THE INTERACTION OF THYROID-STIMULATING ANTIBODIES WITH SOLUBILISED HUMAN THYROTROPHIN RECEPTORS

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1. Introduction

Thyroid receptors for thyrotrophin (TSH) and thyroid-stimulating antibodies (TSAb) are closely related as indicated by the ability of TSAb to inhibit the binding of labelled TSH to thyroid membranes [1–4] and to isolated thyroid cells [5,6]. The effect of TSAb on the TSH–TSH receptor interaction may be due to direct binding of the antibody to the TSH receptor. Alternatively, TSAb may bind to a site different from the TSH receptor in such a way as to induce changes in the thyroid membrane and cause inactivation of the TSH receptor. In order to investigate these two possibilities we have studied the interaction between TSAb and solubilised human TSH receptors. Our data indicate that TSAb inhibits the binding of labelled TSH to soluble TSH receptors and provide evidence for the concept that TSAb is an antibody to the TSH receptor.

2. Methods

A crude membrane fraction was prepared from human thyroid tissue (obtained at partial thyroidectomy for Graves' disease) as described [2,3]. All manipulations were performed at 0–4°C unless otherwise indicated. The membranes were suspended in 50 mM NaCl; 10 mM Tris–HCl, pH 7.4 (Tris/NaCl) containing 0.1% Triton X-100 (2 ml Triton buffer/g equiv. thyroid membranes) and centrifuged at 100 000 × *g* for 1 h. The supernatant, which contained only small amounts of soluble TSH receptors, was discarded and the sediment resuspended in Tris/NaCl containing 0.5% Triton (1.5 ml Triton buffer/g equiv. thyroid membranes) and resedimented by centrifugation at 100 000 × *g* for 1 h. The supernatant which contained considerable TSH receptor activity and about 100 μg/ml protein [7] was used immediately or stored at −70°C. The TSH-binding properties of the Triton extracts were studied using bovine TSH (30 units/mg, a generous gift from Dr J. G. Pierce) labelled with ^{125}I [2,3,8]. Bound and free labelled TSH were separated by chromatography on a 2.6 × 40 cm column of Sepharose 6B in Tris/NaCl, containing 0.05% Triton, or by precipitation with polyethylene glycol (PEG) mol. wt 4000.

Maximum labelled-TSH binding to soluble receptors occurred after an overnight incubation at 0°C. In a typical experiment 50 μl soluble receptor (5 μg protein) were added to 100 μl test material (immunoglobulin or hormone) in Tris/NaCl containing 1 mg/ml bovine serum albumin (Tris/NaCl/BSA). Labelled TSH (5000–10 000 cpm in 50 μl Tris/NaCl/BSA, containing 0.5% Triton) was then added. In addition, reaction mixtures which contained test material and labelled TSH, but with 50 μl Triton–albumin buffer instead of soluble receptor, were included. After 18–24 h at 0°C, 1.5 mg normal human IgG in 500 μl Tris/NaCl was added, followed immediately by 700 μl 30% PEG in 1 M NaCl. The labelled TSH–TSH receptor complex, precipitated together with carrier IgG, was sedimented by centrifugation (20 000 × *g* for 15 min) and counted for ^{125}I. In the absence of soluble receptor, 8–12% of the labelled TSH was associated with the PEG precipitate and this was not affected by the addition of up to 3 μg (0.1 units) unlabelled TSH (1 unit/mg, gift from Armour Pharmaceuticals). In the presence of soluble

receptor, 30–40% of the labelled TSH was precipitated with PEG and this was reduced to 9–12% by addition of 3 μg unlabelled hormone. Results were expressed as the difference between the amount of labelled TSH precipitated in the presence and absence of soluble TSH receptor, except for Scatchard analysis [9]. In this case non-specific binding was taken as the amount of labelled hormone bound in the presence of 3 μg unlabelled TSH.

Immunoglobulin G was prepared from Graves' and normal human sera by precipitation with ammonium sulphate followed by chromatography on Sephadex G-200 [10]. The IgG preparations were monitored for their ability to inhibit the binding of labelled TSH to human thyroid membranes by the method [3]. The binding of labelled TSH to thyroid membranes was also studied by this method except that the incubation of hormone and membranes was carried out at 0°C and IgG and PEG were added prior to separation of bound and free, as described above for the studies with soluble receptors. The thyroid-stimulating activity of the IgGs was studied by the method [11] adapted for use with human thyroid cells.

