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STRUCTURAL CHANGES CAUSED BY THYROTROPIN IN THYROID CELLS AND IN LIPOSOMES CONTAINING RECONSTITUTED THYROTROPIN RECEPTOR

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SUMMARY Thyrotropin causes a rapid and significant increase in the fluorescence polarization of DPH† when this hydrophobic probe is incorporated into a strain of functioning rat thyroid cells (FRTL5). This increase is ligand-specific and is not related to cAMP production. The phenomenon seems to reflect the interaction of thyrotropin with the glycoprotein component of its membrane receptor, as suggested by experiments in which thyrotropin causes increases in DPH fluorescence polarization in liposomes embedded with this receptor component but not with gangliosides. A strain of nonfunctioning rat thyroid cells (FRT), exhibiting no reactivity with monoclonal antibodies to the glycoprotein component of the thyrotropin receptor, requires two orders of magnitude higher concentrations of thyrotropin to exhibit a comparable phenomenon.

It has been proposed that lateral motion of membrane proteins may not be governed solely by the viscosity of the lipid bilayer, that such motion can be important in the expression of a cellular response, and that receptor-mediated perturbations of membrane fluidity are a means whereby cellular response might be initiated and regulated (1-3). Thyrotropin causes receptor-mediated, but cAMP-independent, perturbations of membrane potentials in thyroid cells (4), as well as altered phospholipid metabolism (5, 6). Studies using 6-carboxylfluorescein have indicated that TSH can induce bilayer changes

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†Abbreviations: 1,6-diphenyl-1,3,5-hexatriene, DPH; cyclic adenosine-3',5'-mannophosphate, cAMP; thyrotropin, TSH; human chorionic gonadotropin, hCG; follitropin, FSH; cholera toxin, CT; Fisher rat thyroid cells, strain L5, FRTL5; Fisher rat thyroid (nonfunctioning strain), FRT; Hank's balanced salt solution, HBSS; N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, HEPES.

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in liposomes embedded with crude soluble preparations of the TSH receptor (7). These findings raised the possibility that receptor-mediated modifications of the physical state of the plasma membrane might exist in vivo and be a means of regulating non-cAMP-mediated cellular responses. As a first approximation of the validity of this possibility, we have studied the fluorescence properties of the hydrophobic probe DPH incorporated into thyroid cells as well as into liposomes embedded with components of the TSH receptor.

MATERIALS AND METHODS

Bovine TSH was a purified preparation with an activity of 25 ± 3 IU/mg (8). hCG was purchased from Sigma; CT was from Calbiochem-Behring. DPH (Sigma) was dissolved in distilled tetrahydrofuran at 11 mM and stored in the dark at room temperature before dilution and use in individual experiments.

The cell lines used in this study are clones of epithelial cells derived from normal Fisher rat thyroids (9). The FRT line lacks differentiated thyroid functions, such as thyroglobulin biosynthesis and iodide trapping, and grows in Coon's modified Ham's F-12 medium supplemented with 5% calf serum. The FRTL5 line maintains differentiated thyroid functions at variance with the FRT line. To grow, it requires the addition in the culture medium of a mixture of 6 hormones and growth factors (6H medium) including TSH (8, 9).

