EFFECTS OF LONG-ACTING THYROID STIMULATOR ON THE REORGANIZATION INTO FOLLICLES OF ISOLATED THYROID CELLS AND ON THE BINDING OF RADIOIODINATED THYROTROPIN TO REASSOCIATED CELLS

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1. Introduction

Evidence has been accumulated that long-acting thyroid stimulator (LATS) is an immunoglobulin G having the property to reproduce most of the metabolic and morphologic effects elicited by thyrotropin (TSH) on thyroid tissue [1–4]. Several recent data support the concept that both LATS and TSH exert their stimulatory actions by activating the adenyl cyclase–cyclic AMP system, suggesting a common receptor site on the plasma membrane of the thyroid follicular cells [5]. Previous reports have shown that TSH induces aggregation and reorganization into follicles of isolated porcine thyroid cells via the adenylate cyclase–cyclic AMP system [6–8]. In view of the aforementioned similarity between TSH and LATS the present investigations were undertaken to ascertain whether such a morphogenetic effect could be reproduced by LATS. In addition, the effect of LATS on the binding of radioiodinated TSH by cultured porcine thyroid cells as described recently [9] was investigated.

2. Materials and methods

LATS-positive sera were obtained from patients with active Graves' disease. The potencies of the seven samples used in these experiments ranged from 55 to 401 MRC mU/ml, as assessed in the mouse bioassay by the use of the LATS MRC Research Standard B. In two different assays, 18.5 and 27.1 MRC mU, respectively, were found to be equivalent to 1 Kriss Unit [10]. LATS-negative sera were drawn from 15 patients with various thyroid disorders, including 5 with toxic adenoma, 4 with idiopathic myxedema, 4 with goitrous or non-goitrous congenital hypothyroidism, and 2 with Hashimoto's thyroiditis. Serum TSH levels, determined by radioimmunoassay, were undetectable in all thyrotoxic subjects, moderately elevated in those with Hashimoto's disease and greatly increased (up to 680 μU/ml) in both hypothyroid groups. Pooled human thyroid tissue from surgical specimens was used for neutralization of LATS, as previously described [11]. Briefly, the sediment separated from the whole thyroid homogenate by centrifugation for 1 hr at 105,000 *g*, was washed twice and then added to LATS serum. In the study reported here, appropriate experimental conditions were used to provide a concentration of 0.32 mg of tissue protein per MRC mU. After incubation for 1 hr at 37° and for 12 hr at 4°, the insoluble material was removed by centrifugation. The resulting supernatant was tested for residual LATS activity and then used for further experiments. LATS serum incubated in the absence of thyroid tissue was used as control.

Isolated cells were obtained by trypsinization of porcine thyroid glands as previously described [12] and seeded in 25 cm^2 Falcon plastic flasks at a concentration of 3×10^6 cells per ml of Eagle's minimum essential medium at pH 7.4 containing 20% calf serum, penicillin (200 U/ml) and streptomycin sulphate (50 μg/ml), or in Falcon Microtest II tissue culture plates at a concentration of 7.5×10^5 cells per ml of the same medium (1.5×10^5 cells in 0.2 ml medium per cupule). Both flasks and plates were incubated at 35° in 95% air—5% CO_2. Reorganization of cells into follicles was observed by light microscopy (× 100) as previously described [6,7]. Control TSH- or dibutyryl cyclic AMP-induced reorganized cultures were obtained by the addition of 40 mU RSH/ml or 0.4 mM dibutyryl cyclic AMP at the onset of culturing. Biologically active porcine [^{125}I]TSH was prepared by Dr. P. Jaquet using the lactoperoxidase method of iodide oxidation.

3. Results and discussion

3.1. Effect on aggregation and organization of thyroid cells

Each of the seven LATS-positive sera tested in this study proved to be able to induce an aggregation of isolated porcine thyroid cells followed by rearrangement into histiotypic follicular structures. The morphology and the time course of these changes were similar to those observed in parallel experiments with porcine TSH. Cell agrregation and reassociation into follicles could be usually detected by the second day of culture and lasted for 3 to 6 days in the case of LATS-stimulated cells and 5 to 7 days in the case of TSH-stimulated cells. The minimum concentrations of LATS-serum producing a positive response in Falcon bottles [6] or in Microtest plates as described in [13], i.e., the reorganization of cells into follicles, ranged from 1 to 20 μl/ml, respectively. No effect on cell reassociation was observed with either normal human serum or any of the 15 LATS-negative sera, tested in concentrations of 50 μl/ml. The fact that several sera eliciting no response had elevated TSH levels should not be regarded as surprising, since the maximal concentration of thyrotropin which could be provided by their addition to the cell culture was ≤ 34 μU/ml. Previous studies with porcine THS showed that at least 60 μU/ml were required for a posiive effect on cell reassociation.

