CHAPTER IV

DISCUSSION

Several authors have reported that most thyroid nodules became smaller with thyroid hormone treatment[91,92,93] and also papillary thyroid cancers occasionally regress after full replacement doses of thyroid hormone[94]. The effects of TSH on the thyroid are presumed to be initiated by the binding of TSH to specific receptors on thyroid plasma membrane[4,5,9,11]. It is thought that useful information on human TSH receptor is obtainable from binding studies with ^{125}I-labelled bTSH. Therefore, the binding affinity and binding capacity in normal and neoplastic human thyroid tissues by Scatchard analysis were compared. During these preliminary assessments some important modifications were performed to improve the existing techniques.

Iodination Techniques.

Three different methods for iodination of purified hTSH and bTSH were made and compared. Chloramine-T method[95,96] was found to be an inferior to the Bolton-Hunter conjugation[97] and iodogen methods[87,88] because labelling of TSH with ^{125}I by the chloramine-T method causes extensive damage to TSH. This could be related to chemical damage by the oxidizing (chloramine-T) and reducing (sodium metabisulphite) agents used in this iodination method.

The binding affinity between ^{125}I-labelled TSH and its receptor is reduced and also the pH optimum for receptor binding interaction is shifted by this method. The Bolton-Hunter conjugation and iodogen methods were found to be equally reactive for binding to the TSH receptor but the cost of the former method is much more expensive than the latter method which is a gentle and simple technique. Therefore, radioiodination by iodogen method was chosen and performed throughout of this investigation. Moreover, the highly purified bTSH (Prof. J.G. Pierce, UCLAS of Medicine) was used for iodination in the present study in preference to hTSH because of its availability at much higher purity since bTSH bound to human thyroid membranes better than hTSH and much high binding percentages were obtained with the ^{125}I-labelled hTSH. These results are in agreement with those of the former workers[86].

The quality of the ^{125}I-labelled TSH is a very important factor in the TSH receptor assay. Substitution of ^{125}I more than one atom into the hormone molecule to produce a high specific activity, by either the chloramine-T or the iodogen method, altered the structure of the hormone and a significant reduction in specific binding was found in a high specific activity preparations (see Table 1, page 29). These observations were similar to the report of Kermode et al. 1981[86]. Iodination should be limited to a low specific activity (between 15-66 μCi/μg or 0.6-2.4 Bq/pg) and iodination reaction time for 2 min between ^{125}I and bTSH by iodogen method giving a specific activity of about 43.2-71.3 μCi/μg or 1.6-2.6 Bq/pg was performed in this radioreceptor assay. The maximal

specific binding of ^{125}I-labelled bTSH to its receptor sites was approximately 35.5 ± 5.17 percent as given in Table 1, page 29.

Receptor Purification of ^{125}I-bTSH.

Each preparation of ^{125}I-labelled TSH was subjected to repurification by receptor adsorption since receptor purification of the ^{125}I-labelled TSH led to a dramatic improvement in its ability to bind to thyroid membrane, together with a small reduction in non-specific binding. Three different elution buffers (2M NaCl, 1g BSA/1, pH 5.0, 2M NaSCN, 1g BSA/1, pH 5.5 and 200 mM NaCl, 10 mM Tris-Maleate, 1g BSA/1, pH 5.0) and different elution time and temperature (18 h at 4°C, 1 h at 37°C and 30 min at 37°C) were performed and tested in order to estimate the efficiency of elution. The supernatants of three elution buffers were rechromatographed and selected fractions of the peaks (^{125}I-receptor purified bTSH, see Fig. 8, page 33) which were used in the receptor assay. Elution time for 30 minutes at 37°C in 2M NaCl, 1g BSA/1, pH 5.0 was decided to use because a shorter elution time at high temperature is safe and more suitable than a longer elution time at that temperature. Although the efficiency of elution from 2M NaSCN, 1g BSA/1, pH 5.5 (52.5 ± 8.4 %) for 30 min at 37°C was higher than from 2M NaCl, 1g BSA/1, pH5.0 (43.8 ± 4.8 %) at the same condition (Table 2, page 32), the latter elution buffer was chosen and used throughout of this thesis since the best response curve was obtained from this elution buffer, as shown in Fig.9, page 34

Sources of Normal Human Thyroid Tissues and Their Binding Properties.

