Thyrotropin Receptor-Adenylate Cyclase Function in Human Thyroid Neoplasms

Alan R. Saltiel, Christopher H. J. Powell-Jones, Colin G. Thomas, Jr., and Shihadeh N. Nayfeh

Abstract

The action of thyrotropin (TSH) on plasma membranes was studied to elucidate the mechanism of hormonal regulation of malignant versus normal human thyroid tissue. Thyroid plasma membranes of six specimens of papillary or follicular carcinoma and six of adenoma, as well as adjacent normal tissue obtained from these patients, were evaluated with respect to binding of 125I-labeled TSH and stimulation of adenylate cyclase. Scatchard analysis of TSH binding revealed the presence of two species of binding sites in normal thyroid of different affinities and capacities. In 11 of 12 tumors studied, the high-affinity binding site remained intact; however, the total number of low-affinity sites was markedly lower than normal tissue. Other parameters of binding were not altered in neoplastic thyroid. In each of these tissues, the hormone responsiveness and kinetics of adenylate cyclase activation were essentially identical to those observed in normal tissue, although basal activity was typically greater in the neoplasm. One carcinoma was totally deficient in both 125I-labeled TSH binding and TSH-stimulatable adenylate cyclase, although basal activity was detected. Furthermore, adenylate cyclase of this specimen was not activated by prostaglandin, in contrast to normal thyroid and other thyroid tumors. These results suggest that: (a) clinical behavior of thyroid carcinomas may not be reflected by TSH receptor-adenylate cyclase function; (b) lack of clinical response as manifest by tumor regression cannot be ascribed to the absence of functional TSH receptors or adenylate cyclase; and (c) decreased low-affinity binding present in tumors is not correlated with altered hormone responsiveness of adenylate cyclase but may reflect more general cancer-induced changes in membrane structure or composition.

Introduction

It is well recognized that certain endocrine tumors may be subject to selective hormonal control (14, 30, 33). Thyroid cells are regulated by TSH, a glycoprotein hormone produced in the anterior pituitary. TSH exerts its effects without entering the cell by interacting with a specific cell surface receptor. This interaction results in the activation of adenylate cyclase, the membrane-bound enzyme which catalyzes the production of cAMP, thought to be the intracellular mediator for the hormone (33).

The growth of some well-differentiated thyroid cancers may be dependent upon the presence of TSH. In other tumors, TSH deprivation is followed by no regression. To explain differences in the relative susceptibility of these neoplasms to hormonal manipulation, investigators have evaluated the primary site of TSH action in experimentally generated thyroid tumors (21-23), as well as human thyroid tumors (7, 9, 12, 15, 20, 36). However, no consensus exists as to the relation between the TSH receptor and the functional status of the tumor.

One of the obstacles in evaluating the role of the receptor in diseased thyroid tissue has been the inability to successfully define the TSH receptor itself and to correlate hormone binding with adenylate cyclase stimulation. Using 125I-labeled TSH radioreceptor assays, we have recently defined proper conditions under which specific high-affinity TSH receptors can be differentiated from other binding sites of lower affinity and specificity in human thyroid tissue (31, 32). Comparison of these results with measurements of adenylate cyclase responsiveness to TSH in certain thyroid tissues indicates that the physiologically significant TSH receptor, responsible for activation of the enzyme, is a single, noncooperative class of binding site of high affinity and low capacity (34). Furthermore, evidence suggests that the role of the hormone in modulating adenylate cyclase activity may be to facilitate the interaction of guanyl nucleotides with the regulatory component of the enzyme (35).

In this study, 6 specimens of well-differentiated human thyroid carcinoma and 6 specimens of thyroid adenoma have been evaluated with respect to TSH binding and adenylate cyclase response. Although total specific binding activity of diseased tissue was decreased in comparison to adjacent normal thyroid, the high-affinity receptor and adenylate cyclase response to TSH and guanyl nucleotides were similar to those of normal tissue. Comparison of these biochemical analyses to clinical findings imply that the apparent lack of hormonal control or function of thyroid tumors cannot be ascribed to the absence of TSH receptors which are responsible for stimulation of cAMP production.

Materials and Methods

Materials. All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.) with the exception of Gpp(NH)p and alumina activity I (ICN Corp., Cleveland, Ohio), pyruvate kinase (Boehringer Mannheim Corp., Indianapolis, Ind.), 125I (IMS 30; Amersham/Searle Corp., Arlington Heights, Ill.), and [α-32P]ATP and [2,8-3H]cAMP (New England Nuclear, Boston, Mass.). Highly purified bovine TSH (40 IU/mg) was a gift from Dr. John Pierce (UCLA, Los Angeles, Calif.). Partially purified TSH (NIH-B8) was obtained from the National Pituitary Agency (Baltimore, Md.).

