BINDING OF THYROID STIMULATORS TO THYROID MEMBRANES

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1. Introduction

Thyroid-stimulating hormone (TSH) and the thyroid-stimulating immunoglobulin described as long acting thyroid-stimulator (LATS) have been shown to bind to receptors in the thyroid [1–6]. However, reports on the interaction of immunoglobulins with LATS activity on the binding of labelled TSH to its receptor appear to be conflicting. Fayet et al. [5] have reported that LATS serum incompletely inhibited the binding of labelled TSH to intact thyroid cells whereas Amir et al. [6] were unable to demonstrate any action of LATS on TSH binding to isolated thyroid membranes.

This paper describes the effects of immunoglobulins from several LATS sera on the binding of labelled TSH to thyroid membranes.

2. Methods and results

Highly purified bovine TSH (30 U/mg; a generous gift from Dr. J.G.Pierce), was labelled [7] with ^{125}I to a specific activity of 100 μCi/μg. Bovine serum albumin (10 mg) was added as carrier protein and the mixture chromatographed on a column of Sephadex G-100 in 50 mM NaCl; 10 mM Tris pH 7.5 (Tris–NaCl) to remove small amounts of aggregate and free iodide. The purified label was then stored at -30^0C.

Crude guinea pig thyroid membranes were prepared from tissue slices. The slices were homogenised in a glass homogeniser with cold 10 mM Tris pH 7.5 and the homogenate centrifuged at 500 *g* for 5 min. The supernatant was centrifuged further at 10 000 *g* for 15 min and the sedimented membrane fraction resuspended in Tris–NaCl containing 1 mg/ml of bovine serum albumin (Tris–NaCl –BSA) and stored at 0^0C for up to 12 hr. The membranes obtained from 1 mg of tissue were defined as 1 mg equivalent.

Immunoglobulins were precipitated from serum by addition of 3.75 M ammonium sulphate to a final concentration of 1.6 M. The precipitate was washed twice with 1.6 M ammonium sulphate and dissolved in distilled water (about 50% of the original serum volume). After dialysis against Tris–NaCl, the protein solution was centrifuged at 100 000 *g* for 15 min and stored at 4^0C for up to two months. Preparations Ba, Ro, Co, Wi, Wr, Mi and Ay were from patients with Graves' disease. A pool of serum from 10 healthy donors was used to prepare normal immunoglobulins. Thyroid stimulating activity was determined by the McKenzie method [8] (table 1).

Table 1
McKenzie assay responses to immunoglobulin preparations

Patient	IgG dose (mg./mouse)	Assay response (% with S.E.M.)
Ba	0.25	815 ± 90
Ro	0.25	995 ± 100
Co	1	1166 ± 130
Wi	12	1053 ± 270
Wr	12	358 ± 60
Mi	12	93 ± 6
Ay	12	85 ± 9
Normal	12	64 ± 12

Membranes (20 μl of 200 mg equivalent/ml) were incubated for 5 min at 37°C with 200 μl of Tris–NaCl–BSA containing cold bovine TSH (1 U/mg, kindly supplied by Armour Pharmaceuticals) or immunoglobulins. Labelled TSH (about 30 pg in 100 μl of Tris–NaCl–BSA) was then added and the incubation continued for 1–2 hr. The mixtures were then cooled to 0°C and, after addition of 1 ml of Tris–NaCl–BSA, centrifuged at 15 000 *g* for 15 min. All determinations were made in triplicate. Under these conditions, about 7% of the labelled TSH was bound to membranes in the absence of any cold TSH (fig. 1). Seventy five per cent of the bound TSH could be eluted by treatment with 2 M NaSCN [3] and after removal of the NaSCN by dialysis or gel filteration about 30% of the eluted material was bound to thyroid membranes on reincubation (fig. 1).

This receptor purified material was used in all subsequent experiments. It was stored at −30°C and used within 3 days of preparation.

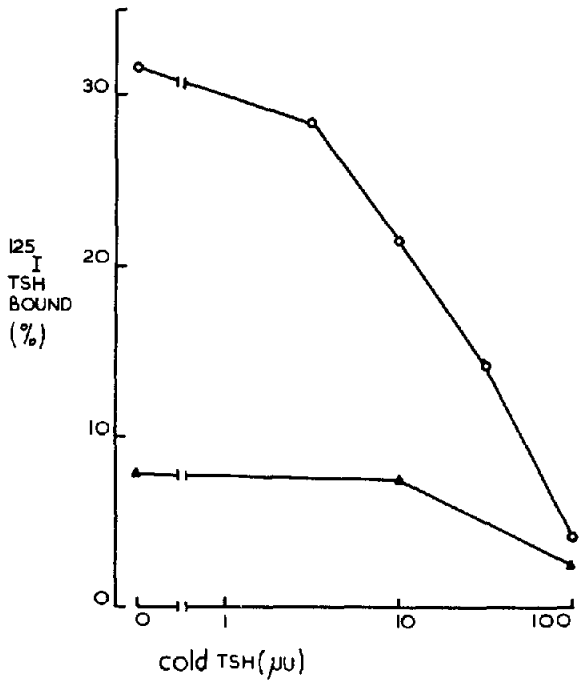

Fig. 1. Binding of ^{125}I-labelled TSH to thyroid membranes. (▲——▲) Label used before receptor purification. (○——○) Receptor purified label.