Human chorionic gonadotrophin (hCG) (gift from National Pituitary Agency) was labelled with ^{125}I under the conditions used to label TSH and run on Sephadex G-100 (40 × 2.6 cm column in Tris/NaCl) to remove aggregates and free ^{125}I. The labelled hormone showed similar testicular-binding properties to those described [12].

3. Results

Chromatography of mixtures of labelled TSH and 0.5% Triton extracts of human thyroid membranes on Sepharose 6B resolved the mixtures into two principal peaks of radioactivity (fig.1). Calibration of the column with different proteins (fig.1) indicated that the two peaks were characteristic of labelled

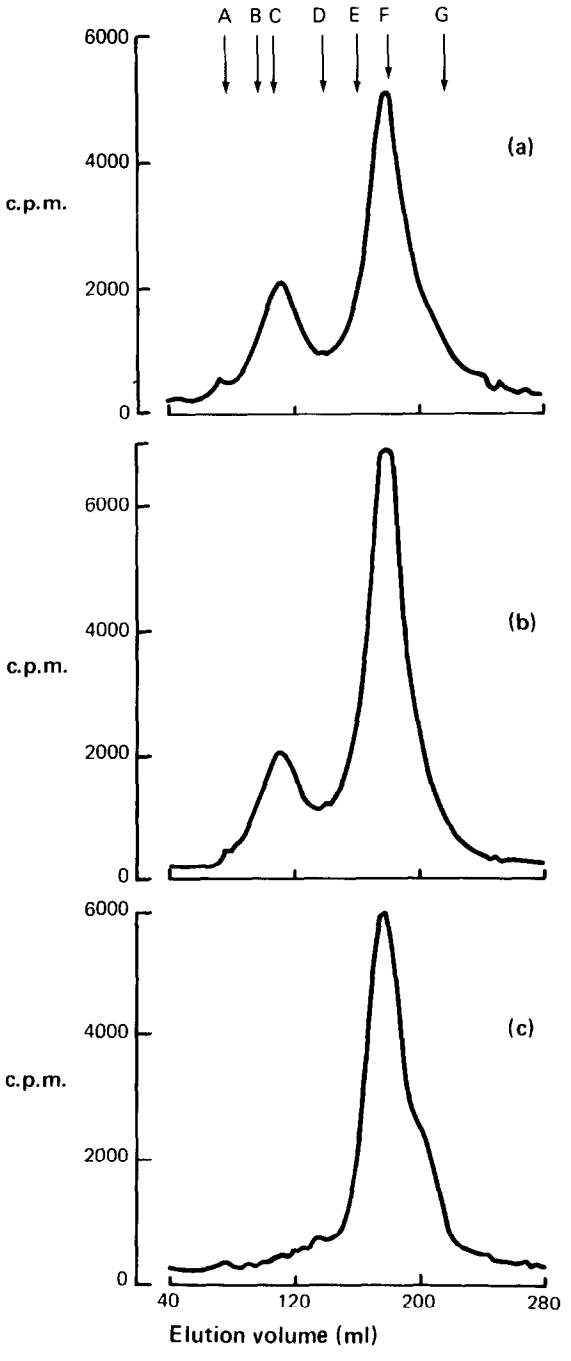

Fig.1. Chromatography of mixtures of labelled bovine TSH (100 000 cpm in 0.5 ml), IgG (0.5 ml) and 0.5% Triton extracts of human thyroid membranes (1 ml) on Sepharose 6B. (a) 100 μg normal human IgG; (b) 100 μg TSAb–IgG; (c) 4 mg TSAb–IgG. Reaction mixtures without IgG or containing 4 mg normal human IgG gave the same elution profile as that shown in (a). Reaction mixtures containing 3 μg unlabelled bovine TSH instead of TSAb gave the same elution profile as that shown in (c). The elution volumes of blue dextran 2000, IgM, IgA dimer, IgG, haemoglobin, TSH monomer and $Na^{125}I$ are shown as A, B, C, D, E, F and G, respectively. Column dimensions 2.6 × 40 cm; buffer Tris/NaCl containing 0.05% Triton; flow rate 20 ml/h.

TSH monomer (mol. wt 3×10^4) and a substance corresponding to approx. mol. wt 3×10^5. The formation of the high molecular weight peak was inhibited in a dose-dependant manner by unlabelled bovine TSH. When the high molecular weight peak was isolated, mixed with 3 μg unlabelled bovine TSH and re-run on the Sepharose column, 95% of the radioactivity eluted as TSH monomer. Using Sepharose, no interaction could be demonstrated between ^{125}I-labelled hCG and soluble TSH receptors.