Fluorescence measurements using DPH were performed as follows. Cells were grown to confluence in 10-cm petri dishes and then switched to 5H medium or maintained in 6H medium as noted. Cells were collected by adding 4 mM EGTA to the culture dish for 45 minutes, followed by gentle scraping with a rubber policeman. Cells were washed 3 times by centrifugation at 1,000 rpm and suspended in HBSS containing 10 mM HEPES at pH 7.2. Cell number was measured using a Coulter counter. DPH was freshly prepared for each experiment by adding appropriate amounts of the tetrahydrofuran solution to 20 mM Tris-acetate buffer, pH 7.4, while the solution was vigorously stirred. Stirring was continued for 30 minutes under a flow of N_2 to allow evaporation of tetrahydrofuran. Cell suspensions and DPH dispersion were combined to obtain 10^6 cells/ml and a concentration of 10^{-6} M DPH. After 30 minutes at 37°C, the cells were washed in HBSS-HEPES buffer and finally suspended in the same buffer at a density of 10^5 cells/ml. Samples of 1.5 ml of this suspension were used for measurements of fluorescence and fluorescence polarization in a Perkin Elmer MPF 44A fluorescence spectrophotometer equipped with a thermostated cell holder, a polarization attachment, and a built-in sample stirrer. Polarization measurements and calculations were performed according to well-established procedures [for review, see (10)]. Controls included additions of buffer prepared from a tetrahydrofuran solution containing no DPH, but identically manipulated.

Phosphatidylcholine-cholesterol liposomes containing either mixed brain gangliosides (ICN Nutritional Biochemicals) or a lithium diiodosalicylate-solubilized preparation of the glycoprotein component of thyroid TSH receptor were prepared as previously described (11, 12). Labeling of liposomes with DPH was carried out following the same procedure as for the cells.

The intracellular cAMP content was measured using a radioimmunoassay according to a procedure detailed previously (13).

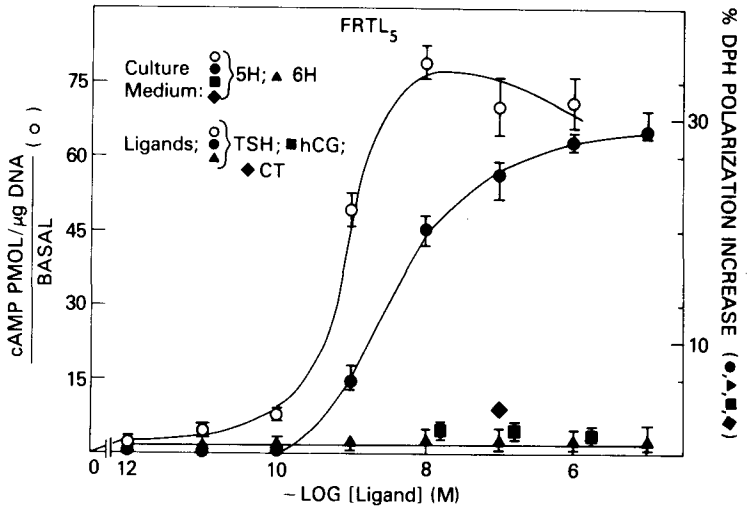

FIG. 1. The effect of various concentrations of TSH on the fluorescence polarization of DPH incorporated into FRTL5 cells maintained in 6H complete medium (▲) or in the 5H medium lacking TSH (●). The significant effect of TSH on 5H cells is compared with an absence of an effect in the same cells by (■) hCG or (◆) cholera toxin (CT). The 5H cells were maintained in 5H medium for 7 days prior to these experiments; analogous to previous studies (18), they are viable if returned to 6H media at that time. Individual aliquots of cells were prepared as detailed in Materials and Methods; each ligand was added at the noted concentration, and the fluorescence measurements were performed after 2 minutes at 37°C under continuous stirring. The figure compares the effect of TSH on the accumulation of cAMP (O) in the 5H FRTL5 cells as previously detailed (18). Cholera toxin at 10^{-10} M and 10^{-9} M increases cAMP production 30- and 50-fold, respectively, over the basal values under the same conditions. Each data point is the average of replicate values in at least 3 different experiments.