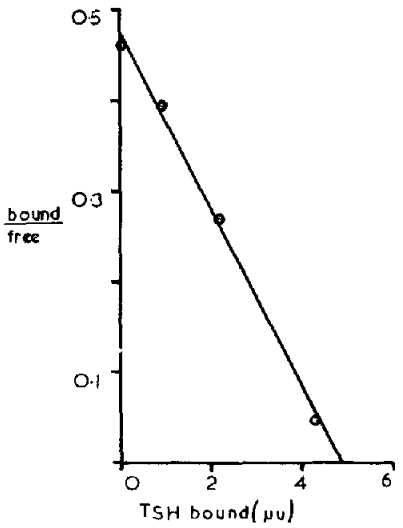

Fig. 2. Binding of ^{125}I-labelled TSH to thyroid membranes shown as a Scatchard Plot. The negative slope gives the association constant (2.6×10^{10} molar^{-1}) and the amount bound at bound/free = 0 gives the binding capacity of the membranes (1.2 μU equivalents/mg equivalent).

The binding of receptor purified label to thyroid membranes was significantly inhibited by less than 3 μU of cold TSH (fig. 1). The Scatchard analysis [9] shown in fig. 2 indicated a single population of binding sites with an association constant of 2.6×10^{10} molar^{-1} and showed that the crude membrane fraction contained 1.2 μU equivalents of receptors in each mg equivalent.

The effects of preincubating the thyroid membranes with immunoglobulins from different sera are shown in fig. 3. Normal immunoglobulins inhibited TSH binding to small extent and in a non dose dependant manner. Immunoglobulin preparation (Ba) with high LATS activity (table 1) completely inhibited TSH binding whereas preparation (Ro) with similar LATS activity to (Ba) was less effective in the receptor assay.

Immunoglobulins (Wi) and (Wr) with far lower LATS titres than (Ro) showed similar effects to (Ro) in inhibiting TSH binding. With preparation (Mi) (LATS negative) increasing the dose of immunoglobulin increased the amount of TSH bound to the membranes and (Ro), (Wi) and (Wr) showed inhibition of TSH binding at low doses and potentiation at high doses. Immunoglobulin (Ay) with no detectable

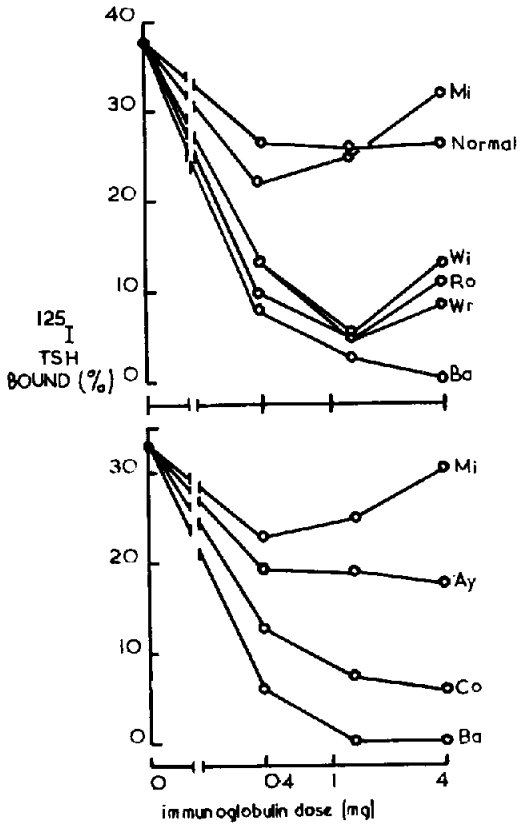

Fig. 3. Effect of immunoglobulins from patients with Graves' disease on the binding of ^{125}I-labelled TSH to thyroid membranes. Two separate experiments are shown. Immunoglobulins (Mi) and (Ba) were included in both studies.

LATS activity showed a small but significant inhibition of binding.

3. Discussion

Immunoglobulins from patients with Graves' disease inhibited TSH binding to thyroid membranes but dose–response lines from different immunoglobulins were non parallel and the ability of particular preparations to inhibit TSH binding did not correlate with their LATS activity as measured by McKenzie bioassay (table 1).

This data suggested that the immunoglobulins contained a population of antibodies directed towards thyroid cell surface sites related to the TSH receptor. Different sera probably contained different antibody concentrations, affinities and specificities and in this context different immunoglobulin preparations might have been expected to give different dose response effects in the TSH receptor assay. Similarly, lack of correlation between the mouse bioassay and the guinea pig receptor system probably reflected species difference and artifacts of the indirect in vitro binding situation.

The ability of some immunoglobulins to enhance TSH binding to thyroid membranes was apparently similar to the reported potentiation of TSH binding to retre-orbital tissue membranes by immunoglobulins from patients with Graves' disease [10]. It was difficult to rationalise these observations but possibly an antigen–antibody interaction close to the TSH receptor induced changes in the membrane which altered availability, specificity or affinity of the TSH receptor sites. A combination of direct competition for the TSH receptor with an indirect enhancing effect could have been responsible for some immunoglobulins showing inhibition of binding at low doses and potentiation of binding at high doses.

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