There are 2 sources of normal thyroid tissues, one from operation and the other was obtained at autopsy. Fresh normal human thyroid tissues were obtained from the adjacent normal thyroid removed from patients who had thyroidectomied for thyroid diseases and these tissues were proved in histologically as normal thyroid tissues. Human thyroid tissues obtained at surgery were superior to tissues obtained at autopsy since the former tissues gave the maximal percentage of binding of ^{125}I-labelled bTSH, as indicated in Table 3, page 35. Owing to small quantities and numbers of fresh normal human thyroid tissues from operation, therefore, the present method was devised to obtain normal tissues at autopsy. The autopsied thyroid tissues were assessed by specific binding test. Some of these thyroid tissues were selected for use as normal thyroid tissues in the receptor assay but lower percentages of the specific binding from the autopsied thyroid materials obtained more than 10 hours after death were rejected.

Radioreceptor Assays for TSH Receptors.

As it has been reported that the suitable condition for TSH membrane radioreceptor assay was performed at physiological pH and temperature in the presence of 50 mM NaCl[98], and binding was unaffected if the tissue was left standing at 37°C for at least 2 h in a standard binding assay[82] and 90% of maximum binding was achieved within 2 h[99]. Therefore, the incubation condition in this investigation was performed at 37°C for 2 h. Under these conditions, pH 7.5 was optimal for binding

of TSH to its receptors. The results showed that unlabelled bTSH was able to displace ^{125}I-receptor purified bTSH from the receptors and produced different standard curves for normal and other thyroid diseases, as presented in Fig. 10, page 37. The means (± SD) of maximum specific bindings for twenty normal subjects (35.57 ± 5.17 %), twelve toxic diffuse goiter patients (35.67 ± 5.26 %) and ten nodular goiter patients (35.64 ± 4.23 %) were similar but the maximal percentage of binding for ten follicular adenomas (33.88 ± 4.48 %) was slightly lower ($P < 0.4$) than the normal mean. It was interesting to note that the maximum specific binding for two patients with papillary carcinoma (14.80 ± 0.08 %) and for three patients with Hashimoto's thyroiditis (11.52 ± 0.73 %) were significantly lower ($P < 0.001$) than those for the normal mean value, as illustrated in Table 4, page 36.

The TSH displacement curves yielded linear Scatchard plots which were used to evaluate the association constant (K_a) and number of binding sites. The binding data have been converted into molar terms by assuming that 1 I.U.bTSH corresponds to approx. 1.0 nmol. The mean (± SD) binding capacities and binding affinities of twenty normal thyroid membrane preparations were 2.17 ± 0.30 pmol/g equiv. and 1.73 ± 0.47 x 10^9 M^{-1}, respectively, which did not differ significantly from those of toxic diffuse goiter and nodular goiter (Table 5, page 40). The normal mean of binding capacity (2.17 ± 0.30 pmol/g equiv.) with use of the present study is comparable to the normal mean value of 1.87 pmol/g equiv. assessed by Kermode et al.[86], but the normal mean of binding affinity

($1.73 \pm 0.47 \times 10^9$ M^{-1}) is much lower than 4.0×10^9 M^{-1} and 6.90×10^9 M^{-1} reported by the previous workers[86,100]. This could be related to some artefacts either in methodology or in analysis of assay results. Some of the discrepancies between the various studies might possibly reflect differences in the nature of the TSH-receptor interaction in thyroid tissues from different species. Two principal artefactual sources of curvature which can distort Scatchard plots are inadequate correction for non-specific binding[101,102] and use of tracer and displacing hormones with different affinities for the receptor[93,103]. Some of those authors who have observed curved Scatchard plots have considered the curvature to be caused either by the presence of a second set of binding sites with low affinity[2,80] or by some form of co-operation in the TSH-receptor interaction[98,104,105]. However, a highly purified bTSH was used in radioiodination in this study and ^{125}I-labelled bTSH of low specific activity was prepared. Moreover, the ^{125}I-receptor purified bTSH was performed before using in the radioreceptor assay throughout of the present investigation so a simple form of TSH-receptor interaction involving a single set of independent binding sites was also obtained.

