THYROID-STIMULATING HORMONE BINDING TO CULTURED THYROID CELLS

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1. Introduction

The suggestion that thyroid stimulating hormone (TSH) interacts with its target tissue at a superficial cell site was made by Pastan et al. [1] on the basis of experiments showing the persistent effect of TSH on [1-^{14}C]glucose oxidation in thyroid slices exposed to the hormone and washed thoroughly in hormone-free medium. Additional support to this idea was given by Yamashita and Field [2] and Wolff and Jones [3] who showed that plasma membranes purified from thyroid gland contained a TSH-sensitive adenylcyclase. Previous investigations demonstrated that TSH induced the reorganization into follicles of cultured isolated thyroid cells (Fayet and Tixier [4], Fayet et al. [5]), via the adenylcyclase-cyclic AMP system (Fayet and Lissitzky [6], Lissitzky et al. [7]) whereas monolayer cultures were obtained in its absence. TSH- or dibutyryl cyclic AMP-stimulated cells showed the organizational [8] and the specific metabolic properties of the gland follicular cells [7].

This communication reports the direct measurement of specific TSH-receptor interaction in this system and some of the properties of the binding sites.

2. Materials and methods

2.1. *Thyroid cells*

Isolated thyroid cells were obtained by trypsinization of porcine thyroid glands as previously described [4, 5]. Freshly isolated cells were seeded at a concentration of 3×10^6 cells per ml of Eagle minimum essential medium pH 7.4 containing 20% calf serum, penicillin (200 U/ml) and streptomycin sulfate (50 μg/ml) (medium E) in 25 or 75 cm^2 Falcon plastic bottles and incubated at 35° in air or in 95% air–5% CO_2. Reorganization of cells into follicles was obtained by the addition of 0.4 mM dibutyryl cyclic AMP or 40 mU TSH/ml at the onset of culturing.

2.2. *TSH*

Porcine TSH was obtained from a side fraction during the purification of LH [9]. The TSH preparation was extensively purified by the method of Liao et al. [10], TSH-α and -β were prepared according to Liao and Pierce [11]. Preparation of LH and its subunits have been described previously [9]. The thyrostimulating activity as determined by the McKenzie bioassay [12] was 37, 0.9 and < 0.01 IU/mg for TSH,

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TSH-α and TSH-β, respectively; that of LH, LH-α and LH-β was less than 0.01 IU/mg in all cases. In all calculations, a M.W. of 28 000 was taken for TSH.

2.3. *[^{125}I]TSH*

TSH labelling with ^{125}I was obtained according to Thorell and Johansson [13] using lactoperoxidase and H_2O_2 as the oxidizing agent. The labelled hormone contained 1.5 to 2.0 iodine atoms/mole with a specific radioactivity of 1.7 to 2.5 Ci/μmole and gave a single peak in SDS–polyacrylamide gel electrophoresis. About 70% of the labelled hormone reacted with an excess of anti-TSH antibodies. Such [^{125}I]TSH preparations contained less than 3% contaminating iodide and retained full biological activity as shown by their potency to induce the reorganization into follicles of isolated thyroid cells. The techniques used for biological activity measurements [14], TSH labelling and properties of the labelled hormone [15] will be described in full elsewhere.

2.4. *Binding studies*

At given time intervals of culture, cells were obtained by a 10 min incubation at 35° with 3 mM EGTA in Earle's salt solution free of Ca^{2+} and Mg^{2+} followed by gentle scraping of the cell layer with a rubber policeman. No difference has been detected in the binding properties of cell suspensions obtained either by scraping alone or by the latter treatment. Cells were recovered by centrifugation at 200 *g* for 5 min and washed twice in cold medium E without calf serum. Cell concentration was adjusted to $4–8 \times 10^6$ cells/ml of medium E buffered with HEPES at pH 7.4. Incubations were performed in 1 × 7 cm polyethylene tubes with shaking at 35° for 15 min. Tubes contained in 0.5 ml of HEPES-medium E, $1–2 \times 10^6$ cells, 10 nM KI, [^{125}I]TSH and other additives when required. At the end of the incubation period, 3.5 ml of cold HEPES-medium E were quickly added and the tubes were centrifuged at 1500 *g* for a total period of 1 min between start and stop of the rotor. The supernatant was aspirated and the cell pellet was washed once with the same medium. The overall time of the procedure was 2.5 min. After addition of 1 ml water, the radioactivity of the cell pellet was estimated in a Packard Autogamma spectrometer with a counting efficiency of 25%.

Specific binding was obtained by subtracting from radioactivity bound the amount which was not displaced by an excess of native TSH (see table 1). Controls of [^{125}I]TSH adsorption on tubes were performed in the same conditions omitting cells. It never exceeded 0.1% of the total radioactivity added between 1×10^4 and 7×10^5 cpm and was subtracted from all values.

