Thyrotropin and norepinephrine stimulate the metabolism of phosphoinositides in FRTL-5 thyroid cells

Nancy J. Philp and Evelyn F. Grollman*

Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and **Laboratory of Biochemistry and Metabolism, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA*

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Hormone-induced changes in phospholipid metabolism were examined in a functioning rat thyroid cell line (FRTL-5). Stimulation of FRTL-5 cells, prelabeled with ^{32}P, with TSH or NE resulted in a rapid decrease in the radioactivity of both phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylinositol 4-monophosphate (PIP). The effects of TSH on phospholipid metabolism and calcium mobilization are independent of those on adenylate cyclase. This suggests that the TSH receptor may be unique in that it activates enzyme cascades involved in cAMP production and Ca^{2+} mobilization.

Thyrotropin *Phosphoinositide* *Inositol phosphate* *Ca^{2+} mobilization* *Phospholipid* *(Thyroid cell)*

1. INTRODUCTION

Regulation of FRTL-5 cells, a continuous line of rat thyroid cells, involves Ca^{2+} as well as cAMP second messengers [1–5]. With respect to the former, we have shown that TSH and NE increase IP_3 formation from phosphoinositides [6], cytosolic Ca^{2+} levels [5] and arachidonic acid levels [7,8]. We have shown further that these events are preludes to TSH and NE stimulation of iodide efflux and the iodination of thyroglobulin [7,9].

The formation of IP_3 has been linked to the hydrolysis of PIP_2 with the formation of diacylglycerol and IP_3 [10,11]. The present report shows that TSH and NE induce the hydrolysis of PIP_2 and PIP in accord with their ability to increase IP_3. This occurs at very early times, consistent with other studies of ligand-mediated phosphoinositide/IP_3/Ca^{2+} signals. The data thus remain consistent with the evidence that TSH and NE modulation of the phospholipid/Ca^{2+} signal system is an important aspect of regulation of thyroid hormone formation.

* To whom correspondence should be addressed

Abbreviations: NE, norepinephrine; TSH, thyroid-stimulating hormone; IBMX, 3-isobutyl-1-methylxanthine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate

2. MATERIALS AND METHODS

The FRTL-5 cells (ATC CRL no.8305) used here were grown on 100 mm dishes (Costar, Cambridge, MA) in Coon's modified Ham's F-12 medium supplemented with 5% calf serum (Grand Island Biological, Grand Island, NY), 1 mM nonessential amino acids (Microbiological Associates, Bethesda, MD), and a mixture of 6 hormones or growth factors (TSH, insulin, cortisol, transferrin, glycyl-L-histidyl-L-lysine acetate, and somatostatin), as described [1,12]. Cells were maintained in an atmosphere of 5% CO_2-95% air at 37°C and the culture medium

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changed every 3–4 days. Cells were used in these studies when they reached 70–80% confluency. In some experiments the medium was switched for 5–7 days to medium supplemented with hormone mixture lacking TSH.

To obtain cell suspensions, after aspirating the medium the cells were incubated in Ca^{2+}-Mg^{2+}-free Hanks' balanced salt solution containing 5 mM EGTA. The cells were incubated for about 20 min in a shaking bath at 37°C and the cells then suspended by gently pipetting. The cells were collected by centrifugation at 500 × *g*, washed and resuspended at a density of 1×10^6 cells/ml in low phosphate medium (10 μM PO_4) containing 10 mM Hepes buffer (pH 7.4) at 37°C. Cells were stabilized in this medium for 15 min before adding $^{32}P_i$ (10 μCi/ml). The time necessary to achieve an isotopic steady state in the inositol-containing phospholipids was determined. Thereafter the cells were routinely labeled for 60 min before the addition of the agonists. At various times after the addition of the agonists a portion of the cell suspension (0.6 ml) was quenched in 2.25 ml chloroform:methanol:HCl (6 N) (100:200:2) and extracted as described by Bligh and Dyer [13] and modified by Lapetina and Michell [14]. Polyphosphoinositides were separated by thin-layer chromatography on silica G plates using a solvent system composed of chloroform:methanol:ammonium hydroxide:H_2O (45:45:3.5:10) [15]. PA, PI, PC, and PE were separated on silica G plates using the two-dimensional solvent system described by Yavin and Sutra [16]. Lipids were visualized by iodine staining and radioautography. The spots corresponding to the lipids of interest were scraped into scintillation vials and 2 ml H_2O added. The radioactivity was quantified from Cerenkov radiation in a liquid scintillation counter. The data are expressed as a percentage of control values so experiments done on different days could be compared. Experiments were repeated at least 3 times with duplicates done within each experiment. The means ± SE are given.

