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EFFECT OF THYROTROPIN ON PHOSPHOLIPID COMPOSITION IN THYROID PLASMA MEMBRANES

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Differences particular in phospholipid levels from pig thyroid membranes incubated in the presence of TSH were observed, but with exception of lysophosphatidylcholine, were not statistically significant. TSH evoked about a twice increase of lysophosphatidylcholine content. A slight decrease of phosphatidylcholine, phosphatidylinositol and phosphatidylserine were found, when at the same time a slight increase of phosphatidylethanolamine and sphingomyelin level was noted. There were no statistical differences in range fluctuation of individual phospholipid levels, but direction of fluctuation (i.e. decrease or increase) were identical for every single phospholipid in all experiments. An increase of lysophosphatidylcholine and a simultaneously decrease of phosphatidylcholine contents suggests the stimulation of phospholipase A_2 activity.

Key words: *phospholipids, thyrotropin, thyroid gland, plasma membranes*

INTRODUCTION

Phospholipids are constituents of cell and are localized predominantly in te membrane. In addition to their structural role, phospholipids are involved in the regulation of membrane enzymes and take part in signal transduction (1, 2, 3). Althought most of the effects of TSH on the thyroid are mediated via activation of the adenylate cyclase-cyclic AMP system, existence of another signalling system involving inositol phospholipids was demonstrated (4, 5). In 1985, the frist evidence that hormones promoted phosphatidylcholine hydrolysis was presented (6). Changes in cellular lipid metabolism are initiated by receptor activation of various phospholipases including phospholipase A_2, phospholipase C and phospholipase D (7). The present experiments were undertaken to examine effect of TSH on phospholipid composition in the membrane of pig thyroid gland.

MATERIAL AND METHODS

All reagents were of highest analytical purity. The solvents were pured by redistillation. The reference compounds: sphingomyelin S 7004 Lot 107F-8356, phosphatidic acid P 9511 Lot 65F-8371, phosphatidylcholine P 5763 Lot 82F-8360, phosphatidylethanolamine P 6387 Lot 122F-8340, lysophosphatidylcholine L 4129 Lot 127F-8420, phosphatidylinositol P 0639 Lot 108F-8416, phosphatidylserine P 7769 and thyrotropic hormone T2026 Lot 58F-0041 (TSH) were obtained from Sigma Chemical Co.,

Preparation of plasma membranes

The pig thyroid glands were obtained from te slaughterhouse immediately after killing the animals. After the removal of connective tissue and fat, the plasma membranes were prepared according to the method described by Ozawa et al. (8) with slight modifications. The finely chopped thyroid glands were homogenized in 10 mM Tri-HCl buffer, pH 7.5 (1 g slices in 9 ml buffer) in a Potter homogenizer. The homogenate was centrifuged at $1,500 \times g$ for 6 min. The sediment was discarded. The supernatant was centrifuged at $15,000 \times g$ for 15 min. The pellet was washed with 10 mM Tris-HCl buffer, pH 7.0 containing 10 mM K_2HPO_4, 0.5 mM $CaCl_2$, 0.9 mM $MgSO_4$, 2 mM ethyleneglycol-bis-(β-aminoethylether) N,N,N′,N′-tetraacetic acid (EGTA) and 110 mM KCl, centrifuged again and suspended in the same buffer. The pellet obtained from 100 g of thyroid was suspended in 24 ml of buffer.

Incubation of plasma membranes with TSH

1 ml membranes suspension and 1 ml of 10 mM Tris-HCl buffer, pH 7.0 was incubated at 37°C for 1 h in the absence or presence of TSH (300 mU/ml). The reaction was stopped by adding 18 ml of mixture methanol-chloroform-1 N HCl (10:5:3, v/v) and the membranes were homogenized.

Extraction and analysis of phospholipids

After homogenization the membrane suspension was transferred to the stoppered tubes. Five ml of chloroform which were used to wash the homogenizer and 4 ml of 2 M KCl were added. Then the mixture was throughly shaken and centrifuged to separate two phases. The lower chloroform phase transferred to separate tube and aqueous phase was washed with 5 ml of chloroform and centrifuged. Chloroform lower phases were collected, mixed with equal volume of 0.1 N HCl, shaking briefly and centrifuged. After centrifugation chloroform phase was neutralized with NH_3 steam and evaporated to dryness. The dried residues was dissolved in 500 µl of benzene-ethanol (4:1, v/v) and stored at −20° until use.

The phospholipids were separated by one-dimensional thin-layer chromatographic method described by Brockmann and Gercken (9), Visualization of lipid spots carried by exposing the plates to iodine vapours. Phospholipids were identified on TLC plates by comparison with standars. Spots of phospholipids were scraped from plates and lipid phosphorus was estimated according to Bartlett (10) after digestion with 72% perchloric acid for 1.5 h at 165°C.

