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Translocation of protein kinase C in porcine thyroid cells following exposure to thyrotropin

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We have previously shown that protein kinase C activators modulate diferentiated thyroid function in vitro; however, how protein kinase C may be activated physiologically is unknown. The present studies were undertaken in order to determine whether TSH could activate protein kinase C in vitro. Following exposure of porcine thyroid cells to TSH, translocation of protein kinase C from the cytosol to its membrane-bound form was observed. Maximal translocation occurred at the lowest TSH concentration able to trigger this response (10 mU/ml) but persisted at higher concentrations (20–100 mU/ml). Time-course studies revealed that translocation of protein kinase C was seen only after 40 min. TSH could also produce a similar translocation in human neutrophils (known to have TSH receptors). In thyroid cells pre-treated with TSH, modulation of phorbol-mediated protein kinase C translocation was noted. These results indicate that TSH causes the translocation of protein kinase C in porcine thyroid cells (and possibly other TSH receptor-containing cells) and therefore may regulate the action of protein kinase C on differentiated thyroid function.

Protein kinase C; Thyrotropin; Phorbol ester; (Thyroid cell)

1. INTRODUCTION

A signal transduction system involving receptor-mediated phospholipid turnover, diacylglycerol accumulation and activation of a Ca^{2+}- and phospholipid-dependent enzyme, protein kinase C, plays a role in many cellular responses [1]. Recently, evidence which implies that protein kinase C may be involved in the regulation of differentiated thyroid function has emerged. Tumor-promoting phorbol esters which are known activators of protein kinase C [1,2] have been shown to modulate differentiated thyroid function in vitro [3–6]. We have demonstrated that under identical conditions, the phorbol ester, 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) can cause the translocation of protein kinase C from the cytosol to its membrane-bound form in thyroid cell extracts [6]. However, how protein kinase C may be activated in the thyroid remains unknown. Thyroid hormone synthesis is normally stimulated by the pituitary hormone, thyrotropin (TSH) via a receptor-linked cyclic AMP-responsive system [7]. Recently, TSH has been shown to stimulate inositol 1,4,5-trisphosphate (IP_3) generation in human thyroid slices independently of adenylate cyclase stimulation [8]. The present studies were to determine whether TSH could activate protein kinase C in vitro as demonstrated by its subcellular redistribution in porcine thyroid cells.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

TPA, ATP, H1 histone type IIIS, 1,2-diolein, phosphatidylserine, collagenase (type IA), PMSF and Nonidet P40 were obtained from Sigma. [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from New England Nuclear. DEAE-cellulose (DE52) was from Whatman. Bovine TSH (Thytropar) was supplied by Armour Pharmaceuticals.

2.2. *Methods*

2.2.1. Isolation of porcine thyroid cells

Porcine thyroid cells were isolated using a discontinuous trypsinization process that has been previously described [9]. Cells were incubated with TSH in serum-free medium for 40 min (unless otherwise stated) at 37°C prior to washing, resuspension and assessment of protein kinase C activity.

2.2.2. Protein kinase C extraction

Protein kinase C was measured as described [6]. Briefly, 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 were according to Lowry et al. [10].

2.2.3. DE52 chromatography

A modification of the procedure of Kraft and Anderson [11] 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 histone H1. 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. Protein kinase C activation in human neutrophils

Isolated human neutrophils were incubated with TSH (100 mU/ml) or TPA (100 nM) for 40 min at 37°C prior to assessment of protein kinase C. The measurement of protein kinase C activation in human neutrophils has been previously reported by the present authors [12]. All assays were performed in duplicate.

2.2.6. Statistical analyses

All statistical analyses were performed using a one-way analysis of variance on the Epistat computer program.

3. RESULTS AND DISCUSSION

The ability of TSH to induce translocation of protein kinase C is illustrated in fig.1. At 10 mU/ml TSH cytosolic protein kinase C decreased by 27% and membrane-bound protein kinase C increased by 63%. The difference is likely due to recruitment of protein kinase C that was previously loosely bound to the plasma membrane. Persistent translocation of protein kinase C was observed at higher TSH concentrations but this was noted to be no greater than that seen at 10 mU/ml. At lower concentrations of TSH, no redistribution of protein kinase C was noted (not shown). These findings are similar to those of Tanabe et al. [13] who studied the effect of TSH on protein kinase C in bovine thyroid slices; however, in their studies, cytosolic and membrane extracts were exposed to different TSH concentrations. The concentration of TSH required to cause protein kinase C translocation is high yet similar to

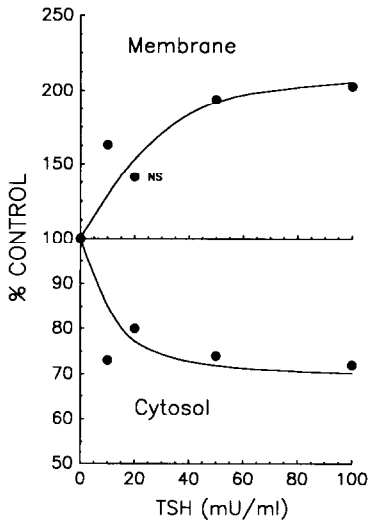

Fig.1. Effect of TSH on the translocation of protein kinase C in porcine thyroid cell extracts. Each point represents the mean of 7 separate experiments. All values are statistically significant compared to controls with a minimum p value of <0.02 except where noted.

that noted to produce IP_3 generation in human thyroid slices [8]. Although it is conceivable that our TSH preparation could contain a protein kinase C activator other than TSH, IP_3 generation in human thyroid slices was produced by the same type of TSH preparation as used in this study with similar results when purified bovine or human TSH was used [8].

