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Protein synthesis in rat cardiac myocytes is stimulated at the level of translation by phorbol esters

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12-*O*-Tetradecanoylphorbol 13-acetate acutely stimulated the rate of protein synthesis maximally by about 43% in terminally differentiated myocytes freshly isolated from adult rat hearts. Stimulation was rapidly expressed (within 20 min). The relative effects of four phorbol esters on protein synthesis was consistent with a specific effect on protein kinase C. Inhibition of transcription with actinomycin D had no effect on the absolute stimulation of the protein synthesis rate by 12-*O*-tetradecanoylphorbol 13-acetate. We conclude that protein kinase C may be involved in the regulation of the translational process.

Protein synthesis; Phorbol ester; Translational control; (Rat heart myocyte)

1. INTRODUCTION

The activity of Ca/phospholipid-dependent PKC is stimulated in vivo by diacylglycerol derived from agonist-stimulated hydrolysis of membrane phosphatidylinositols. The tumour-promoting phorbol esters such as TPA act as diacylglycerol analogues both in vitro and in vivo (review [1]) and have been invaluable in implicating PKC in the regulation of cellular processes. In neonatal rat cardiac myocyte cultures, chronic (>24 h) treatment with TPA resulted in cell hypertrophy but the level at which TPA acted (RNA/protein synthesis or degradation) was not investigated [2]. TPA also increased DNA synthesis in cultures of terminally differentiated cardiac myocytes [3] and enhanced c-*myc* protooncogene expression in neonatal rat heart myocyte cultures [4]. In non-cardiac cell cultures (GH_3 pituitary tumour cells), TPA stimulated protein synthesis at the level of translation initiation. This stimulation was sensitive to the transcriptional inhibitor, actinomycin D [5]. It was suggested that TPA stimulated the synthesis of an mRNA species that encoded an initiation factor whose concentration was rate-limiting. Others have also detected a small and as yet ill-defined stimulation of protein synthesis by TPA in 3T3 fibroblast cultures [6]. Effects of TPA at the level of transcription required pre-exposure of cells to the ester for 2 h or more before maximum stimulation is attained [5]. We have now examined whether phorbol esters have more acute effects on protein synthesis in freshly-isolated terminally differentiated myocytes prepared from adult rat hearts.

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Abbreviations: AM-D, actinomycin D; BSA, bovine serum albumin; DMSO, dimethyl sulphoxide; K-H, Krebs and Henseleit; PDB, phorbol 12,13-dibutyrate; PDD, phorbol 12,13-didecanoate; PKC, protein kinase C; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; TCA, trichloroacetic acid

2. EXPERIMENTAL

Materials were obtained from standard sources [7] unless stated. Myocytes were prepared from hearts of 250–300 g fed male rats. Heart were perfused retrogradely at 37°C with K-H

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buffer [8] containing 0.1% BSA (Sigma fraction V) and a reduced concentration (10 μM) of added Ca. After 6 min, the concentration of added Ca was increased to 50 μM and collagenase (Worthington Type I) was added to a concentration of 1 mg/ml. After 5 min, the softened heart was removed from the cannula and myocytes were liberated by four consecutive incubations each of 5 min at 37°C with low-Ca K-H buffer containing 1 mg/ml collagenase and 2% BSA. No Ca was added to the first incubation medium and the three following incubations each contained 50 μM added Ca. Myocytes from the third and fourth digestions were pooled and washed 3 × in collagenase-free K-H buffer containing 2% BSA and 50 μM added Ca. They were examined by phase-contrast microscopy before use. They were quiescent and the proportion of rod-shaped cells was 75–95%.

Freshly isolated myocytes (0.35–0.55 mg myocyte protein/tube) were preincubated with phorbol esters (diluted with medium from a 1 mM stock solution in DMSO) at 37°C for 20 min in a total volume of 200 μl of low (50 μM) added Ca K-H buffer supplemented with 10 mM glucose and 2% BSA in siliconised glass tubes. In experiments with AM-D (diluted with medium from a stock solution of 5 mg/ml in DMSO), myocytes were preincubated with the inhibitor (5 μg/ml) for 20 min before the preincubation with TPA or insulin. In each experiment, myocytes from a single heart were incubated in quadruplicate with each agent. The mean from quadruplicate incubations was taken as one experimental observation. Measurement of protein synthesis was initiated by the addition of 40 μl of K-H buffer (no Ca added) containing 2.5 mM [U-^{14}C]Phe (spect. act. 4.5 dpm/pmol) and all the other amino acids necessary to support protein synthesis each at a concentration of 1.25 mM. Incubations were performed at 37°C under an atmosphere of 95% O_2:5% CO_2 and myocytes were gently shaken at 10 min intervals. Protein synthesis was terminated at the times indicated by addition of 1 ml of ice-cold 5% TCA. Protein pellets were washed four times with 2 ml of 5% TCA and dissolved in 1 ml NCS (Amersham International) after neutralization with 50 μl of 0.5 M NaOH. Radioactivity incorporated was measured in a toluene-based fluor. Control experiments showed that DMSO had no effect on protein synthesis at the concentration present. In the absence of myocytes, radioactivity was also linearly incorporated into TCA-precipitable material at about 15% of the control rate (results not shown). This blank was subtracted. Protein was measured by the biuret method [9] in a sample of cells washed free of BSA. ATP was measured in TCA supernates by the luciferase method [10]. Statistical significance was assessed by a 2-tailed Student's t test with $P < 0.05$ taken as being significant.

