I$ (2 μCi) in 10^{-7} M NaI was then added and incubation continued for a further 18 h. The cells were then washed with 1 ml EBSS, extracted with 0.1 M NaOH and protein bound ^{125}I determined following precipitation with trichloroacetic acid.

2.2.2. Protein kinase C extraction

The cells were sonicated in 20 mM Tris-Cl, pH 7.6, containing 2 mM EDTA, 2 mM PMSF, 0.5 mM EGTA, 0.33 M sucrose, pH 7.6 (Tepes buffer), and centrifuged at 115000 × g for 1 h. The supernatant was used as the cytosolic fraction and the pellet was resuspended in Tepes buffer containing 1% NP40. Protein determinations used the method of Bradford [13].

2.2.3. DE52 chromatography

A modification of the procedure of Kraft and Anderson [2] was employed. DE52 was washed and then equilibrated in 20 mM Tris-HCl, pH 7.6, 0.5 mM EGTA, 1 mM PMSF, 2 mM EDTA (buffer A). The DE52 was then poured into a small plastic column (6.5 × 0.7 cm) and washed with 2 ml buffer A. Cytosol or particulate extract in 1% NP40 from 1 × 10^8 cells was applied and 0.1 M NaCl in buffer A was used to elute protein from the column. Each collected fraction was immediately assayed for protein kinase C activity.

2.2.4. Protein kinase C assay

Protein kinase C was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into H1 histone. The standard assay mixture (250 μl) contained 20 mM Tris-HCl, pH 7.6, 50 μg histones, 100 μM ATP (~50 cpm/pmol), 75 mM Mg acetate, 50 μl sample and 5 mM $CaCl_2$, 25 μg phosphatidylserine and 0.8 μg 1,2-diolein. In the negative control samples, the latter 3 reagents were omitted and 0.5 mM EGTA was added. After incubation for 10 min at 30°C, 30% trichloroacetic acid was added to stop the reaction. The protein precipitate was collected on a 0.45 μm membrane filter (Millipore) and the incorporation of ^{32}P was measured by scintillation counting in a Beckman LS 6800. Enzymatic activity was expressed as pmol ^{32}P incorporated into histone/min per mg protein. All assays were performed in quadruplicate.

2.2.5. Statistical analyses

All statistical analyses were performed using a one-way analysis of variance on the Epistat computer program. All experiments were performed on at least 3 separate occasions and a representative experiment of closely-agreeing assays is shown.

3. RESULTS AND DISCUSSION

The effects of TPA (10^{-8}–10^{-6} M) on TSH-stimulated iodine organification in porcine thyroid cells is shown in fig.1. The non-tumour promoting phorbol ester, 4β-phorbol had no effect. The dramatic inhibition of iodine organification in the cells exposed to TPA is similar to that noted by others in cultured thyroid cells [8–10]. To determine if the mechanism of action of TPA could include protein kinase C activation, the ability of phorbol esters with different protein kinase C activating abilities were studied in a similar manner and the results are shown in fig.2. The tumour-promoting phorbol esters TPA, phorbol 12,13-didecanoate and phorbol 12,13-dibutyrate inhibited TSH-stimulated iodine organification by 98, 96 and 45%, respectively. The non-tumour

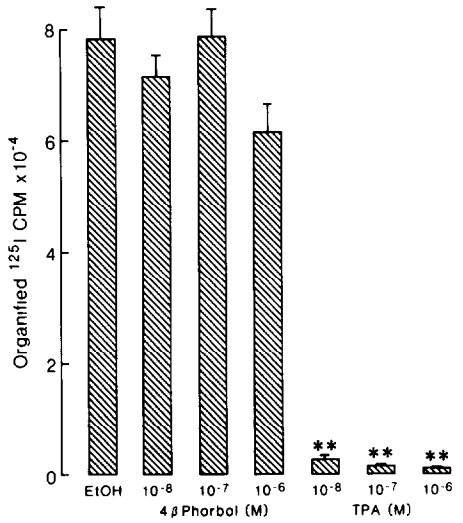

Fig.1. The effects of TPA and 4β-phorbol on TSH-stimulated iodine organification (expressed as organified ^{125}I cpm) in cultured porcine thyroid cells. The phorbol esters were dissolved in absolute ethanol at 2 mM and the final concentrations used are shown, ** $p < 0.001$.

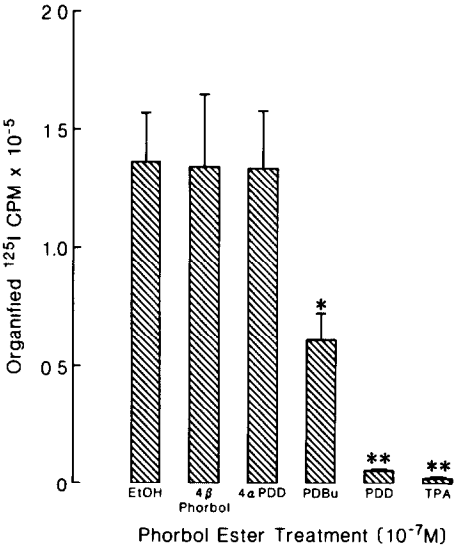

Fig.2. The effects of various phorbol esters on TSH-stimulated iodine organification in cultured porcine thyroid cells. All agents were used at a concentration of 10^{-7} M (final). TPA, 12-*O*-tetradecanoylphorbol 13-acetate; 4αPDD, 4α-phorbol 12,13-didecanoate; PDBu, phorbol 12,13-dibutyrate; PDD, phorbol 12,13-didecanoate; * $p < 0.005$, ** $p < 0.001$.

