THYROTROPIN RELEASING HORMONE STIMULATES METABOLISM OF PHOSPHATIDYL INOSITOL IN GH_3 CELLS

A possible mechanism in stimulus–response coupling

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1. Introduction

In a wide variety of cell types, stimulation through surface receptors by various ligands (peptide hormones, catecholamines, neurotransmitters) of metabolic or secretory activity is accompanied by an increased metabolism of phosphatidylinositol (PI), a membrane phospholipid with specific functions [1,2]. Following hormonal stimulation there is an acceleration of the breakdown of PI to diacylglycerol and inositol phosphate followed by the rapid resynthesis of PI and this change in PI metabolism has been termed the 'PI response' [1]. If cells are pre-labelled with [^{32}P]phosphate a PI response is manifest through an increase of ^{32}P-labelling of PI due to the resynthesis from radioactive ATP [2].

In all the cases where a PI response can be observed, Ca^{2+} is involved in stimulus–response coupling [2]. Therefore the increased turnover of this phospholipid may account for the increase of Ca^{2+} permeability of the plasma membrane [2]. In clonal pituitary cell strains, i.e., GH-cells, the hypothalamic peptide thyrotropin releasing hormone (TRH) stimulates the acute release of both prolactin (PRL) and growth hormone (GH); in addition, PRL synthesis is increased whereas GH synthesis is reduced in the same cells [3]. GH cells thus provide an attractive model system to study molecular mechanisms involved in the action of TRH. It has been shown that the TRH stimulation of PRL release depends on extracellular Ca^{2+} [3,4]. Ca^{2+} influx into GH_3 cells brought about by depolarization of the plasma membrane with high potassium, or by Ca^{2+}-ionophores mimics a TRH response [4]. Electrophysiological experiments show an increase in calcium action potentials following TRH stimulation [5–7]. The mechanisms by which TRH modifies intracellular [Ca^{2+}] are not known yet. We have therefore investigated whether a PI response can be implied in stimulus–response coupling for TRH in GH_3 cells.

Abbreviations: PI, phosphatidylinositol; TRH, thyrotropin releasing hormone; PRL, prolactin; GH, growth hormone

2. Materials and methods

GH_3 cells were obtained from the American-type Culture Collection, repository number CCL 82.1, batch F 1685 or from Dr U. Eppenberger, Basel. Culture media, trypsin, and sera were from GIBCO, culture flasks and dishes from Falcon or Sterilin, carrier free [^{32}P]phosphate was from Amersham. Thin-layer chromatography plates were from Merck (silica gel 60 precoated glass plates, layer thickness 0.5 mm). Purified hormones, reference preparations and antibodies for the radioimmunoassays for rat PRL were obtained from Dr Parlow through the hormone distribution program of NIAMDD. Pure lipids for reference in the thin-layer chromatography were from Supelco. TRH (1-*N*-[5-oxo-L-prolyl)-L-histidyl]-L-prolinamide) was from Roche.

2.1. *Cell culture*

GH_3 cells were grown in Ham F-10 supplemented with 15% horse serum and 2.5% fetal calf serum [3].

2.2. ^{32}*P-Incorporation into phospholipids*

Culture medium was withdrawn and replaced by Ham F-10 containing carrier free [^{32}P]orthophosphoric acid (2–2.5 μCi/ml). Incubations were carried out in

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a humidified atmosphere of 5% CO_2 and 95% air at 37°C and incubation as well as washing solutions were preincubated for a minimum of 30 min prior to use. TRH and/or KCl were added in a small volume at 50–100-fold the final dilution. Incubations were stopped by withdrawal of the incubation medium, and cells were washed twice with 2 ml Ham F-10. Then 4 ml ice-cold Ham F-10 was added and the culture flasks were chilled on ice. Cells were detached by rinsing the culture flask with a pasteur pipette, collected by centrifugation (500 × *g* for 10 min), frozen and conserved at −70°C prior to extraction. This method of detachment which avoids the use of trypsin or chelators yields 85–95% of the cell protein.