The TSH used in these studies was the same as in [1,3] and had a specific activity of 26 ± 3 IU/mg in the McKenzie assay [17]. Crude TSH used in the tissue culture medium was obtained from Armour (Chicago, IL) and had a specific activity of about 0.3 IU/mg protein. Norepinephrine was purchased from Sigma (St. Louis, MO). [^{32}P]Orthophosphoric acid (carrier-free in 0.02 N HCl) was purchased from ICN (Irvine, CA). All other chemicals were obtained from commercial sources and the highest purity available.

3. RESULTS

Incorporation of $^{32}P_i$ radioactivity into PIP, PIP_2 and PA is very rapid in FRTL-5 cells grown in the presence of TSH (fig.1). Isotopic equilibrium occurs within 1.5 h. In the absence of TSH, incorporation of $^{32}P_i$ is reached at the same time, but the $^{32}P_i$ incorporation attained is 25–40% of that in cells grown in the presence of TSH.

Previous studies have shown that TSH increases incorporation of ^{32}P into PI and PA [18–20]. Here, we show that TSH causes a similar increase in FRTL-5 cells (fig.2). In this experiment, the cells were maintained without TSH for 6 days to increase sensitivity to TSH acutely. 1 h after readdition of TSH, labeling of PI was increased by 200% and PA by 138% over that of unstimulated cells. The effect of NE on PI was more dramatic (fig.2), increasing PI labeling by 360% and PA 130%. There was no change in PC from control values but both TSH and NE increased labeling of PE 110

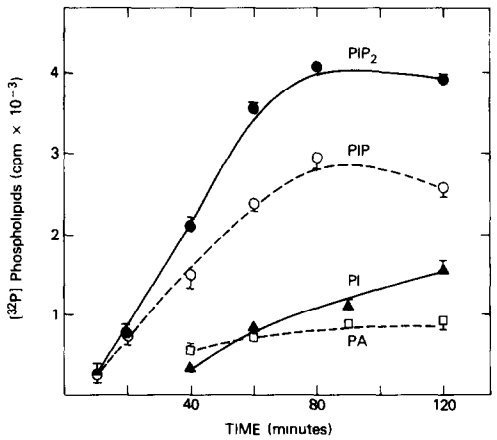

Fig.1. Time of incorporation of $^{32}P_i$ into various phospholipids. $^{32}P_i$ (10 μCi/ml) was added to FRTL-5 cells at time zero, and the incorporation into PI (▲), PA (□), PIP (○), and PIP_2 (●) determined as described in section 2 at the times indicated. Each point is the value of the mean ± SE of a minimum of 3 separate experiments.

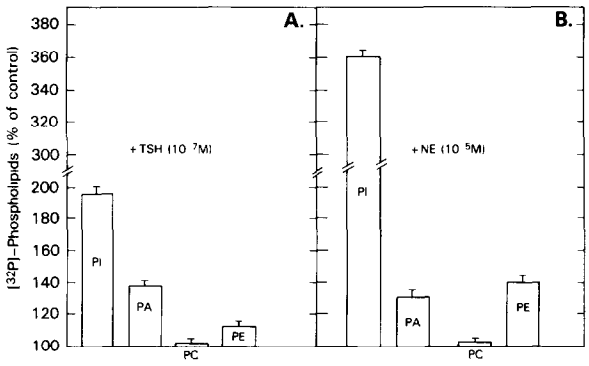

Fig.2. Effect of TSH and NE on $^{32}P_i$ incorporation into various phospholipids. $^{32}P_i$ (10 μCi/ml) was added for 180 min to FRTL-5 cells that had been maintained in medium without TSH for 6 days. TSH or NE at the indicated final concentrations was added and the incubations continued for 60 min. $^{32}P_i$ incorporated into PI, PA, PC and PE was determined as described in section 2. The mean ± SE is given. Experiments were repeated at least 2 times.

