THE INTERACTION OF GRAVES' IgG WITH THE THYROTROPHIN RECEPTOR

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Received 24 February 1981

1. Introduction

Sera from patients with Graves' disease contain antibodies which inhibit the binding of thyrotrophin (TSH) to thyroid membranes [1–4]. The effect could be due to a direct interaction between the antibody and the TSH receptor or the antibody might inactivate the receptor indirectly by interacting with a different membrane component. Here, we describe attempts to distinguish between these two possibilities. Our investigations demonstrate that Graves' IgG has similar inhibiting effects on labelled TSH binding to membrane bound receptors, crude detergent solubilised receptors and detergent solubilised receptors purified by Sepharose–TSH affinity chromatography. Furthermore, the effects of antibody are independent of detergent concentration and no evidence could be obtained for the formation of significant amounts of a complex consisting of antibody, solubilised receptors and TSH. These observations suggest that Graves' IgG contain antibodies which interact directly with the TSH receptor.

2. Methods

2.1. *Preparation of IgG and solubilisation of TSH receptors*

IgG was prepared from serum by ammonium sulphate precipitation (final conc. 1.6 M) followed by gel filtration of Sephadex G-200 in 50 mM NaCl; 10 mM Tris–HCl (pH 7.5) (Tris/NaCl) [3,5]. IgG concentrations were determined spectrophotometrically [6] and adjusted by ultrafiltration (Amicon UM-10 membrane). Crude thyroid membranes were prepared from porcine or human (Graves') tissue and solubilised using Lubrol 12A9 (1% w/v) in Tris/NaCl containing 15 mM iodoacetamide (Lubrol buffer) [3,4,7]. Porcine or human thyroid tissue (1 g) gave a membrane preparation (1 gm equiv.) containing ~50 mg protein [8] and 1 ml solubilised receptors contained ~1 mg protein [9]. Highly purified bovine TSH (30 units/mg), a generous gift from Dr J. G. Pierce, was labelled with ^{125}I using the iodogen method [10] and receptor purified [2,3,11]. Of the labelled material, 65–75% could be bound by an excess of thyroid membranes and showed similar biological activity to the native material [11].

2.2. *Analysis of labelled TSH binding*

The binding of labelled TSH to crude thyroid membranes was studied by incubating the membranes (100 μl) for 15 min at 20°C with 100 μl aliquots of test material (Graves' IgG, TSH or other hormones diluted in normal pool IgG so that each 100 μl aliquot contained a total of 0.5 mg IgG). Labelled TSH (100 μl; 5000 cpm) diluted in Tris/NaCl containing 1 mg bovine serum albumin/ml (Tris/NaCl/BSA) was then added and incubation continued for 1 h at 37°C. Separation of bound and free labelled TSH was then achieved by centrifugation after addition of 0.5 ml ice-cold Tris/NaCl/BSA. All determinations were made in triplicate. In the case of studies with purified and non-purified detergent solubilised TSH receptors (100 μl aliquots) a similar protocol was used except that bound and free labelled TSH were separated by precipitating receptor bound hormone by addition of polyethylene glycol (PEG; M_r 4000) to 15% (w/v) final conc. [4,7]. In experiments involving increased Lubrol concentrations, the detergent was added to give 15% (w/v) final conc. in the reaction mixtures. In some experiments bound and free hormone were also separated by gel filtration (15 ml/h at 4°C) on Sephacryl S-300 (40 × 2.5 cm) in Lubrol buffer. Non-specific binding of labelled

TSH (~5% amount added) was taken as the % bound in the presence of 10 mU unlabelled TSH (Armour, 1 unit/mg) and all data were corrected for this parameter.

2.3. *Immunoprecipitation of labelled TSH–TSH receptor complexes*

Aliquots (100 μl) of Lubrol (1%, w/v) solubilised human or porcine TSH receptors with or without 10 mU unlabelled TSH were incubated with 100 μl labelled TSH (10 000 cpm) or ^{125}I-labelled IgG [12, 13] (20 000 cpm) for 12 h at 0°C. Serum samples (100 μl diluted 1:10 in Tris/NaCl) were then added and incubation continued for 12 h at 0°C. The mixtures were then examined for the presence of TSH–TSH receptor complexes by precipitation with PEG or for the presence of TSH–TSH receptor–antibody complexes by precipitation with anti-human IgG. This was carried out by adding 100 μl of concentrated anti-human IgG and incubating for a further 12 h at 0°C. All determinations were made in triplicate.