Extraction of phospholipids and isolation of PI by thin-layer chromatography was carried out according to [8] with PI added as a carrier prior to extraction. Two-dimensional thin-layer chromatography, yielded values for ^{32}P-incorporation into PI that were within 15% (range) of the values obtained with the standard one-dimensional analysis.

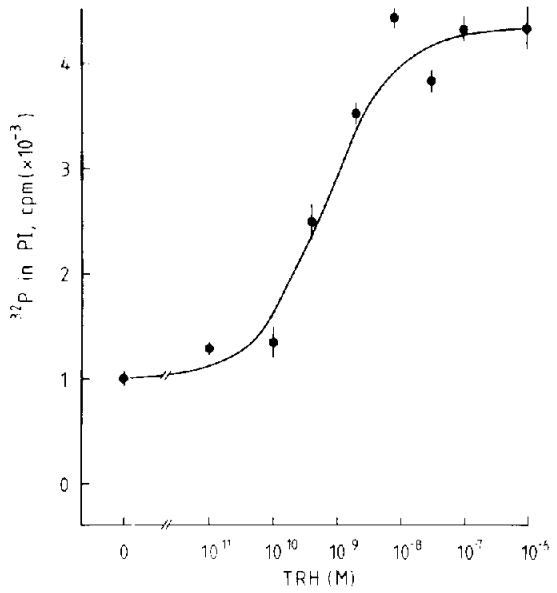

Fig.1. Dose-response curve for TRH activation of [^{32}P]phosphate incorporation into phosphatidylinositol (PI). GH_3 cells were preincubated for 120 min with [^{32}P]phosphate (2.3 μCi/ml) and then exposed to increasing concentrations of TRH for 60 min. ^{32}P-Incorporation into PI was determined as in section 2 and is shown as the radioactivity in PI/culture flask (mean of triplicate flasks ± SEM). Each flask contained 3.1 × 10^6 cells (960 μg protein). ^{32}P-Incorporation into the lipid extract was 3.2 × 10^3 cpm/flask for control and 8.0 × 10^3 cpm/flask for 10^{-7} M TRH.

3. Results

3.1. *TRH dose–response curve*

Incorporation of [^{32}P]phosphate into PI of GH_3 cells is stimulated by TRH in a dose-dependent manner (fig.1). When GH_3 cells which have been preincubated in the presence of [^{32}P]phosphate are exposed to increasing concentrations of TRH, ^{32}P-labelling of PI increases to a maximum of 4-times the control levels. A significant increase is seen at 0.4 nM TRH, the effect is saturable and the concentration of TRH to produce half-maximal stimulation is 0.8 nM. By repeating the experiment shown in fig.1 with GH_3 cells from the 2 different sources the extent of stimulation ranged from 3.8–4.5-fold whereas the minimum concentration of TRH to give a significant increase was unchanged.

3.2. *Time course*

As fig.2 shows, the increase in ^{32}P incorporation into PI due to TRH is most pronounced during the first 30 min. Significant effects of the hormone can be seen as early as 10 min after addition (table 1). At >30 min the rate of TRH stimulated ^{32}P-incorporation levels off, but it remains higher than the control rate.