Preparation of Thyroid Tissue. Specimens of diseased and normal thyroid tissue were obtained from patients undergoing thyroidectomy. Thyroid plasma membranes were prepared as previously described (36). Unlabeled TSH was obtained from the Hormone Distribution Program, National Institutes of Health.
normal adjacent thyroid tissue were collected from patients undergoing thyroidectomy, immersed in liquid N₂ within 5 min, and stored at -90°C. A portion of each specimen was saved for histological identification. Plasma membranes were prepared by a modification of the procedure of Neville (28) and Amir et al. (2), as described previously (31, 32). The preparation used for equilibrium binding studies was performed by incubating membranes with increasing concentrations of unlabeled bovine TSH (NIH-B8) in the presence of a constant amount of ¹²⁵I-labeled TSH. The rate of dissociation of ¹²⁵I-labeled TSH was measured as described previously (31). Separation of bound from free hormone was carried out on Millipore cellulose acetate filters (EHEP02500), and corrections were made to account for filter and nonspecific binding. A molecular weight of 28,000 for TSH was used for nonlinear least-squares curve fit for a Scatchard plot, kindly provided by Dr. David Rodbard of NIH, Bethesda, Md.

Measurement of TSH Binding. Purified bovine TSH was labeled with ¹²⁵I to a specific activity of 50 to 100 μCi/μg, using lactoperoxidase, as described previously (31). Binding of ¹²⁵I-labeled TSH to membranes (10 to 50 μg protein per ml) was measured in 50 mM Tris-acetate buffer, pH 7.4, containing 0.25% bovine serum albumin (31). Equilibrium binding experiments were performed by incubating membranes with increasing concentrations of unlabeled bovine TSH (NIH-B8) in the presence of a constant amount of ¹²⁵I-labeled TSH. The rate of dissociation of ¹²⁵I-labeled TSH was measured as described previously (31). Separation of bound from free hormone was carried out on Millipore cellulose acetate filters (EHEP02500), and corrections were made to account for filter and nonspecific binding. A molecular weight of 28,000 for TSH was used for nonlinear least-squares curve fit for a Scatchard plot, kindly provided by Dr. David Rodbard of NIH, Bethesda, Md.

Adenylate Cyclase Assay. Adenylate cyclase activity was assayed at 30°C for 15 min in a final volume of 0.10 ml of 50 mM Tris-HCl buffer, pH 7.6, as described previously (34). Reactions contained 5 mM MgCl₂, 0.5 mM 3-isobutyl-1-methylxanthine, 0.5 mM dithiothreitol, 1.0 mM EDTA, 0.2 mM ATP, 5 to 10 × 10⁶ cpm [α-³²P]ATP, and 1 to 5 × 10⁶ cpm [²⁻³H]cAMP, and an ATP-regenerating system consisting of 5 mM phosphoenolpyruvate and 10 units pyruvate kinase per ml. Reactions were started by addition of membrane protein (50 to 100 μg) to prewarmed reaction mixture and terminated at the designated intervals by immersion in boiling water for 30 sec, followed by dilution with 1.0 ml of 25 mM Tris-HCl buffer, pH 7.6. The cAMP was separated on neutral alumina, and the percentage of recovery was calculated from total [²⁻³H]cAMP in the column eluate. Results were expressed as the means of triplicate determinations. In general, variability was less than 10%.

RESULTS

Characteristics of TSH Action in Plasma Membranes of Diseased Thyroid Tissue. TSH action was evaluated in thyroid tissue obtained from 12 patients. Table 1 contains a summary of relevant information. Of 6 patients with follicular carcinoma, all were clinically euthyroid. Four had been on TSH-suppressive therapy prior to operation, without clinical evidence of tumor regression. In only one patient (L. S.) did the thyroid carcinoma demonstrate an avidity for radionuclide preoperatively. Following total thyroidectomy, the metastases (2 in lung and one in lymph node) in 3 of these patients demonstrated radioiodide uptake in association with an increased level of endogenous TSH. In all 6 patients with thyroid adenoma (benign nodule) tested, none demonstrated uptake of radioiodide in vivo nor regressed with TSH-suppressive therapy.