Table 1

Time course of TSH-stimulated protein kinase C translocation in porcine thyroid cell extracts

Time (min)	PKC activity (pmol ^{32}P/min per mg protein)	
	Cytosol	Membrane
TSH (100 mU/ml)		
0	771 ± 48	427 ± 46
1	765 ± 45	441 ± 34
5	744 ± 13	384 ± 18
15	745 ± 44	491 ± 88
40	679 ± 16[b]	628 ± 70[a]
TPA (10^{-7} M)		
40	631 ± 8[a]	640 ± 59[a]

[a] $p < 0.001$ vs control
[b] $p < 0.002$ vs control

TPA was included as a positive control. Data shown are representative of 2 closely agreeing experiments

Table 2

Effects of TSH on the translocation of protein kinase C in human neutrophils

	PKC activity (pmol ^{32}P/min per mg protein)	
	Cytosol (%)	Membrane (%)
Control	359 –	56.5 –
TSH (100 mU/ml)	187 (58.8)	64.6 (114)
TPA (100 nM)	211 (52.1)	69.0 (122)

Data shown are representative of 2 closely agreeing experiments

Time-course studies revealed in table 1 showed that the redistribution of protein kinase C occurred only after 40 min. A similar prolonged time course was observed for IP_3 generation by TSH [8]. To determine whether the effect of TSH was organ-specific, the ability of TSH to cause translocation of PKC in human neutrophils was assessed and the results are shown in table 2. Surprisingly, TSH produced changes in the redistribution of protein kinase C similar to those induced by TPA. Previous reports have demonstrated TSH receptors in human neutrophils [14] and further studies to determine whether TSH can activate PKC in other non-thyroidal tissues containing TSH receptors are in progress.

To determine whether TSH and phorbol esters activate protein kinase C via a similar mechanism,

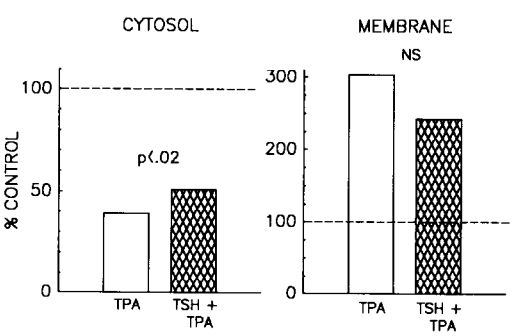

Fig.2. Effect of TSH pre-exposure on protein kinase C translocation induced by TPA in porcine thyroid cell extracts. The results shown have been pooled from 5 separate experiments. No effect was seen if TSH was added after TPA (not shown).

porcine thyroid cells were pre-incubated with TSH (100 mU/ml, 37°C, 40 min), resuspended and exposed to TPA (100 nM, 37°C, 40 min) prior to protein kinase C assay. The results are shown in fig.2. In control cells pre-treated with buffer, TPA caused a significant ($p < 0.001$) translocation of protein kinase C (open bars) as previously described [6]. However, in cells pre-treated with TSH, modulation of the phorbol-mediated translocation of protein kinase C was noted. In the cytosolic component, less protein kinase C was mobilized (hatched bar, $p < 0.02$) compared to cells treated with TPA only. A corresponding change was evident in the membrane component which just failed to reach statistical significance. These findings suggest that TSH and TPA act on similar pools of cellular protein kinase C. It is interesting to note that concanavalin A which can bind TSH prevents the phorbol-mediated redistribution of protein kinase C in rat glioma C6 cells [15].

The mechanism by which thyroidal protein kinase C is activated is the subject of ongoing investigation. Although previous studies have shown that TSH promotes ^{32}P incorporation into phosphatidylinositol [16,17], TSH stimulation of inositol phosphate generation has not been demonstrated in dog [18], calf [19] or ovine thyroid [20] but has been recently observed in human thyroid [8]. In porcine thyroid tissue, TSH transiently stimulated diacylglycerol formation with concomitant changes in PI [21]. Whether protein kinase C is activated in porcine thyroid cells via this mechanism remains to be elucidated.

In summary, our studies have demonstrated that TSH causes the translocation of protein kinase C in porcine thyroid cell extracts. These results provide direct evidence for the existence of a TSH-dependent signal transduction system involving protein kinase C activation in porcine thyroid. The role of this process in the regulation of thyroid function and growth remains to be explored.

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