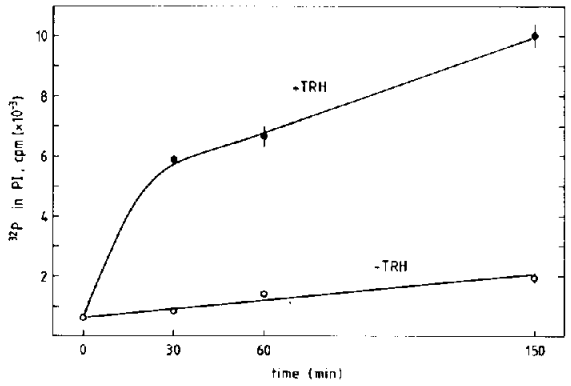

Fig.2. Time course of incorporation of [^{32}P]phosphate into phosphatidylinositol. GH_3 cells were preincubated in Ham F 10 with 2.3 μCi/ml of [^{32}P]phosphate for 90 min. At time zero TRH, 10^{-7} M (closed symbols) or vehicle (open symbols) was added and the incubation was continued for the time indicated. Detachment of cells, extraction and chromatography were as in section 2. Shown are the means (± SEM) of cpm ^{32}P/flask incorporated into PI from triplicate incubations. Each flask contained 2.3 × 10^6 cells (corresponding to 920 μg cell protein). Radioactivity of the lipid extract increased from 2.3 × 10^3 cpm/flask at time zero to 4.1 × 10^3 cpm/flask after 60 min for control (−TRH) *vs* 1.2 × 10^4 cpm/flask for TRH-stimulated cells.

Table 1
Effects of KCl *vs* TRH on [^{32}P]phosphate incorporation into PI (cpm ^{32}P/culture flask, mean ± SEM, $n = 6$)

Condition	Incubation time	
	10 min	20 min
Basal	803 ± 21	1199 ± 95
KCl (50 mM)	751 ± 76	1012 ± 76
TRH (10^{-7} M)	1697 ± 136[a]	3237 ± 262[a]

[a] Significantly different from basal condition, $p < 1\%$

GH_3 cells (8.9×10^5 cells/culture flask) were preincubated with [^{32}P]phosphate (2.5 μCi/ml) for 90 min; TRH, KCl or vehicle was added and the incubation continued for 10 or 20 min after which the cells were harvested, extracted and ^{32}P-incorporation into PI was determined as in section 2

3.3. *Ca^{2+} independence*

The results in table 1 show that when cells, pre-labelled with [^{32}P]phosphate, are exposed to depolarizing concentrations of K^+ in the presence of Ca^{2+} there is no change in ^{32}P incorporation into PI. In the same experiment TRH causes a significant increase in PI labelling already after 10 min. It is known that K^+ is able to stimulate PRL release in GH_3 cells to the same extent as TRH by causing a Ca^{2+} influx [4] and we have repeated this finding with the cells used in our experiments (not shown). The data in table 1 therefore demonstrate that changes in intracellular [Ca^{2+}] that are sufficient to mimic a TRH response do not affect PI metabolism.

4. Discussion

Acceleration of the metabolism of PI in GH_3 cells following stimulation by TRH could be one of the important mechanisms in the mediation of the secretory and/or other responses of GH_3 cells to TRH. The sensitivity of the PI response to TRH stimulation in GH_3 cells is compatible with physiological conditions (fig.1) and, following stimulation by the releasing hormone a significant increase in PI labelling can be observed as early (table 1) as the increase in the release of PRL and GH [4]. It is known that the PI reponse occurs always in connection with an obligatory role for Ca^{2+} for stimulus–response coupling [2], however, the exact function for an increased turnover of PI is barely known. If the role of the PI response was to mediate the calcium influx, it should be independent of Ca^{2+}. For the TRH stimulation in GH_3 cells this is the case. PI metabolism is not changed by an influx of Ca^{2+}, induced by depolarization of plasma membrane with high K^+. In addition the PI labelling is unaffected by isobutylmethylxanthine (an inhibitor of cAMP phosphodiesterase) at concentrations that stimulate PRL release (not shown). Therefore the PI response is neither a consequence of increased intracellular Ca^{2+} nor increased cAMP levels. This suggests the postulate that it is an early event in stimulus–response coupling for TRH. Finally, a PI response to TRH seems to be generally observable since we could repeat all of our experiments with very similar results with GH_3 strains from the two different sources and there is recent preliminary evidence of a TRH effect on phospholipid turnover in GH_4C_1 cells [9]. However it remains to be seen whether and how TRH affects phospholipid and in particular PI metabolism in normal non-transformed pituicytes.

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