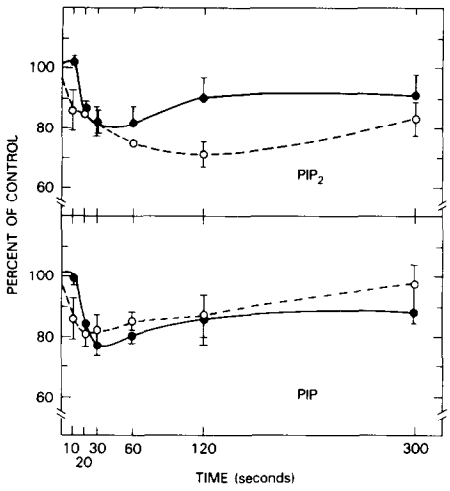

Fig.3. Effect of TSH and NE on $^{32}P_i$ incorporation in polyphosphoinositides. $^{32}P_i$ was added to cells for 1 h prior to the addition of 10^{-7} M TSH or 10^{-5} M NE. The incorporation of $^{32}P_i$ into PIP and PIP_2 were determined as described in section 2 at the indicated times after addition of TSH (●) or NE (○). The control value was obtained just prior to TSH or NE addition. The data are expressed as a percentage of control values so that experiments done on different days could be compared. Results are the means ± SE of at least 5 separate experiments.

and 140% over control values, respectively. The addition of 8-bromo cyclic AMP and the phosphodiesterase inhibitor IBMX did not affect lipid labeling in parallel experiments (not shown).

As shown in fig.3, NE stimulation of FRTL-5 cells grown in the presence of TSH resulted in a rapid decrease in the amount of ^{32}P radioactivity found in PIP and PIP_2. The maximum decrease is seen after about 1 min with a gradual return to baseline levels. The NE effect was an α-adrenergic response since it was unaffected by propanolol but blocked by equimolar concentrations of phentolamine. TSH, when used at high concentrations (1×10^{-7} M), also stimulated breakdown of PIP_2 and PIP. The maximum decrease in these phospholipids was at about 30 s (fig.3). Agonists which increase intracellular levels of cAMP in these cells [3] (cholera toxin, Graves IgG) did not alter PIP_2 or PIP labeling (nor did 8-bromo cAMP, dibutyryl cAMP, or IBMX). When the cells were labeled to equilibrium in the absence of TSH, the effect of 1×10^{-7} M TSH on [^{32}P]PIP_2 and [^{32}P]PIP levels was similar to that shown for TSH in fig.3. The maximum decrease was at 1 min; for PIP_2 the mean ± SE ($n = 6$) was 76.4 ± 9% of control and for PIP 76.1 ± 7%. The levels of PIP_2 and PIP returned to prestimulation values at 5 min.

4. DISCUSSION

In this report we show that exposure of FRTL-5 cells to either TSH or NE causes an increase in phospholipid metabolism as measured by an increase incorporation of $^{32}P_i$ into PA and PI. The results establish that the enhanced turnover of inositol-containing phospholipids observed in thyroid tissues both in vivo and in vitro is preserved in this functional cell line. In addition we show that NE and TSH produce rapid breakdown of PIP_2 and PIP which can be measured within seconds. It is not surprising that NE stimulates breakdown of polyphosphoinositides since α-adrenergic receptor activation has been associated with increase cytosolic calcium due to the breakdown of PIP_2 in other systems [11]. The TSH concentration needed to effect a change in phospholipid metabolism is high (1×10^{-7}) compared to that needed to increase cAMP levels [1]. These results are however consistent with studies

showing high concentrations of TSH are necessary to increase IP_3 levels, Ca^{2+} levels, arachidonic acid release, iodide efflux, and iodination of thyroglobulin [4,5–8]. The early effect of TSH and NE on PIP_2 and PIP breakdown is consistent with Ca^{2+}/PI turnover as an important biological signal for iodide efflux [4] thyroglobulin iodination [9], and activation of thyroid peroxidase [21].

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