2.4. *Purification of TSH receptors by affinity chromatography*

Partially purified bovine TSH (Armour; 1 unit/mg) was coupled to CNBr-activated Sepharose (Pharmacia) using the procedure recommended by the manufacturers. The final product contained ~2 units TSH/ml settled gel and leakage of hormone from the gel could not be detected by TSH receptor assay. Lubrol (1%)-solubilised porcine TSH receptors (10 ml containing ~10 mg protein) were run (4 ml/h) through 5 ml columns of Sepharose–TSH at 20°C. The columns were then washed with 15 ml 1% Lubrol buffer and then 15 ml 1% Lubrol buffer adjusted to 2 M in NaCl, 2 ml fractions being collected throughout. Each fraction was dialysed (at 0°C) for 24 h against Lubrol buffer (2 × 11) and 2 × 100 μl aliquots assayed for TSH binding activity and total protein [9]. A typical affinity column elution profile is shown in fig.2. The 2 fractions eluted with 2 M NaCl and containing the highest TSH binding activity were pooled and used for further analysis.

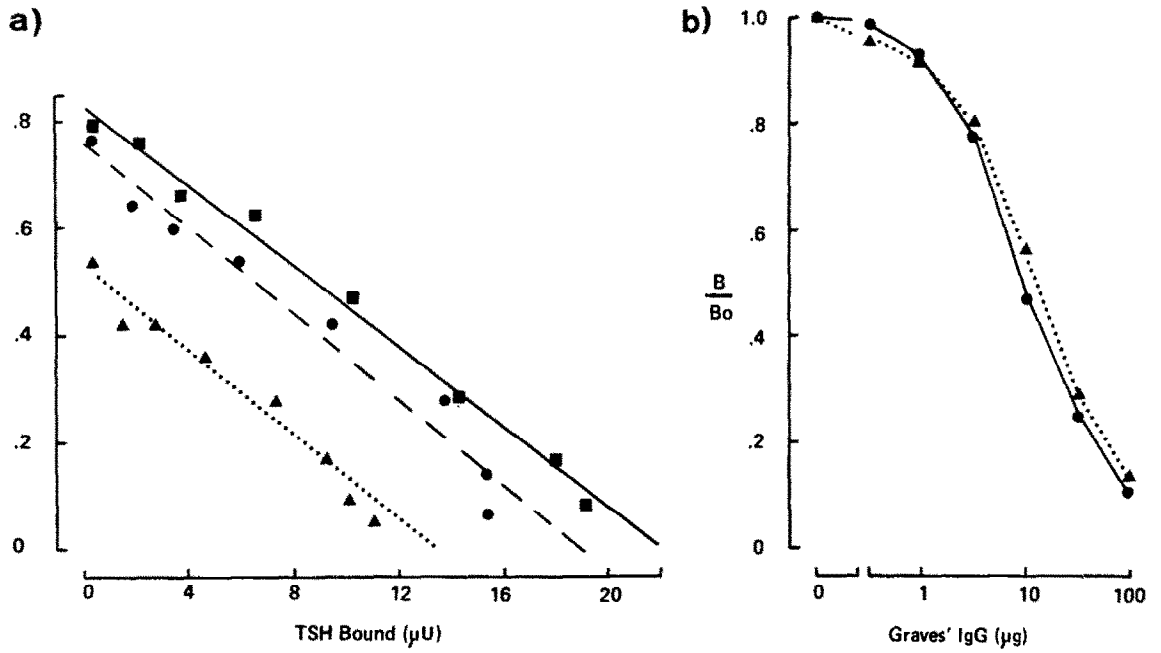

Fig.1. (a) Scatchard analysis of TSH binding to: affinity-purified porcine receptors in 1% Lubrol (▲); non-purified receptors in 1% Lubrol (●) and non-purified receptors in 15% Lubrol (■). The data shown are typical of experiments with 2 different preparations of porcine TSH receptors. The slopes of the 3 lines were similar ($P > 0.1$) and could be interpreted as indicating an association constant of 10^{10} M^{-1}. (b) Effect of Graves' IgG on labelled TSH binding to non-purified porcine, TSH receptors in 1% Lubrol (●) and 15% Lubrol (▲). Similar results were obtained with IgG's from 6 different Graves' patients using porcine or human receptor preparations. B_0 = % ^{125}I-labelled TSH specifically bound in the presence of normal pool IgG (40–50%); B = % labelled TSH bound in the presence of increasing amounts of Graves' IgG.

