TIME-DEPENDENT STABILISATION OF THE TSH–TSH RECEPTOR COMPLEX

Alan BRENNAN, Vaughan B. PETERSEN, Meryl M. PETERSEN, Bernard REES SMITH and Reginald HALL
Endocrine Unit, Departments of Medicine and Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, England

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1. Introduction

Until recently the interaction between polypeptide hormones and their receptors on the surfaces of target cells was considered to be a transient, readily reversible process [1]. However, studies with several hormones [2,3] now indicate that hormone–receptor complexes can be internalised suggesting that a more permanent association between hormone and receptor occurs. Here we report an investigation of the interaction between thyrotrophin (TSH) and the TSH receptor which indicates that the hormone–receptor complex becomes more stable with time and the process occurs at different rates in different receptor preparations.

2. Methods

Pierce bovine TSH (30 units/mg) was iodinated to spec. act. ~1 atom/molecule using the iodogen method [4] and receptor purified [5]. Porcine thyroid membranes were solubilised [5–7] using 0.1% Lubrol 12A9 in 50 mM NaCl; 10 mM Tris (pH 7.4). Thyroid cells were obtained by enzymatic disaggregation of thyroid tissue and cultured on plastic supports for 1–2 days [8,9]. After removal from the supports with EGTA [8,10] single cells were obtained by centrifugation through a Ficoll-Hypaque gradient [11]. The thyroid preparations were incubated with labelled TSH (10^5 cpm/ml) at 37°C for various times and then dissociation of label followed after addition of an excess (10 mU) of unlabelled TSH. Dissociation of TSH from thyroid membranes (200 mg equiv./ml) was usually studied in 50 mM NaCl; 10 mM Tris (pH 7.4) containing 0.1% bovine serum albumin (BSA) and 0.1% bacitracin and bound and free hormone separated by centrifugation (10 min at 20 000 × *g*). Studies with solubilised membranes (100 μl aliquots) were carried out in the same buffer with the addition of 0.1% Lubrol and bound and free hormone separated by precipitation with polyethylene glycol [6,7]. Experiments with isolated cells (2×10^6/ml) employed 250 mM sucrose, 1 mM $KClO_4$, 1 μM KI, 0.5% BSA, 10 mM Hepes (pH 7.4) and bound and free TSH were separated by centrifuging (1 min at 10 000 × *g*) the cell suspension through 1 ml ice-cold buffer. Non-specific binding was determined by running parallel studies in which an excess of unlabelled TSH (10 mU) was added to the various thyroid preparations before addition of labelled TSH. All determinations were made in triplicate and each complete experiment carried out on at least 2 separate occasions. In some studies, TSH–TSH receptor complexes were homogenised in 2 ml 0.1% Lubrol and analysed on Sepharose 6B. Complexes formed between labelled TSH and pre-solubilised membranes were run directly on the Sepharose column. Also, labelled TSH bound to thyroid membranes was sometimes extracted with 2 M NaCl, centrifuged (100 000 × *g*; 2 h) and analysed on Sephadex G-100.

3. Results

The rate of dissociation of labelled TSH from crude thyroid membranes decreased with increasing time of contact (preincubation) between membranes and hormone (fig.1A). The dissociation $t_{1/2}$ at 37°C was 13.5 min after a 15 min preincubation of TSH and membranes and this increased to 200 min after