3. Results

3.1. *Binding of [^{125}I]TSH to isolated thyroid cells cultured for 14–20 hr*

The rate of [^{125}I]TSH binding to unstimulated cells is shown in fig. 1. Specific binding which represents, according to cell preparations, between 65 and 90% of the total radioactivity bound is a time-dependent process. Saturation was consistently obtained from 10–12 min incubation at 35°. In contrast, binding not displaced by native TSH does not tend to plateau in the time period investigated. Specific uptake as a function of [^{125}I]TSH concentration (fig. 2) reached a plateau between 8 and 10 ng [^{125}I]TSH for a volume of incubation of 0.5 ml and 2×10^6 cells. Scatchard plot [16] (fig. 3) of the data shows a linear relation between bound and bound/free hormone suggesting a single type of noninteracting binding sites. The equilibrium association constant (K_a) derived from five experiments is 1.0×10^9 M and the number of sites per cell is 520 ± 25 (S.D.) cal-

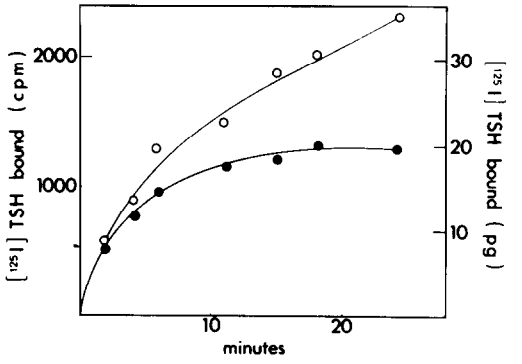

Fig. 1. Kinetics of [^{125}I]TSH binding to unstimulated isolated thyroid cells cultured for 15 hr: 2×10^6 cells in 0.5 ml were incubated at 35° with 3×10^{-10} M [^{125}I]TSH (65 000 cpm/ng). (o—o—o) Total counts; (•—•—•) total counts minus counts not displaced by 25 μg native TSH.

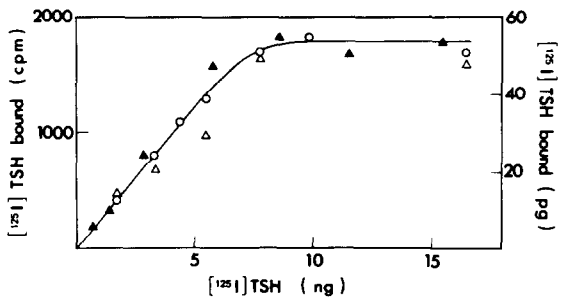

Fig. 2. Specific interaction of [^{125}I]TSH with thyroid cells as a function of [^{125}I]TSH concentration. 2 × 10^6 cells per tube in a final volume of 0.5 ml were incubated at 35° for 15 min. Data plotted are the difference between total uptake and radioactivity not displaced by 25 μg native TSH for every concentration of the labelled hormone. Cells cultured for 15 hr in the absence (○) or for 15 hr (△) and 84 hr (▲) in the presence of dibutyryl cyclic AMP. [^{125}I]TSH: 34000 cpm/ng.

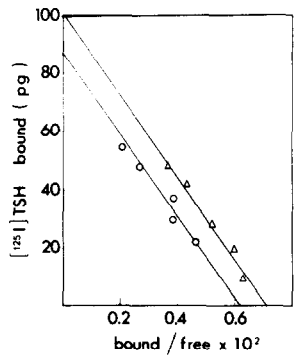

Fig. 3. Scatchard plot of binding affinity of [^{125}I]TSH to unstimulated (○) or dibutyryl cyclic AMP-stimulated (△) thyroid cells cultured for 15 hr.

Table 1
Effect of native TSH and other polypeptides hormones on the binding of [^{125}I]TSH to cultured isolated thyroid cells.

Addition	Inhibition of [^{125}I]TSH binding (%)	Addition	Inhibition of [^{125}I]TSH binding (%)
None	0	LH, 25 μg	63
TSH, 10 ng	40	LH-α, 25 μg	0
TSH, 20 ng	45	LH-β, 25 μg	0
TSH, 50 ng	45	TSH-α, 25 μg	76
TSH, 100 ng	71	TSH-β, 25 μg	55
TSH, 1 μg	76	FSH, GH, TRH,	
TSH, 5 μg	91	Insulin, glucagon,	
TSH, 10 μg	91	ACTH (25 μg)	0
TSH, 25 μg	90		

Cells (2 × 10^6) were incubated in 0.5 ml HEPES-medium E at 35° with 3.3 ng [^{125}I]TSH (110 000 cpm) and increasing amounts of native TSH or other hormones. Total counts bound to the cells was 1470. Bovine GH (0.81 U/mg), ovine FSH (5 mU/mg) and human ACTH (140 U/mg) were kindly donated by the National Institutes of Health (USA), Dr. M. Jutisz (France) and Dr. C.H. Li (USA), respectively. Crystalline bovine insulin and bovine glucagon were obtained from Eli Lilly Co. (USA) and synthetic TRH from Hoffman La Roche (Switzerland). For TSH, LH and their subunits see the Methods section.

culated from the number of [^{125}I]TSH molecules bound at saturation, if one site binds one molecule of TSH.