3. Results

In preliminary experiments the interaction of immunoglobulins (prepared by ammonium sulphate precipitation only [3]) from 36 different Graves' sera with membrane-bound human TSH receptors were compared with their effects on non-purified detergent solubilised human TSH receptors. Inhibition of labelled TSH binding was similar with both receptor preparations, linear regression analysis giving $r = 0.83$ ($P < 0.001$). This study was then extended in order to assess whether increased dispersion of the thyroid membrane components using high concentrations of detergent could influence the interaction between Graves' IgG and the TSH receptor. As shown in fig.1, increases in Lubrol concentration to 15% (w/v) did not influence the alibity of the Graves' IgG to inhibit the TSH–TSH receptor interaction. TSH binding to the solubilised receptors showed similar characteristics at both detergent concentrations (fig.1).

The possibility that detergent solubilised TSH receptor preparations might interact with both TSH and Graves' IgG to form a complex containing both hormone and antibody was investigated by immunoprecipitation but as can be seen from table 1, anti-human IgG only precipitated very small amounts of labelled TSH from mixtures of labelled TSH, soluble receptors and Graves' or Hashimoto sera. Precipitation with polyethylene glycol instead of anti-IgG demonstrated the presence of TSH–TSH receptor complexes and also showed that sera with high levels of receptor antibody activity induced some dissociation of the complex (table 1).

When solubilised porcine TSH receptors were run on Sepharose–TSH in Lubrol buffer, most of the protein together with a small amount of TSH binding activity were unretarded (fig.2). Subsequent elution with 2 M NaCl eluted a small amount of protein with considerable TSH binding activity. SDS gel electrophoresis of this preparation under reducing conditions [14] showed the presence of 8 major peptide units of 20 000–150 000 M_r. Scatchard analysis [15] of TSH binding to both affinity purified and non-purified soluble porcine TSH receptors gave linear plots ($r = 0.97$ and 0.98, respectively; fig.1). Conventional interpretation of this type of analysis suggested that the purified receptors bound 7 mU TSH/mg protein compared with 0.15 mU TSH/mg protein for the non-purified preparations (representing a 50-fold purification). The tissue and hormonal specificities of these

Table I

Solubilised human[a] TSH receptors incubated with labelled TSH followed by:	% Total labelled TSH specifically precipitated with	
	Anti IgG	PEG
Normal human serum pool	0.0	18.1
Sera from Graves'[b] patients		
1	0.0	7.1
2	1.2	10.1
3	0.7	9.5
4	0.0	13.4
Sera from Hashimoto[b] patients		
a	0.0	20.1
b	0.6	18.5
c	0.7	18.8
Solubilised human TSH receptors incubated with labelled IgG followed by normal human serum pool.	98 (% labelled IgG precipitated)	

[a] Similar results were obtained using porcine TSH receptors
[b] Hashimoto sera had high levels of microsomal and thyroglobulin antibodies and IgGs prepared from the Graves' sera readily inhibited labelled TSH binding to particulate or solubilised receptors

preparations were similar to those in [3,4,16] labelled TSH showing no detectable binding to non-thyroid preparations. In addition, hormones other than TSH did not influence labelled TSH binding to the various thyroid preparations except for large amounts (20 units) of human chorionic gonadotrophin (Organon; 1000 units/mg) which inhibited labelled TSH binding to purified and non-purified TSH receptor preparations by ~20% in agreement with [16].

When labelled TSH was incubated with non-purified porcine TSH receptors or affinity-purified receptors and the reaction mixture analysed on Sephacryl S-300, similar ^{125}I elution profiles were obtained similar to those in [4,7]. Radioactivity was eluted as a small aggregate peak ($K_{av} = 0.00$) an intermediate peak ($K_{av} = 0.24$) and a more included component ($K_{av} = 0.51$). The aggregate and intermediate peaks consisted of TSH–TSH receptor complexes as their formation was completely inhibited by addition of 10 mU unlabelled TSH to the receptors prior to la-