3. RESULTS

The rate of [U-^{14}C]Phe incorporation into protein (fig.1) was linear both under control conditions (rate = 459 pmol/h per mg protein, $r = 0.996$ by regression analysis) and in the presence of 1 μM TPA (661 pmol Phe incorporated/h per mg protein, $r = 0.996$, stimulation = 44%). The linearity

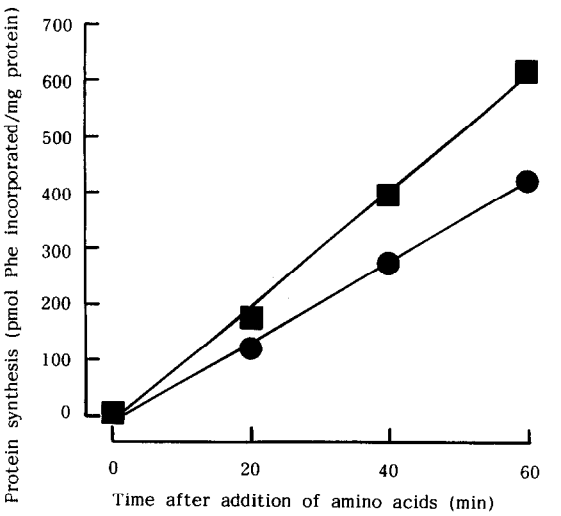

Fig.1. Time course of protein synthesis in myocytes in the absence (●) and presence (■) of 1 μM TPA.

with TPA indicates that the preincubation period was sufficient for it to produce a maximal effect. Results obtained in a second independent experiment were similar. Longer incubation times were not investigated.

The dependence of myocyte protein synthesis stimulation on TPA concentration is shown in fig.2. The upper limit of the TPA concentration was set by the necessity to dissolve it in DMSO. The stimulation of protein synthesis by 1 μM TPA was apparently maximal (fig.2 and other results not shown) and was 43 ± 1% (mean ± SE for 11 independent observations). The stimulation was always less than that induced by maximally effective concentrations of insulin (67 ± 5% at 6.7 nM insulin, mean ± SE for 6 independent observations). The effects of 1 μM TPA and 6.7 nM insulin were not additive (results not shown). The concentration of TPA giving half-maximal stimulation of protein synthesis was 5 ± 1 nM (mean ± SE for 6 independent observations). This value is similar to that reported for the stimulation of purified PKC by TPA [11]. Further experiments investigated the relative potencies of three additional phorbol esters (table 1). There was no stimulation of protein synthesis detectable at phorbol ester concentrations of 0.1 nM (results not shown). The rank order of potency at 10 nM phorbol ester was 4-βPDD ≥ TPA > PDB. The maximal stimulation attained was the same for all three

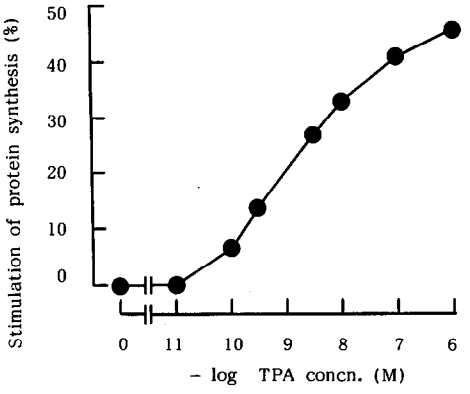

Fig.2. Concentration-dependence of the stimulation of protein synthesis by TPA. Results from a single, representative experiment are shown. Similar results were obtained with 5 other separate preparations of myocytes. Protein synthesis was measured in a 1 h incubation.