The specific binding of [^{125}I]TSH is directly proportional to thyroid cell concentration in the range 0.3 to 2.5 × 10^6 cell per 0.5 ml (not illustrated). Binding is also temperature dependent. At 0° the amount of [^{125}I]TSH bound is only 25% of that bound at 35°. No binding is observed at 45°.

That [^{125}I]TSH binds to superficial cell sites was assessed from the following experiments: i) immediately after trypsinization, cells fail to bind [^{125}I]TSH; a period of 12 hr in culture is required for the recovery of TSH-binding sites. ii) Incubation of cells cultured for 20 hr with 0.02% TPCK-trypsin for 1 min at 20° causes 65% inhibition of [^{125}I]TSH binding. iii) The corresponding 1500 *g* fraction of thyroid cell homogenates binds [^{125}I]TSH to about the same extent as whole cells.

Table 1 shows the displacement by increasing amounts of native TSH of [^{125}I]TSH bound to the cells: 50% inhibition of labelled-TSH binding occurs in the presence of a 10-fold excess of native TSH. The specificity of the TSH–cell interaction is also assessed from the absence of displacement of [^{125}I]TSH from cells by other polypeptide hormones used at high concentrations (table 1). Porcine LH at a concentration of 25 μg per tube causes about 60% inhibition of [^{125}I]TSH binding which is likely related to a small contamination of the preparation by TSH. In contrast, LH-α and LH-β do not compete with TSH

for the receptor sites. TSH-α and TSH-β (25 μg) cause displacement of [^{125}I]TSH. This effect could be produced by TSH contamination amounting to about 4 and 0.3% of the α- and β-subunits preparation, respectively. On the basis of unpublished combined data obtained with *in vivo* and *in vitro* bioassays as well as multiple radioimmunoassay systems, it has been shown that it was indeed the case, and that TSH-α and TSH-β are devoid of intrinsic binding activities. This agrees with the fact that LH-α which is structurally closely related to TSH-α causes no inhibition of [^{125}I]TSH binding.

3.2. *Evolution of binding properties with time and nature of the culture*

Dibutyryl cyclic AMP-stimulated cells which reassociate into follicles after 2 days of culture [6] have the same binding properties as one day-old unstimulated cells. The same K_a and the same number of available sites were derived for cells cultured in the presence of dibutyryl cyclic AMP between 14 and 96 hr (fig. 2). In contrast, the number of sites of monolayer cells decreases with time of culture: about 500 at 14 hr, 100–200 at 4–5 days and 40 at 11 days. These results agree with previous observations [5] that addition of TSH to monolayer cells from 24 hr of culturing fails to stimulate reorganization of cells into follicles. Thyroid cells cultured in the presence of TSH (40 mU/ml) show negligible specific binding of [^{125}I]TSH which suggests the almost complete saturation of available binding sites in cells cultured in the continuous presence of excess TSH.

4. Discussion and conclusions

The properties of TSH binding to cultured isolated thyroid cells are consistent with those of stereospecific receptors with high affinity (K_a of 1×10^9 at 35°) and low capacity (about 500 sites per cell). The value of K_a found is not very different from that previously determined indirectly in dog thyroid slices (3×10^8 at 1°) [1]. That these properties could be taken to describe those of cells in the gland is rendered likely by the fact that our measurements have been made on reassociated cells showing all the basic morphological and metabolic properties of the follicular cell *in vivo* [6–8]. The small number of sites per cell is striking as compared to other systems such as, for instance, isolated rat adipocytes which show about 10 000 sites per cell for insulin [17].

For the minimal concentration of TSH in the culture medium still able to induce thyroid cell reorganization into follicles (1×10^{-11} M) it can be calculated, if each site binds 1 mole of TSH, that as few as 1% of TSH binding sites are occupied. It is interesting to note that this concentration corresponds to the resting level of plasma TSH in both man [18] and pig (unpublished observations). For elevated TSH concentrations (1×10^{-9}M), as found in plasma in conditions of full pituitary stimulation through the feed-back mechanism, 100% of cell binding sites should be occupied.

As for the mechanism of action of other hormones whose effect is mediated by cyclic AMP, it can now be ascertained on the basis of the present data and previous observations [1, 7] that the physiological effects of TSH depend on the primary specific interaction of the hormone at the cell surface.

Whereas dibutyryl cyclic AMP-stimulated cells preserve the same number of binding sites during the whole time period of histiotypic reassociation in culture, a progressive decrease with time of available binding sites is observed in unstimulated cells. Whether this is due to the absence of synthesis of hormonal receptors or to its masking or inactivation by changes in membrane conformation remains to be determined.

Acknowledgements

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