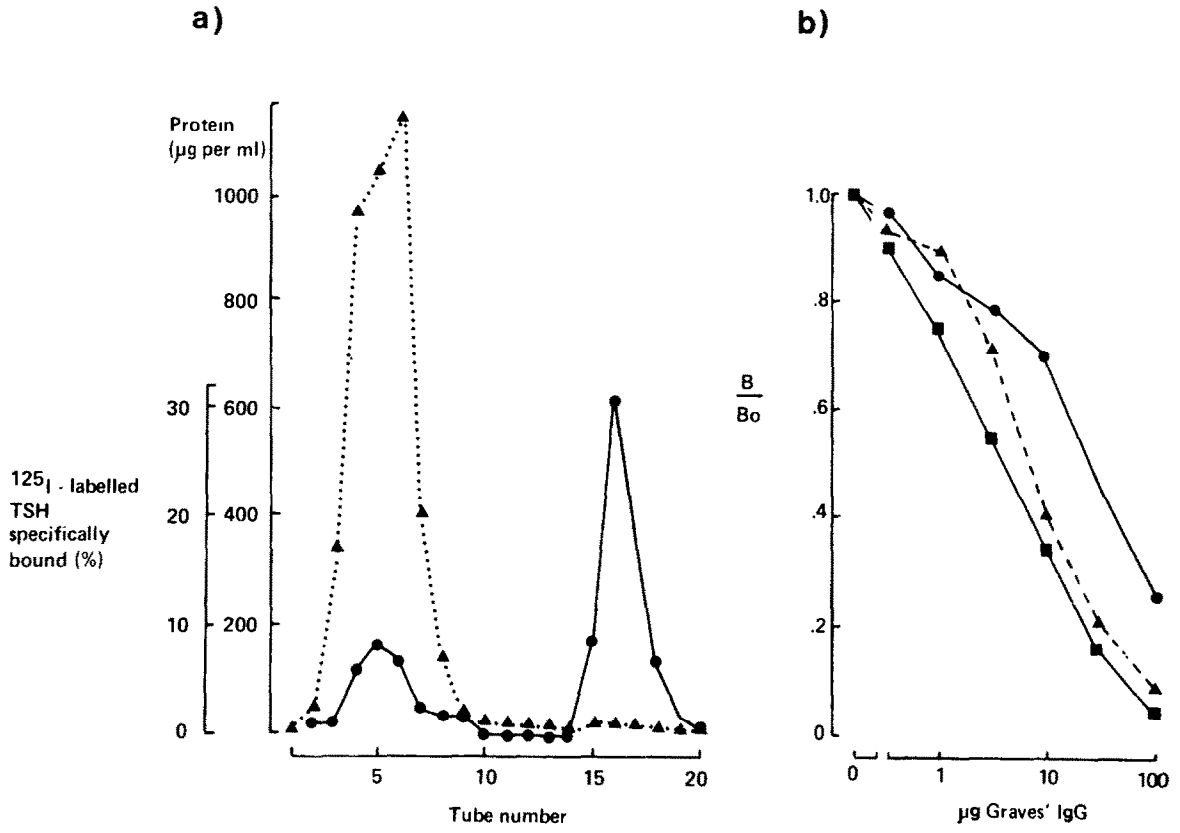

Fig.2. (a) Affinity chromatography of detergent solubilised porcine TSH receptors on Sepharose–TSH: protein content of each fraction (▲); labelled TSH specifically bound (●). The elution profile shown is typical of 6 separate expt. (b) Interaction of Graves' IgG with various porcine TSH receptor preparations. affinity purified receptors (■); non-purified solubilised receptors (▲); membrane bound receptors (●). Similar results were obtained with IgG preparations from 2 other Graves' sera. See fig.1 legend for definition of B and B_0.

belled hormone. The more included component (K_{av} = 0.51) corresponded to uncomplexed TSH.

Graves' IgG inhibited labelled TSH binding to the purified receptor preparations in a similar manner to the membrane bound and non-purified receptors except that a slightly steeper dose-inhibition relationship was observed with the purified preparations (fig.2).

4. Discussion

Our studies demonstrate that the ability of Graves' IgG inhibit labelled TSH binding to TSH receptors in thyroid membranes is maintained when:

(i) The receptors are dispersed in detergent micelles;
(ii) The concentration of detergent is increased to effect greater dispersion;
(iii) The receptors are purified by affinity chromatography on TSH–Sepharose.

Furthermore, the binding of Graves' IgG and TSH to the receptor appears to be mutually exclusive with no evidence for the formation of significant amounts of the type of antibody–receptor–hormone complexes observed with insulin receptor antibodies [17] and acetyl choline receptor antibodies [18]. Consequently, the effects of Graves' IgG on TSH binding appear to be due to the interaction of the antibody with a membrane component which is firmly bound to the TSH receptor and as such would appear to be a component of the TSH receptor molecule itself. The antibody and hormone may well interact with different sites on the TSH receptor but these sites are closely related as evidenced by the inability of the receptor to bind antibody and hormone simultaneously.

Acknowledgements

This work was supported by grants from the Medical Research Council and the Welsh Office. We are most grateful to Imperial Chemical Industries Limited for generous gifts of Lubrol 12A9.

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