From the results listed in table 1, it is apparent that the activities of the different LATS sera observed in the present system, expressed in terms of minimal effective concentrations, showed no satisfactory correlation with the LATS activities measured on the same specimens by the mouse bioassay, expressed in terms of MRC mU. A possible explanation for this discrepancy may reside in the different sensitivity of the two assay systems, involving the use of separate animal species, i.e., porcine and murine, respectively. Recent evidence indicates that the sera from patients with Graves' disease may contain variable amounts of thyroid-stimulating immunoglobins which are effective on human, but not on murine thyroid tissue

Table 1

Activities of different specimens of LATS serum on the reassociation of isolated porcine thyroid cells and the levels of LATS as assessed by the mouse bioassay

Specimen no.	LATS level (MRC mU/ml)	Minimal concentration producing cell-reassociation (μl/ml of medium)
1	401	20
2	351	10
3	234 (136)*	10 (20)*
4	217	10
5	167	1
6	162	2
7	55	20

* Values observed after incubation of LATS serum with human thyroid sediment (0.32 mg of tissue protein per MRC mU).

[14,15]. Thus, it is conceivable that the relative concentration of human immunoglobins cross-reacting with either porcine or murine thyroid cells, or both, may vary in different serum specimens. Consistent with this interpretation is the apparent parallelism of the two assay systems observed in an individual sample after partial neutralization of LATS by human thyroid tissue. Preincubation of LATS serum No. 3 with appropriate amounts of human thyroid sediment led to a 58 ± 12% loss of activity in the mouse bioassay, and increased from 10 to 20 μl/ml the minimal concentration required for reassociation of isolated porcine thyroid cells.

Preliminary experiments showed that LATS-induced reassociated cells were able to take up and organify iodide after two days of culture as shown previously for TSH-stimulated cells [7].

3.2. Effect on binding of [^{125}I]TSH to reassociated thyroid cells

Recent experiments [9] showed that one-day old unstimulated cells or dibutyryl cyclic AMP-induced reassociated cells cultured for 2 to 4 days were able to bind specifically radioiodinated TSH with the same K_a. Cells cultured in both conditions exhibited the same number of sites per cell. Cell bound radioactivity could be displaced by increasing amounts of native TSH, complete displacement being observed with an excess of the latter. A dose-related inhibition of [^{125}I]TSH binding to dibutyryl cyclic AMP-reassociated cells was also observed when LATS-serum rather than native TSH was added to the system (fig. 1). In the presence of excess LATS serum 64 ± 9% (mean ±S.D.) inhibition was observed whereas, under similar conditions, an excess of native TSH produced at least 90% inhibition. Among other possibilities, the difference in animal species between the target cells and radioiodinated TSH on the one side and the LATS specimen on the other, may account for the failure to obtain complete displacement with an excess of LATS serum.

The present data provide further support to the concept that LATS and TSH might compete for the same or closely related binding sites on the plasma membrane of the thyroid epithelial cell. Indirect evidence favoring this hypothesis have previously been reported by Burke [16] who demonstrated that preincubation of thyroid microsomes with TSH limited

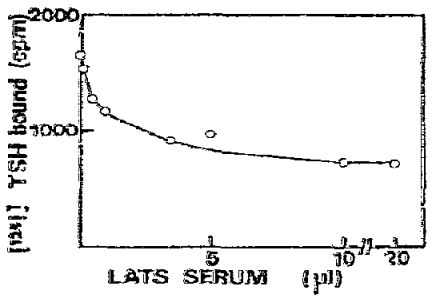

Fig. 1. Effect of LATS on the binding of [^{125}I]TSH to cultured isolated thyroid cells. Cells (2.8×10^5) were incubated in 0.5 ml HEPES-medium E [9] for 15 min at 35° with 4.3 ng [^{125}I]TSH (specific activity: 2.5 Ci/μmole) and increasing amounts of LATS serum no. 4. Cells were washed twice with the same medium as described in [9] and radioactivity counted. In the presence of 10 μg of native porcine TSH, 220 cpm remained bound to cells. Typical experiment on three. Controls including normal human serum and LATS-negative sera from hyperthyroid patients were unable to displace [^{125}I]TSH.

their ability to neutralize LATS. From a study of the combined effects of TSH and LATS on iodide trapping by isolated thyroid cells, he also showed [17] that the thyroidal sites of action of both stimulators might be similar or identical. However, Yamashida and Field [18] recently reported that LATS inhibited non-competitively the TSH-induced stimulation of adenylate cyclase in thyroid plasma membranes. They interpreted this finding as suggesting that LATS might act on the latter by changing their conformation rather than binding to a specific receptor site. The present data do not allow to exclude this possibility.

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