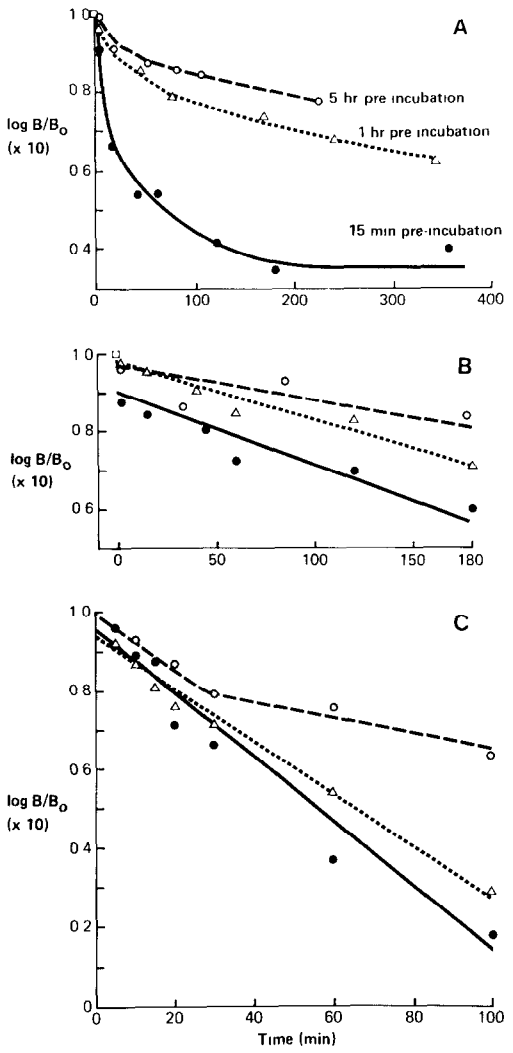

Fig.1. Dissociation of labelled TSH from thyroid membranes (A), detergent solubilised membranes (B) and isolated cells (C). Preincubation of hormone and thyroid preparation for 15 min (●——●); 1 h (△– – –△) 3 h for cells and 5 h for other preparations (○– – –○). Data shown are typical of 2 separate experiments (A) or 3 separate experiments (B or C). B_0 = % labelled TSH specifically bound initially and B = % bound at various times during the dissociation process.

1 h preincubation ($t_{1/2}$ values are means of closely agreeing determinations made on 2 separate occasions) and in the region of 400 min after a 5 h preincubation. This progressive decrease in dissociation rate was independent of [NaCl] over 0–50 mM but the effect was reduced at lower temperatures. Incubation for 1 h at 37°C prior to addition of labelled TSH did not influence the hormone binding or dissociation characteristics (data not shown).

Labelled TSH bound to detergent solubilised membranes showed a dissociation $t_{1/2}$ of 121 ± 10 min (mean ± SEM of determinations made on 3 separate occasions) and this was significantly ($p < 0.01$) increased to 184 ± 7 min after 1 h and 740 ± 90 min after 5 h (fig.1B). Sepharose 6B analysis of the complexes formed between labelled TSH and detergent solubilised membranes gave similar elution profiles to those shown in fig.2. There was no evidence of aggregation of hormone–receptor complexes after 15 min or 1 h incubations but analysis after a 5 h incubation indicated that >50% of the complex was eluted in the column void volume and this probably reflected the relative instability of the soluble hormone–receptor complex [7].

Thyrotrophin bound to isolated thyroid cells for 15 min or 1 h dissociated with $t_{1/2}$ of 33 ± 4.8 min and 35.3 ± 1.2 min, respectively, (means ± SEM of determinations made on 3 separate occasions) and these values were not significantly different ($p > 0.6$). After preincubations of cells and hormone for 3 h, dissociation of TSH showed complex kinetics with $t_{1/2}$ values of 70 min and 90 min in 2 separate experiments.

Lubrol extracts of TSH- TSH receptor complexes on crude membranes or isolated thyroid cells were resolved into 2 major components during chromatography on Sepharose 6B (fig.2A,B). These corresponded to Lubrol-solubilised TSH–TSH receptor complexes (eluting first) and non-complexed TSH. Complex formation was completely inhibited in the presence of excess (10 mU) unlabelled TSH. The elution profiles of extracts obtained after incubation of membranes with labelled TSH for 15 min or 1 h were virtually identical (fig.2A). In addition, hormone–receptor complexes extracted from isolated cells after 1 h or 3 h incubations gave similar elution profiles (fig.2B).

Labelled TSH bound to thyroid membranes for up to 5 h was readily dissociable with 2 M NaCl. Analysis on Sephadex G-100 of 2 M NaCl extracts obtained after incubation of TSH and membranes for 15 min, 1 h or 5 h indicated that the extracts contained almost entirely TSH monomer with no significant amounts of higher or lower molecular weight material. Similarly, ~80% of the labelled TSH which did not bind to thyroid membranes during the incubation chromatographed on G-100 as TSH monomer.