The thyroid membrane from twelve patients with toxic diffuse goiter (Graves' disease) was found to have a high affinity ($1.59 \pm 0.42 \times 10^9$ M^{-1}) and high capacity or high number of TSH receptor (2.33 ± 0.55 pmol/g equiv.) which were similar to the normal membrane preparations. These results reflect the manifestations of accelerated metabolism

since in Graves' disease T_3 and T_4 levels are abnormally high and TSH levels are low or undetectable. It is increasingly evident that most or all patients with Graves' disease have circulating immunoglobulins that bind to and stimulate the TSH receptors, resulting in the pathologic hormone excess[75]. These antibodies, previously bioassayed and known as long acting thyroid stimulator (LATS)[106,107] and LATS-protector[108], were more recently assayed for their ability to inhibit binding of TSH to its receptor on human thyroid membrane[11,75]. Interestingly, patients with Graves' disease who were treated with surgery had a marked reduction of antibodies, indicating that these antibodies may be produced in thyroid. Therefore, thyroidectomy was the first logical attempt to reduce hyperthyroidism and then radioiodine (^{131}I) therapy was used.

The numbers of TSH receptors and their binding characteristics for thyroid membranes obtained from nodular goiter and toxic diffuse goiter were the same (Table 5, page 40 and Fig. 11, page 39), but the treatment of nodular goiter patients is different from the patients with toxic diffuse goiter. It was known that hormonal action can be modified by alterations in either receptor number (capacity) and affinity, and that a variety of factors may regulate the hormone receptor interaction. There was no problem about nodular goiter which TSH was believed to play an important role in the genesis and growth of such goiters[109,110] and administration of exogenous thyroid hormone can block TSH production and suppress its secretion[92]. Greer and Astwood[111] reported that, in 67 % of patients with solitary thyroid nodules, the nodules decreased

in size when the patients were given full replacement doses of thyroid hormone, and nodules disappeared in 39 %. Subsequent studies also confirmed that most thyroid nodules became smaller with thyroid hormone treatment, but complete regression was unusual[92,112]. Surgery is advised if the swelling continues or if there is any suspicion of neoplastic degeneration.

The number of TSH receptors (1.76 ± 0.31 pmol/g equiv.) in ten follicular adenomas was significantly lower ($P < 0.005$) than in normal thyroid membrane although the binding affinity ($2.04 \pm 0.28 \times 10^9\ M^{-1}$) in these patients was slightly higher ($P < 0.1$) than in the normal mean (Table 5, page 40). This result is in agreement with that of Clark and Castner[99]. The abnormality of low TSH receptors in patients with follicular adenoma might be related to impaired TSH attachment to the cells of the adenoma or a subsequent impairment in the expression of cyclic AMP action on iodine metabolism[113]. The specific biochemical defect responsible, however, remains to be elucidated.

The association rate constant ($2.14 \pm 0.00 \times 10^9\ M^{-1}$) of papillary carcinoma was found to be slightly higher ($P < 0.4$) than that of the normal, but the number of high affinity binding site (0.57 ± 0.00 pmol/g equiv.) and specific binding percentage (14.80 ± 0.08%) of two papillary carcinomas in the present investigation were distinctly lower than those of the normal ($P < 0.001$), as indicated in Table 5, page 40 and Table 4, page 36, respectively. These findings emphasize the fact that few TSH receptor sites for this type of carcinoma would take up radioactive

iodine hardly during subsequent ^{131}I-treatment, so alternative treatment should be used (eg. surgery, drugs or external radio-therapy). However, some more data of assay results are needed to confirm these preliminary results. Treatment of papillary carcinoma of thyroid gland, if the diagnosis is an incidental finding in a solitary nodule, is lobectomy, since this will remove all of the disease. The patient must take T_4 for like, to suppress any further stimulation from TSH.