Statistical analysis were performed using Student's t-test.

RESULTS AND DISCUSION

For the determination of TSH effect on phospholipid composition the thyroid membranes were incubated with increasing doses of TSH. The changes in percent phospholipid composition in the presence of TSH at concentration of 150 mU/ml were negligible. More evident effect of TSH was observed at a concentration of 300 mU/ml.

At this concentration an increase of lysophosphatidylcholine (LPC) content and simultaneously a decrease of phosphatidylcholine (PC) was observed *(Table 1)*. Differences in particular phospholipids were noted, but with exception of LPC were not statistically significant. TSH evoked about

Table 1. Effect of TSH on phospholipid composition in pig thyroid membranes. The values are expressed in mol per 100 mol of phosphorus recovered.

Phospholipid	TSH concentration		
	—	—	300 mU/ml
	Incubation time (h)		
	0	1	1
lysophosphatidylcholine LPC	0.7±0.3	0.8±0.3	1.9±0.4*
phosphatidylcholine PC	52.2±4.5	51.6±4.0	48.2±4.2
sphingomyelin SPH	10.8±2.1	10.9±2.0	12.2±1.8
phosphatidylinositol phosphatidylserine PI+PS	11.2±2.2	11.0±2.5	9.4±2.3
phosphatidylethanolamine PE	19.6±3.0	20.1±2.8	21.4±3.2
Others	5.5±1.5	5.6±1.5	5.9±1.4
Total lipid phosphorus µg/g of tissue	218±31.6	197±40.5	205±41.8

*$p < 0.001$ compared to values without TSH

twice increase of LPC content. A slight decrease of phosphatidylcholine phosphatidylinositol (PI) and phosphatidylserine (PS) were found, when at the same time slight increase of phosphatidylethanolamine (PE) and

sphingomyelin(SPH) levels were noted. However, there were no statistical differences in range fluctuation levels but direction of fluctuation levels (i.e. decrease or increase) were identical for every single phospholipid in all experiments.

The experiments described in this report show that TSH causes insignificant changes in phospholipid composition of thyroid membranes with exception of LPC. An increase of LPC and a simultaneously decrease of PC contents suggests stimulation of phospholipase A_2 activity. Because PC is the principal phospholipid in thyroid (about 50% of all phospholipids), its hydrolysis by phospholipase A_2 may be important source of arachidonic acid which is subsequently metabolised to a variety of functionally significant eicosanoides. Phospholipase A_2 hydrolyses several phospholipids including PI, PC and PE to liberate arachidonic acid and the respective lysophospholipids. De Wolf et al (11) demonstrated that in bovine thyroid phospholipase A_2 activity was found mainly in homogenate-sediment, and PC was hydrolised to much greater extend than PE. Hayet et al. (12, 13) observed the direct stimulation of phospholipase A_2 activity in thyroid homogenate by TSH and an increase of free arachidonic acid and prostaglandins in pig thyroid slices. Two enzymatic pathways may be involved in the mobilization of arachidonic acid from membrane lipids: phospholipase A_2 pathway, and sequential action of phospholipase C and digliceride lipase. Arachidonic acid may be liberated indirectly in the phosphoinositide cycle. It was shown that TSH stimulates PI turnover and hydrolysis of inositol phospholipids in isolated thyroid cells and slices (4, 5). In this study a small decrease of PI in the presence of TSH was observed. A decrease of PI content may result both from phospholipase A_2 and phospholipase C activities stimulated by TSH.

Beguinot et al. (14) reported that TSH-dependent effects on FRTL-5 cells (cultured rat cell line) was associated with marked perturbation of membrane physical properties and lipid composition. The effect of TSH correlated with a decrease of PC and an increase of PE, a process which has been linked to change in cell membrane fluidity. In this work we observed similar efect of TSH on major phospholipids contents.

The changes in phospholipids composition were noticed at relatively high doses of TSH, i.e. 300 mU/ml. Similar high amount of TSH was required for stimulation of inositol lipid hydrolysis and was approximately 3 orders of magnitute greater than that necessary for augmentation of cyclic AMP (4). Corda et al. (15) observed that TSH elevated the intracellular Ca^{2+}concentration in the rat thyroid cells (FRTL-5). This effect like that on inositol phospholipid hydrolysis, required much larger amount of TSH that needed for cAMP accumulation. It is very likely that the increase in Ca^{2+} in these cells is a function of TSH- stimulated inositol trisphosphate production. The level of Ca^{2+} may be important for Ca^{2+}- dependent phospholipases. In most tissues,

agonist stimulation of inositol phospholipids can be detected very rapidly, while the effect of TSH on hydrolysis is considerably slower (4). The reason for this difference is not clear. The discrepancy in the amount of TSH required for inositol phospholipid hydrolysis compared with cAMP formation could reflect involvement of two different receptors (4, 5).

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