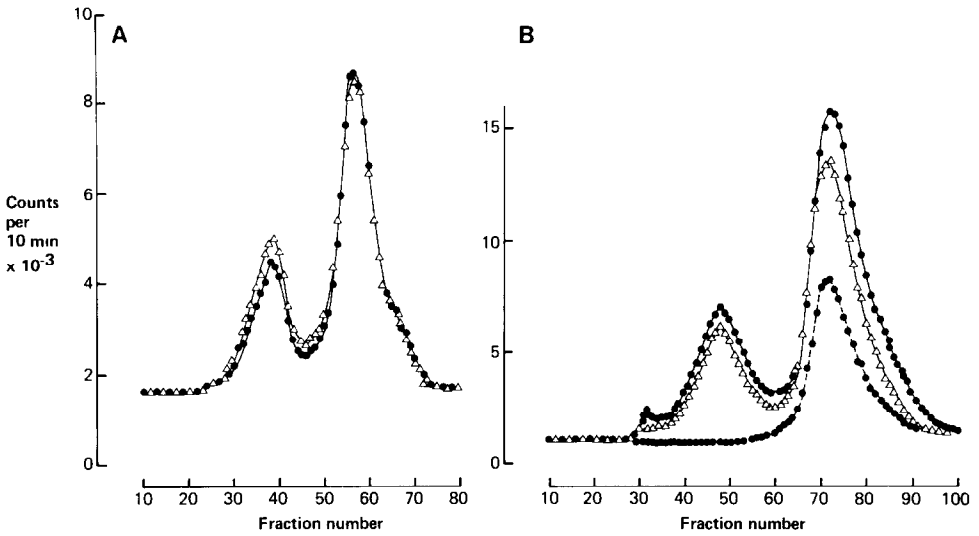

Fig.2. Analysis on a column of Sepharose 6B (40 × 2.6 cm run at 4°C in 0.1% Lubrol) of labelled TSH–TSH receptor complexes extracted with detergent from thyroid membranes (A) or intact thyroid cells (B). Incubation of label and membranes for 15 min prior to extraction (●——●) and for 1 h (△——△). Incubation of intact cells and labelled TSH alone for 1 h prior to extraction (●——●) and for 3 h (△——△). Cells incubated with labelled TSH and excess unlabelled TSH (10 mU) for 1 h prior to extraction (●– – –●). Data shown in both A and B are typical of 2 separate expt.

4. Discussion

The rate of dissociation of labelled TSH bound to porcine thyroid membranes decreased when TSH was bound to the membranes for longer periods. This phenomenon did not appear to involve aggregation or degradation of TSH as judged by analysis of extracts of membrane-bound and free hormone.

Aggregation of intact membrane-bound TSH–TSH receptor complexes could have been important in the process of increased stability but no time-dependent change in molecular size of the hormone–receptor complex occurred as judged by Sepharose 6B analysis of TSH–TSH receptor complexes. The formation of outside-in membrane vesicles with TSH bound to receptors inside could also have contributed to the decreased rate of TSH dissociation. This was investigated by attempting to induce outside-in vesicle formation by incubating membrane suspensions for 1 h at 37°C in the presence of small amounts (10 μU/ml) of unlabelled TSH. Membranes treated in this way showed similar labelled TSH binding and dissociation characteristics to untreated membranes thus providing no evidence for significant ouside-in vesicle formation. Furthermore, TSH bound to detergent solubilised membranes showed increasing stability with increasing incubation time and the rate of dissociation from both membranes and soluble receptors was similar after a 1 h preincubation. Consequently a configurational change in the TSH–TSH receptor complex leading to a more stable form was a likely mechanism for the observed progressive increase in complex stability. This can be represented:

$$\text{TSH} + \text{R} \rightleftharpoons (\text{TSH–R}) \rightleftharpoons (\text{TSH–R})'$$

where initially a readily dissociable hormone–receptor complex (TSH–R) is formed which rearranges into a more stable form (TSH–R)′.

Our observations on the dissociation of TSH from detergent solubilised membranes are consistent with this model and the relatively slow dissociation rate of TSH-soluble membrane complexes even after short incubations suggests that the complex initially formed (TSH–R) rearranged relatively rapidly into (TSH–R)′. With isolated cells, the relatively rapid first order dissociation process with $t_{1/2}$ independent of incubation time over 15 min to 1 h suggested that the complex formed initially between hormone and cells (TSH–R) was only slowly converted into the more stable form (TSH–R)′ with detectable amounts being present after a 3 h incubation.

The results described here are in agreement with studies of TSH binding to thyroid membranes and isolated cells. Complex hormone dissociation characteristics with binding becoming less readily reversible with time have been observed with TSH receptors in guinea pig thyroid membranes [12]. Also, dissociation of TSH bound to porcine thyroid membranes or intact cells has demonstrated both fast and slow components [10].

The complex (TSH–R) formed initially between TSH and its receptor is rapidly converted to the more stable form $(TSH–R)'$ in detergent solubilised membranes, less rapidly in untreated membranes and relatively slowly in intact cells. This may reflect conformational restraints imposed on the receptor by an intact cell structure which are progressively reduced as a result of membrane disruption and replacement of the lipid bilayer with detergent micelles.

Thyroid cell stimulation by TSH occurs very rapidly [9,13] and consequently the formation of (TSH–R) is quite sufficient to initiate cell stimulation. The role of $(TSH–R)'$ in the action of TSH is uncertain but it may be important in events which involve prolonged contact of hormone and receptor such as shedding or internalisation of TSH–TSH receptor complexes.

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