A higher binding capacity (2.13 $\pm$ 0.26 pmol/g equiv.) and a definitely lower binding affinity (0.41 $\pm$ 0.00 x 10^9 M^{-1}) were observed in thyroid membranes obtained from Hashimoto's thyroiditis than those in the normal thyroid membrane preparations (Fig. 11, page 39 and Table 5, page 40), indicating some abnormalities between the reaction of TSH and its receptor in these patients. Confirmatory experiments again are required to support these findings. In Hashimoto's thyroiditis, radioactive iodine therapy should be avoided because of a high risk of hypothyroidism. The treatment of Hashimoto's thyroiditis in asymptomatic patients is controversial. According to other authors, suppressive therapy is indicated even in these patients, since they are likely to benefit from this treatment in terms of reduction of goiter and prevention of further thyroid enlargement.

In conclusion, normal and neoplastic thyroid tissues appear to have specific TSH binding sites. This helps to explain why some tumors regress in patients given exogenous thyroid hormone and lends support to the notion that all patients with differentiated thyroid tumors should receive full replacement doses of thyroxine after thyroidectomy.

SUMMARY

The numbers of TSH receptors and their binding characteristics for thyroid membranes obtained from different thyroid tissues were measured and compared. The initial part of this work mostly devoted to develop and to establish the existing techniques of radioreceptor assay for TSH. Several important modifications for thyroid membrane preparations, radioiodination of bTSH with Na ^{125}I, receptor purification of ^{125}I-labelled bTSH and optimum conditions of the assay system were found and used to improve the assay results. The maximum binding percentages of thyroid membranes from papillary carcinoma (14.80 ± 0.08 %) and from Hashimoto's thyroiditis (11.52 ± 0.73 %) were significantly ($P < 0.001$) lower than those of normal thyroid mean (35.57 ± 5.17 %). This is in contrast to the thyroid membranes from toxic diffuse goiter (35.67 ± 5.26 %), nodular goiter (35.64 ± 4.23 %) and from follicular adenoma (33.88 ± 4.48 %) which were similar to the mean of normal.

The binding capacity and binding affinity of each thyroid specimen were assessed by Scatchard analysis. The results indicated that capacities for toxic diffuse goiter (2.33 ± 0.55 pmol/g equiv.), nodular goiter (2.33 ± 0.26 pmol/g equiv.) and for Hashimoto's thyroiditis (2.13 ± 0.26 pmol/g equiv.) were nearly identical to the normal value (2.17 ± 0.30 pmol/g equiv.), but the binding capacities of

thyroid membranes from follicular adenoma (1.76 ± 0.31 pmol/g equiv.) and from papillary carcinoma (0.57 ± 0.00 pmol/g equiv.) were distinctly lower than those of the normal mean. With regard to the binding affinity, there was no significant difference between the mean values of the normal ($1.73 \pm 0.47 \times 10^9\ M^{-1}$), toxic diffuse goiter ($1.59 \pm 0.42 \times 10^9\ M^{-1}$), nodular goiter ($1.59 \pm 0.30 \times 10^9\ M^{-1}$), follicular adenoma ($2.04 \pm 0.28 \times 10^9\ M^{-1}$), and papillary carcinoma ($2.14 \pm 0.00 \times 10^9\ M^{-1}$), but the lowest binding affinity was observed in three patients with Hashimoto's thyroiditis ($0.41 \pm 0.00 \times 10^9\ M^{-1}$).

The present suudies support the view that patients with thyroid carcinomas should be treated after thyroidectomy with full replacement dose of thyroid hormone. The measurements of TSH receptors on thyroid carcinoma would provide the useful informations which act as a predictor of radioactive iodine therapy. However, confirmatory experiments are required to confirm these findings.