Furthermore, there was no interaction between ^{125}I-labelled TSH and 0.5% Triton extracts of membranes prepared from a human testis (obtained at autopsy a few hours after death). Data obtained with the Sepharose method of separating bound and free TSH were in good agreement with those obtained with the PEG method. Results of studies using the PEG method are shown in fig.2. Labelled-TSH binding was inhibited in a dose-dependant manner by unlabelled TSH whereas hCG, insulin, and ACTH showed no significant effect. Similarly, no interaction between labelled TSH and Triton extracts of human testis could be demonstrated using the PEG method. Studies with the PEG method indicated that soluble TSH receptor activity was heat labile. Inactivation was complete after heating for 1 h at 56°C and the preparations showed only a small proportion of their initial TSH-binding activity after 1 h at 37°C or 18 h at 20°C. This was in contrast to membrane-bound TSH receptors which were stable for several hours at 37°C. The soluble receptors were stable for several days at 4°C and for several weeks at −70°C.

Labelled TSH binding to soluble and membrane-bound receptors showed similar kinetics at 0°C and maximal binding to both preparations occurred between 6 h and 18 h. Satisfactory binding data were not obtained with soluble receptor preparations at 37°C presumably because the soluble receptors were rapidly inactivated at this temperature. Scatchard analysis [9] of the TSH-soluble TSH–receptor interaction gave curved plots similar to those observed for the TSH–membrane-bound TSH-receptor interaction (fig.3). Comparison of binding data obtained from several different thyroid glands suggested that 0.5%.

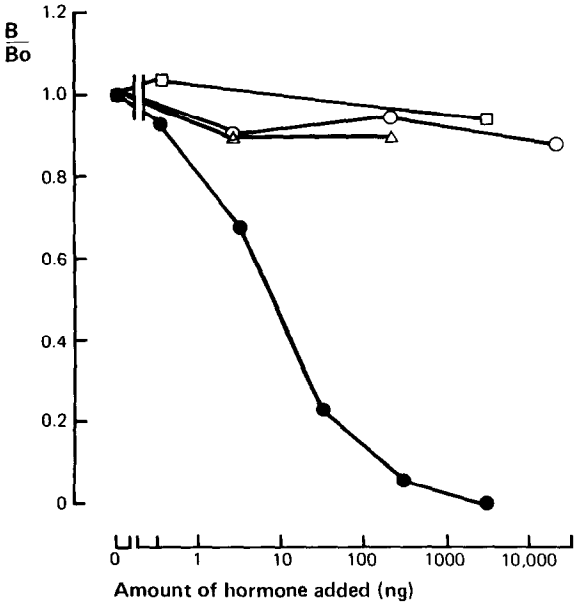

Fig.2. The effects of unlabelled TSH (•), insulin (○), ACTH (△) and hCG (□) on the binding of labelled TSH to soluble TSH receptors. Bound and free labelled TSH were separated by PEG precipitation. Binding data were expressed as:

$$\frac{B}{B_0} = \frac{\%\text{ labelled TSH bound in the presence of unlabelled hormone}}{\%\text{ labelled TSH bound in the absence of unlabelled hormone}}$$

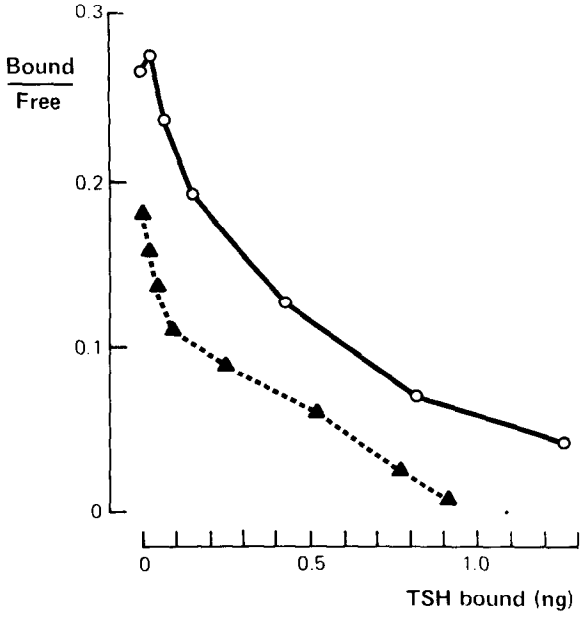

Fig.3. Scatchard analysis [9] of labelled TSH-binding to 15 mg equiv. human thyroid membranes (▲) and to 50 μl soluble TSH receptors (○).