RESULTS

The fluorescence polarization of DPH incorporated into functioning thyroid cells of the strain FRTL5 increases upon the addition of TSH in a concentration-dependent manner between 10^{-9} M and 10^{-5} M (Fig. 1). To observe this phenomenon, however, it is necessary that the FRTL5 cells be maintained without TSH in the culture medium (5H medium) for a few days (7 days in the experiments reported in Fig. 1). The same cells kept in a medium containing TSH (6H medium) do not show any fluorescence polarization changes when challenged with identical concentrations of TSH. The polarization changes occur within 1 or 2 minutes from the addition of the hormone and remain stable for at least 30 minutes at 37°C. The effect of TSH is specific since it cannot be duplicated by a number of other substances, such as bovine serum albumin, insulin, glucagon, bovine growth hormone and human placental lactogen (data

not shown), cholera toxin, and hCG (Fig. 1); the latter is a glycoprotein hormone which is structurally related to TSH.

The concentration of TSH necessary to measure a change in DPH fluorescence polarization differs substantially from that necessary to cause cAMP accumulations but is compatible with concentrations of TSH which affect two non-cAMP-mediated FRTL5 cells' activities, such as growth (5×10^{-10} M to 5×10^{-9} M) and iodide efflux (10^{-9} M) [(8, 9) and Weiss, S. J., Philp, N. J., and Grollman, E. F., submitted to Endocrinology]. Thus a cAMP increase is measurable at 10^{-12} M TSH, whereas fluorescence polarization changes become meaingful at 10^{-9} M TSH, and above 10^{-8} M the TSH effect on cAMP decreases, whereas DPH fluorescence polarization is only slightly above its half maximal value.

Additional support for the specificity of the TSH effect on fluorescence polarization as well as for the absence of a direct relationship between TSH effect on fluorescence polarization and stimulation of cAMP accumulation evolved from the following studies. Cholera toxin which has no effect on FRTL5 cell DPH fluorescence polarization at 10^{-9} M through 10^{-7} M does, in contrast, increase cAMP levels in these cells at these concentrations (Fig. 1). "Nonfunctioning" FRT cells, with a defective glycoprotein component of the TSH receptor, as measured by monoclonal antibodies (14), are insensitive to TSH in cAMP assays (Fig. 2) and do not require TSH as an obligatory growth factor. As can be seen in Fig. 2, TSH effects on DPH fluorescence polarization are shifted to higher TSH concentrations by 2 orders of magnitude. FRT cells are, in contrast, extremely sensitive to cholera toxin as evidenced by the ability to increase significantly cAMP production over the basal level (Fig. 2), the increase being half maximal between 10^{-11} M and 10^{-10} M. The toxin does not, however, cause appreciable changes of DPH fluorescence polarization at concentrations which maximally stimulate cAMP production (Fig. 2).

The effect of TSH on DPH fluorescence polarization in thyroid cells can be reproduced, although at higher hormone concentration, in phosphatidylcholine-cholesterol liposomes in which the glycoprotein component of the TSH

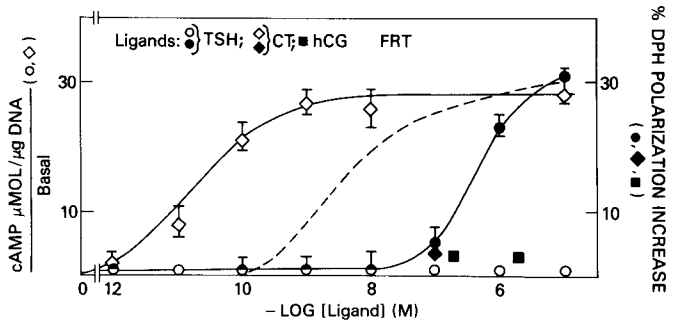

FIG. 2. The effect of various concentrations of TSH on the fluorescence polarization of DPH incorporated into FRT cells (●) is compared to the effect in the same cells by (■) hCG or (◆) cholera toxin (CT). Experimental details are in Fig. 1 and in Materials and Methods. FRT cells are not sensitive to TSH in the cAMP assay (○), whereas they are extremely sensitive to (◇) CT. The dashed line reproduces the TSH effect on DPH fluorescence polarization in FRTL5 cells in 5H as depicted in Fig. 1. Each data point is the average of replicate samples from at least 3 different experiments.

