# COMPARISON OF THYROTROPHIN RECEPTORS IN MEMBRANES PREPARED FROM FAT AND THYROID TISSUE

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SUMMARY: Crude membranes prepared from adipose and thyroid tissue were found to have similar thyrotrophin binding properties. Binding sites for thyrotrophin were not found in harderian or liver tissue membranes.

INTRODUCTION: The principle action of thyrotrophin (TSH) appears to be on thyroid tissue and adenyl cyclase linked TSH receptors have been demonstrated in thyroid cell membranes (1-4). The thyroid-stimulating antibodies (TSAb) responsible for hyperthyroidism in Graves' disease also appear to have their principle effect on the thyroid (5) and recent studies suggest that thyroid-stimulating antibodies are antibodies to the thyrotrophin receptor (4, 6-9).

Specific receptor sites for several hormones have been demonstrated in adipose tissue (10, 11) and TSH and TSAb have been shown to stimulate lipolysis in isolated fat cells (12-14). Also, harderian plasma membranes have been reported to bind TSH (15).

In this paper we describe a comparison of the TSH and TSAb binding properties of membranes prepared from adipose, thyroid, harderian and liver tissue.

METHODS: Epididymal fat, thyroid, harderian and liver tissue were removed from guinea pigs killed by nitrogen asphyxiation and immediately cooled to 0°. The tissue was then cut finely with scissors,

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minced with a razor, homogenised with 10 volumes of 10mM Tris pH 7.5 using a glass homogenizer and centrifuged for 10 min. at 500xg. The supernatant was centrifuged further for 15 min at 15,000xg and the sedimented crude membrane preparation resuspended in 50mM NaCl, 10mM Tris pH7.5 containing 1mg/ml bovine serum albumin (Tris-NaCl-BSA).

Highly purified bovine TSH (30 units/mg; generous gifts from Dr. J.G. Pierce and Dr. J. Fawcett) was labelled with $^{125}I$ to a specific activity of about 100 μCi/μg and receptor purified using human thyroid membranes (7).

Thyroid-stimulating immunoglobulins were isolated from the serum of patients with Graves' disease by precipitation with ammonium sulphate (4). Normal immunoglobulins were prepared from a pool of serum from healthy donors.

Membranes in 50 μl of Tris-NaCl-BSA were incubated with cold bovine TSH (1 unit/mg; a generous gift from Armour Pharmaceutical Company U.K.) in 200 μl of Tris-NaCl-BSA or TSAb in 200 μl of 50mM NaCl; 10mM Tris pH7.5. After 10 min at 37°, labelled TSH (5000 cpm in 50 μl of Tris-NaCl-BSA) was added and incubation continued for 2 hr at 37°. After counting, 1.0 ml of ice cold Tris-NaCl-BSA was added and the tubes centrifuged at 20,000xg to separate bound and free hormone. The amount of membrane bound labelled hormone was determined and expressed as a percentage of the total label added.

RESULTS: The kinetics of labelled TSH binding to guinea pig adipose and thyroid membranes are shown in figure 1. Under the conditions used, binding to both fat and thyroid preparations reached a maximum between 30 and 100 min and half maximal binding occurred after about 3 min. The effects of cold TSH on the binding of labelled TSH to thyroid and fat membranes were similar (Fig. 2). Harderian and liver membranes bound only small amounts of labelled TSH and this was not readily displaced by cold TSH (Fig. 2).

Normal immunoglobulins inhibited the binding of labelled TSH to both fat and thyroid membranes (Table 1). When diluted in normal immunoglobulins, thyroid-stimulating immunoglobulins (TSAb) showed a specific inhibiting effect on the binding of labelled TSH to both fat and

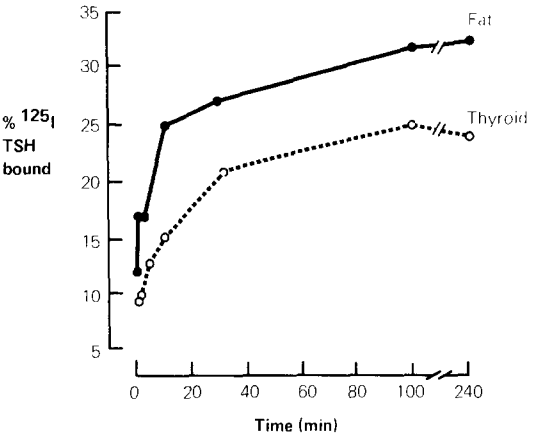


Figure 1.