phorbol esters. The 4-α stereoisomer of PDD (which does not stimulate PKC [11]) did not stimulate protein synthesis (table 1). None of the phorbol esters at a concentration of 1 μM altered the myocyte ATP content (control: 18 nmol/mg protein) at the end of the incubations (results not shown), indicating that phorbol esters do not adversely affect energy metabolism. These results

Table 1

Relative potencies of phorbol esters in stimulating protein synthesis

	Phorbol ester concentration	Rate of protein synthesis (% control)
TPA	1 nM	103 ± 2
	10 nM	128 ± 1
	1 μM	144 ± 1
4-β PDD	1 nM	109 ± 3
	10 nM	132 ± 3
	1 μM	150 ± 3
PDB	1 nM	101 ± 1
	10 nM	114 ± 4
	1 μM	141 ± 5
4-α PDD	1 nM	n.d.
	10 nM	101 ± 3
	1 μM	103 ± 1

Protein synthesis rates in a 1 h incubation are expressed relative to the rate of protein synthesis in the absence of phorbol ester (497 ± 72 pmol Phe incorporated/h per mg protein, mean ± SE) and are the means ± SE of 3 independent experiments. n.d., not determined

Table 2

Effect of AM-D on the stimulation of myocyte protein synthesis by TPA or insulin

	Rate of protein synthesis (pmol phenylalanine incorporated/h per mg protein)			
	− AM-D		+ AM-D (5 μg/ml)	
	Absolute rate	Increase over control	Absolute rate	Increase over control
Control	337 ± 19	n.a.	214 ± 2[c]	n.a.
TPA (1 μM)	474 ± 22[a]	138 ± 5	336 ± 8[a,c]	122 ± 6
Insulin (6.7 nM)	542 ± 38[a]	205 ± 20	405 ± 19[a,b]	191 ± 19

[a] $P < 0.01$ vs control
[b] $P < 0.05$
[c] $P < 0.01$ vs value in the absence of AM-D

Protein synthesis was measured for 1 h. Results are the means ± SE of three independent experiments. n.a., not applicable

suggest that the stimulation of protein synthesis by phorbol esters specifically involves the activation of myocyte PKC.

The acuteness of the stimulation of protein synthesis by TPA (fig.1) suggests that TPA is stimulating translation rather than transcription and this was confirmed in experiments using AM-D (table 2). AM-D had no effect on myocyte ATP content (results not shown). Although AM-D had no effect on the absolute stimulation of protein synthesis by either TPA or insulin (the latter serves as a control since it exerts its acute effects on protein synthesis in heart and other cells at the level of translation [6,12]), it did reduce protein synthesis by the same absolute amount (about 130 pmol Phe incorporated/h per mg protein equivalent to 35–40% of the control rate) under all experimental conditions (table 2). This result suggests that incubation of cardiac myocytes alone induces synthesis of an mRNA species encoding a factor which stimulates protein synthesis independently of phorbol esters and insulin.

4. DISCUSSION

The site in the translational process which is sensitive to PKC is uncertain. There are several

possibilities. Increased initiation of protein synthesis translation (e.g. by insulin [13]) is often accompanied by an increase in the extent of ribosomal protein S6 phosphorylation (review [14–16]). Insulin may stimulate an S6 kinase activity [17]. S6 is also a substrate for PKC in vitro [15]. In cultured murine T lymphocytes, diacylglycerol stimulates S6 phosphorylation, albeit indirectly [18]. Pretreatment of chick embryo fibroblasts with TPA stimulates S6 kinase activity in lysates prepared subsequently [19]. Thus the effects that we have observed may be related to the S6 phosphorylation state. Other stages of the translational process may be sensitive to PKC. Thus, elongation factor 2 phosphatase activity is enhanced in response to topical application of TPA on mouse epidermis [20]. However, the effect we report here may not involve a direct action of PKC on the translational machinerey. We have recently shown that cardiac protein synthesis is stimulated by an increase in intracellular pH [21]. TPA induces intracellular alkalinization by stimulation of Na^+/H^+ antiporter activity (review [22]). Thus, phorbol esters, by raising intracellular pH, could stimulate protein synthesis. The results described here suggest that phorbol esters should promote cardiac protein accretion (see also [2]).

α_1-Adrenergic agonists stimulate phosphatidylinositol hydrolysis (review [23]). They also stimulate protein synthesis [24] and induce hypertrophy [2,25] in cell cultures of neonatal rat heart myocytes (although it was not established whether the effect was exerted transcriptionally or translationally). Furthermore, these agents enhance c-*myc* protooncogene expression [4] in the same system. Taken together, these findings imply a possible involvement of PKC in the regulation of cardiac myocyte protein synthesis and in the development of some forms of cardiac hypertrophy.

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