receptor of bovine thyroid plasma membranes has been incorporated (11) (Table I). Receptorless liposomes, i.e., liposomes which do not contain components of the TSH receptor, do not undergo the same changes when challenged with the hormone. Liposomes containing gangliosides, which bind TSH (12), exhibit

TABLE I. *Effect of various substances on the fluorescence polarization of DPH incorporated into liposomes. T = 37°C.*

	Fluorescence polarization of liposomes containing:					
Challenge with	Gangliosides		Glycoprotein receptor component		Neither component (control)	
	degrees of polarization					
Buffer	0.330	(± 0.005)	0.260	(± 0.003)	0.229	(± 0.003)
10^{-7} M bovine serum albumin	0.328	(± 0.005)	0.262	(± 0.005)	0.228	(± 0.003)
10^{-7} M hCG	0.331	(± 0.006)	0.260	(± 0.008)	0.228	(± 0.003)
10^{-7} M TSH	0.280 *	(± 0.004)	0.283 *	(± 0.006)	0.222	(± 0.004)

Dipalmitoyl-phosphatidylcholine-cholesterol liposomes were prepared as detailed previously (22, 23) and contained either mixed brain gangliosides in 0.1 molar ratio to phosphatidylcholine or the glycoprotein component of the TSH receptor in a 2 : 1 weight ratio to phosphatidylcholine. Control liposomes contained dicethylphosphate in a 0.1 molar ratio to phosphatidylcholine. In each experiment 10^6 particles were suspended in 1.5 ml of HBSS containing 10 mM HEPES at pH 7.2.

*Changes in polarization are significant within each liposome group ($p < 0.01$) compared to liposomes challenged with buffer, bovine serum albumin, or hCG.

changes of DPH fluorescence polarization which are opposite to those which occur in FRTL5 cells or in liposomes containing the glycoprotein component of the TSH receptor (Table I).

DISCUSSION

The TSH-specific effect on the fluorescence polarization of DPH incorporated in FRTL5 cells is comparable to the rapid action of insulin in studies of liver plasma membranes (15) rather than the FSH effect on ovarian granulosa cells (16) which occurs after several hours. The effect involves the ability of TSH to interact with the glycoprotein component of its receptor, since FRT cells with no reactivity toward monoclonal antibodies to the glycoprotein component of the TSH receptor (14) require a much higher concentration of the hormone to exhibit comparable changes of DPH fluorescence polarization and since the effect can be duplicated in liposomes in which the glycoprotein component of the TSH receptor is reconstituted. The phenomenon does not depend on cAMP production, as shown by the following: (i) Changes in DPH fluorescence polarization and cAMP accumulation in FRTL5 cells exhibit different dependence on TSH concentration. (ii) Cholera toxin, which stimulates cAMP production in FRTL5 cells, has no significant effect on DPH fluorescence polarization. (iii) Cholera toxin, which is a potent cAMP stimulator in FRT cells, does not induce comparable fluorescence polarization changes in FRT cells. (iv) Liposomes embedded with the glycoprotein component of the TSH receptor do not possess TSH-sensitive adenylate cyclase, yet display an increase in fluorescence polarization when challenged with TSH.

Although the relationship between DPH fluorescence polarization and fluidity of plasma membranes has been disputed (3, 17), changes of DPH fluorescence polarization do reflect perturbation of the molecules surrounding the probe (18). The present data indicate that the interaction of TSH with the glycoprotein component of its receptor can account for such perturbation "in vivo" in a physiologically relevant situation.

Since TSH effects on DPH fluorescence polarization in FRTL5 cells cannot be measured in cells grown in the presence of TSH, a link to the mechanism of

desensitization is suggested, i.e., the mechanism whereby cells no longer respond biologically to an acute challenge with TSH (8).

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