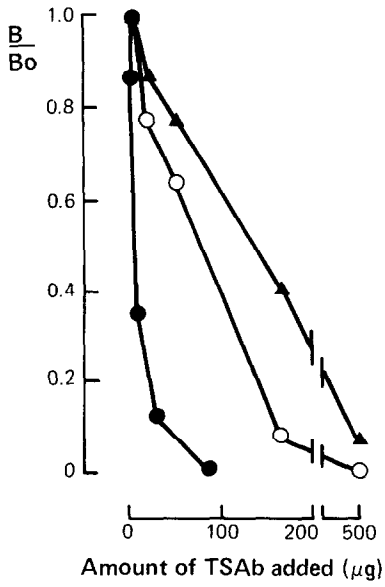

Fig.4. The effect of TSAb from 3 different patients on the labelled TSH-binding to soluble TSH receptors. Bound and free labelled TSH were separated by PEG precipitation and results expressed as:

$$\frac{B}{B_0} = \frac{\%\text{ labelled TSH bound in the presence of TSAb}}{\%\text{ labelled TSH bound in the presence of normal IgG}}$$

TSAb was diluted in normal human IgG so that the total IgG concentration in each reaction mixture was the same.

Triton extracts of human thyroid membranes contained of the order of 20–50% original membrane-TSH binding activity.

Immunoglobulin G with thyroid-stimulating antibody activity inhibited the interaction between labelled TSH and soluble TSH receptors in a dose-dependant manner as judged by chromatography on Sepharose 6B (fig.1) or PEG precipitation (fig.4). Analysis by gel filtration or PEG precipitation of mixtures which contained TSAb and labelled TSH only, showed that no direct interaction between the IgG and labelled TSH occurred.

4. Discussion

The studies with ^{125}I-labelled TSH and 0.5% Triton extracts of human thyroid membranes indicated that the Triton extracts contained a heat labile substance which interacted specifically and reversibly with TSH to form a complex which appeared to have approx. mol. wt 3×10^5. However chromatography of a solution containing 0.5% Triton (in Tris/NaCl) only on Sepharose 6B in 0.05% Triton (in Tris/NaCl) and monitoring the column eluant by extinction at 280 nm indicated that the Triton solution itself contained three principal components with mol. wts 10^5, 2×10^5 and 3×10^6. These components were presumably different sized Triton micelles [13] and when Triton was used to extract human thyroid membranes, hydrophobic membrane components, including the TSH receptor, were probably incorporated into the micelles. Consequently, the Triton micelles would be expected to make a considerable contribution to the app. mol. wt 3×10^5 observed for the TSH–TSH receptor complex.

These observations can be compared with [1] where Triton extracts of guinea-pig thyroid membranes/labelled TSH mixtures were found to contain 2 species of soluble, labelled-hormone–receptor complex with mol. wts 5×10^5 and 1.5×10^5. Lithium diiodo-salicylate extracts of bovine thyroid membranes contain TSH-receptor activity with mol. wts ranging from $1.5 \times 10^4 - 2.8 \times 10^5$ [14]. Furthermore, treatment of this mixture with trypsin converted the high molecular weight components to a component with mol. wt $1.5 \times 10^4 - 3 \times 10^4$. The apparent differences between the soluble TSH-receptor activities described in these and our own studies probably reflected species differences and differences in the conditions used to extract and analyse the receptors.

The binding of labelled TSH to soluble receptor preparations was inhibited in a dose-dependant manner by TSAb (figs.1, 4). Gel filtration and PEG precipitation studies indicated that no direct interaction between TSAb and labelled TSH occurred (i.e., TSAb did not show any TSH-binding activity). Also, mixtures of TSAb, soluble TSH receptor and labelled TSH did not contain any material with a molecular weight characteristic of a trimolecular complex, consisting of TSAb, soluble TSH receptor and labelled TSH (fig.1). A similar study [1] was unable to demonstrate a trimolecular complex of TSAb, soluble TSH receptor and labelled TSH in Triton extracts of mixtures of TSAb, guinea pig thyroid membranes and labelled TSH.

These observations suggest that TSAb binds directly

to the human TSH receptor and provide evidence for the concept that thyroid-stimulating antibodies are TSH receptor antibodies.

The relationship between the TSH/TSAb receptor activity solubilised by Triton, as described here, and the TSAb receptor activity solubilised by freezing and thawing of human thyroid membranes [15,16] has yet to be investigated.

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