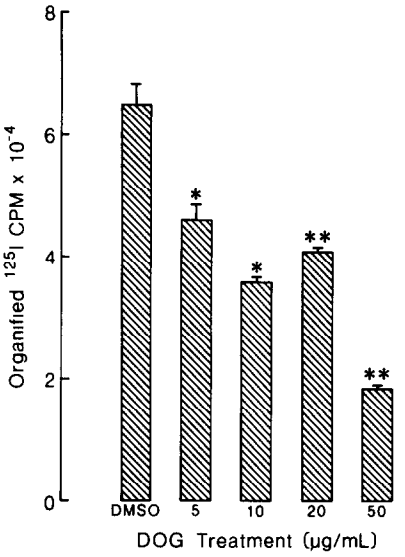

Fig.3. The effect of DOG on TSH-stimulated iodine organification. DOG was stored at −70°C as a 10 mg/ml solution in chloroform. Immediately before use, the chloroform was evaporated and 50 μg/ml solutions were prepared in 1% dimethylsulfoxide (DMSO). Control experiments used 1% DMSO, * $p < 0.005$, ** $p < 0.001$.

promoting phorbol esters, 4β-phorbol and 4α-phorbol 12,13-didecanoate had no effect. The effects of these phorbol esters to modulate differentiated thyroid function in vitro correlates with their reported abilities to activate protein kinase C [14]. The moderate inhibition of iodine organification seen with the potent protein kinase C activator PDBu likely relates to its less hydrophobic properties when compared to TPA. To ensure these effects were not due to a unique property of phorbol esters, thyroid cells were exposed to the non-phorbol diacylglycerol analogue, DOG prior to study. The results are shown in fig.3. Although significant inhibition was also seen with DOG, this agent could not fully mimic TPA action. Similar preliminary results with DOG were noted by Bachrach et al. [8]. This may represent altered access to the cell or different metabolism of DOG. $(Bu)_2$ cAMP could not reverse the inhibition noted following any protein kinase C activator (not shown) implying a post-receptor locus of action for these agents. The principal finding of these studies is shown in table 1. Under identical conditions of TPA exposure that leads to altered TSH-stimulated iodine organification, TPA was capable of causing significant translocation of protein kinase C activity from thyroid cytosol to its active membrane-bound form. Although protein kinase C has been previously described in bovine thyroid [15–17], this is the first study to relate protein

Table 1

Protein kinase C activity in thyroid cell extracts (pmol/min per mg protein)

	Treatment		
	4β-Phorbol	TPA	p
Cytosol	170 ± 5	53 ± 15	$p < 0.001$
Membrane	111 ± 28	466 ± 55	$p < 0.005$
	N.S.	$p < 0.001$	

The results are the mean ± SE of quadruplicate values. Negative controls always showed protein kinase C activity of less than 21 pmol/min per mg protein

kinase C activation to alterations in differentiated thyroid function in vitro. How protein kinase C activation leads to changes in thyroidal metabolism is the subject of ongoing experiments. In conclusion, these studies indicate that phorbol esters modulate differentiated thyroid function in vitro proportionately to their known ability to activate protein kinase C. In the thyroid protein kinase C activation by TPA occurs in conjunction with alterations in differentiated cell function. These data suggest that the action of phorbol esters on thyroid function in vitro is mediated, at least in part, via protein kinase C.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Alberta Heritage Foundation for Medical Research. The authors wish to thank Mrs V. Frazer for excellent secretarial assistance and the staff of Gainer's Inc., Edmonton, for supplying porcine thyroid glands.

REFERENCES

[1] Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[2] Kraft, A.S. and Anderson, W.B. (1983) Nature 30, 621–623.
[3] Nishizuka, Y. (1984) Nature 308, 693–698.
[4] Blumberg, P.M. (1980) Cr. Rev. Tox. 8, 153–197.
[5] Yamamoto, S., Gotoh, H., Aizu, E. and Kato, R. (1985) J. Biol. Chem. 260, 14230–14234.
[6] Trevilyan, J.M., Perisic, O., Traugh, J.A. and Byus, C.V. (1985) J. Biol. Chem. 260, 3041–3044.
[7] Kreutter, D., Caldwell, A.B. and Morin, M.J. (1985) J. Biol. Chem. 260, 5979–5984.
[8] Bachrach, L.K., Eggo, M.C., Mak, W.W. and Burrow, G.N. (1985) Endocrinology 116, 1603–1609.
[9] Roger, P.P., Reuse, S., Servais, P., Van Heuverswyn, B. and Dumont, J.E. (1986) Cancer Res. 46, 898–906.
[10] Haye, B., Aublin, J.L., Champion, S., Lambert, B. and Jacquemin, C. (1985) Biochem. Pharmacol. 34, 3795–3802.
[11] Plannels, R., Fayet, G., Lissitzky, S., Hennen, G. and Closset, J. (1975) FEBS Lett. 53, 87–91.
[12] Ginsberg, J., Shewring, G. and Rees Smith, B. (1983) Clin. Endocrinol. 19, 305–311.
[13] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[14] Kikkawa, U., Takai, Y., Tanaka, Y., Miyake, R. and Nishizuka, Y. (1983) J. Biol. Chem. 258, 11442–11445.
[15] Kasai, K. and Field, J.B. (1982) Biochim. Biophys. Acta 718, 125–134.
[16] Friedman, Y., Poleck, T., Henricks, L. and Burke, G. (1985) Biochem. Biophys. Res. Commun. 130, 971–980.
[17] Tanabe, A., Nielsen, T.B., Sheela Rani, C.S. and Field, J.B. (1985) Arch. Biochem. Biophys. 243, 92–99.