Kinetics of binding of labelled $^{125}$I-TSH to membranes prepared from 30 mg equivalents of adipose tissue and 4 mg of thyroid tissue.

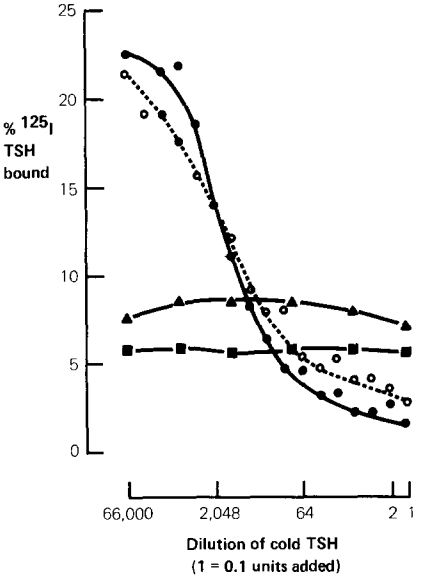


Figure 2.

Effect of cold TSH on the binding of $^{125}$I labelled TSH to membranes prepared from 30 mg of adipose tissue ( ● ), 4 mg of thyroid ( o ), 30 mg of liver ( ▲ ) and 30 mg of harderian gland ( ■ ).

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thyroid membranes (Fig. 3). The effects of TSAb on fat and thyroid membranes were similar.

TABLE 1. The effect of normal immunoglobulins on the binding of labelled TSH to membranes prepared from 30 mg of fat and 4 mg of thyroid

| Amount of normal immunoglobulin added (mg) | % $^{125}$I TSH bound | |
|---|---|---|
| | Fat | Thyroid |
| 0 | 19 | 23 |
| 0.5 | 15 | 17 |
| 1 | 15 | 11 |
| 2 | 13 | 11 |
| 4 | 8 | 11 |

DISCUSSION: Thyrotrophin binding sites in crude membranes prepared from adipose and thyroid tissue were found to be very similar on the basis of kinetics of hormone binding, effect of cold TSH on labelled hormone binding and effect of TSAb on labelled hormone binding (Figs. 1 - 3).

Normal immunoglobulins were found to inhibit the binding of labelled TSH to fat and thyroid membranes (Table 1). Consequently, in order to study the specific effect of thyroid-stimulating immunoglobulins (TSAb) it was necessary to dilute TSAb in normal immunoglobulins.

The recovery of TSH binding activity from unit weight of epididymal fat was 10-20% of that recovered from thyroid tissue. However, the epididymal fat pads alone weighed about 2gm compared with about 60mg for the thyroid. This indicated that in the epididymal fat pads there were more TSH binding sites than in the thyroid. The physiological significance of TSH receptors in adipose tissue is unknown at present and clearly warrants further investigation.

Thyroid-stimulating antibodies have been shown to compete with labelled TSH for membrane bound and soluble thyroidal TSH receptors (4, 6-9) and this data suggests that TSAb is an antibody to the TSH receptor. Our observations on the effect of TSAb on the binding of labelled TSH to

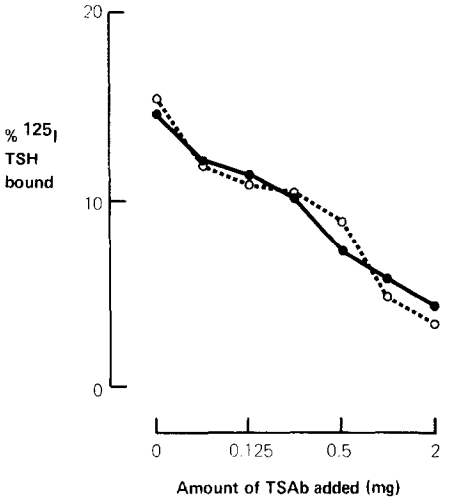


Figure 3.

The effects of TSAb on the binding of $^{125}$I TSH to membranes prepared from 30 mg of adipose tissue ( ● ) and 3 mg of thyroid tissue ( O ). Immunoglobulin containing TSAb was diluted in normal immunoglobulin so that the total amount of immunoglobulin in each tube was 2 mg.

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membranes prepared from adipose tissue provide additional evidence for this suggestion.

Membranes prepared from harderian and liver tissue were not found to have TSH binding sites comparable with those found in fat and thyroid. This is in apparent contrast to a report by Winand and Kohn (15).

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