To evaluate the biochemical significance of these clinical findings, the binding of TSH to plasma membranes prepared from these tissues was examined. Equilibrium binding analysis, performed in 50 mM Tris-acetate buffer, pH 7.4, was used to determine the equilibrium dissociation constant (Kₐ) and total number of binding sites. Scatchard plots of ¹²⁵I-labeled TSH binding are shown in Chart 1. Normal thyroid membranes exhibited 2 species of binding site of different affinities, as has been demonstrated previously (31, 32). Binding analysis of neoplastic and adjacent normal thyroid tissue revealed that the total number of low-affinity binding sites was consistently lower in the diseased tissue. In 6 of 10 cases, however, there was no significant change in the Kₐ or total number of binding sites of the high-affinity component. A characteristic example is shown in Table 1. Evaluation of TSH action in specimens of thyroid carcinoma and thyroid adenoma.
detected in the tumor, whereas normal adjacent tissue ex
cancerous tissue from Patient J. R. clearly illustrates dimin
tractions of NaCl. Furthermore, the dilution-induced dissociation
virtually identical in all cases but one. tumors, the relative degree of stimulation achieved by TSH was
similarity in adenylate cyclase responsiveness to TSH (Table
components within the normal range.
however, neither the high- nor the low-affinity component was
hibited a typical nonlinear Scatchard plot with both binding
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an effect equivalent to that seen in normal membranes, yieldingsimilar Ka.;,'s. Results of a typical experiment are shown in
Gpp(NH)p dose-response curve was observed (Chart 3B). The
Kact for TSH stimulation of adenylate cyclase was also deter
in Chart 1A; Scatchard plots of TSH binding to normal and
cancerous tissue from Patient J. R. clearly illustrates dimin
low-affinity binding in the tumor, with no corresponding
interaction with the high-affinity site. In Patient J. H. (Chart 1B),
neither the high- or the low-affinity component was
ehibited by hormone, has been observed in porcine thyroid (35)
and other adenylate cyclase systems (4). It is thought to reflect
the kinetic enhancement by hormone of the dissociation of
ary nucleotides from the regulatory component of the en-
zyme (17).
It was of special interest to further evaluate adenylate cyclase
activity of Patient J. H. In contrast to adjacent normal thyroid
tissue (Chart 3A), the tumor obtained from this patient exhibited
no TSH binding (Chart 1B) or adenylate cyclase response to
TSH (Table 1; Chart 3B). Basal activity in this tumor, however,
was greater than that of adjacent normal tissue, indicating that
which TSH stimulation was detected, the Kact was estimated to
be 10^-10 M (data not shown).
The effects of TSH and guanyl nucleotides on the production
of cAMP were examined as a function of time, in both normal
and neoplastic membranes (Chart 4). Although activity ob-
tained in the tumor of Patient E. H. was 5-fold higher than that
of normal tissue, the relative effects of hormone and nucleo-
tides were identical. There was no effect of GTP on the enzyme
in the presence or absence of TSH. TSH alone produced a
slight increase in activity. When Gpp(NH)p was added, activity
increased 3- to 4-fold; however, a significant time lag persisted
up to 10 min. Addition of TSH with Gpp(NH)p abolished the lag
period, resulting in an increase in total cAMP produced. The
appearance of a Gpp(NH)p-induced time lag, which is
abolished by hormone, has been observed in porcine thyroid (35)
and other adenylate cyclase systems (4). It is thought to reflect
the kinetic enhancement by hormone of the dissociation of
guanyl nucleotides from the regulatory component of the en-
zyme (17).

Characterization of adenylate cyclase responsiveness to TSH and nucleotides was measured in thy-
roid tumors and normal thyroid tissue. As has been demonstrated
in porcine thyroid membranes (35), Gpp(NH)p, the
nonhydrolyzable analog of GTP, increased enzyme activity of
normal human thyroid in a dose related manner (Chart 2A). The
apparent activation constant (Kact) for Gpp(NH)p was estimated
to be 0.5 μM. The addition of TSH caused an increase in total
activity and a leftward shift of the dose-response curve (Kact
= 0.05 μM). In all tumors tested, the addition of Gpp(NH)p had
an effect equivalent to that seen in normal membranes, yielding
similar Kact's. Results of a typical experiment are shown in
Chart 2B. In the tumor obtained from Patient J. H. (Chart 3), in
which no TSH binding was detected (Chart 1B), Gpp(NH)p was
capable of stimulating the enzyme, but no effect of TSH on the
Gpp(NH)p dose-response curve was observed (Chart 3B). The
Kact for TSH stimulation of adenylate cyclase was also deter-
mained in normal and neoplastic tissues. In all specimens in

Chart 2. Effect of TSH on the sensitivity of adenylate cyclase to Gpp(NH)p.
Plasma membranes (0.5 mg/ml) were assayed for adenylate cyclase activity for
15 min at 30° in the presence of increasing concentrations of Gpp(NH)p with or
without 10 nm TSH. A, pooled normal human thyroid; B, carcinoma from Patient
E. H.

Plasma membranes from normal adjacent thyroid (A) and thyroid carcinoma of
Patient J. H. (B) were assayed for adenylate cyclase activity, as described in
Chart 2.
the loss of responsiveness was not due to tissue harvesting or preparative procedures. To determine whether the lack of adenylate cyclase response to TSH in the carcinoma of Patient J. H. was a selective defect or part of a more generalized membrane alteration, the effects of PGE₁, a known regulator of thyroid adenylate cyclase (6, 39), NaF, and Gpp(NH)p were assayed and compared to adjacent normal tissue (Table 2). Both NaF and Gpp(NH)p stimulated cAMP production in the tumor, indicating the presence of a functional enzyme containing an intact guanyl nucleotide regulatory component. PGE₁ stimulated the enzyme in normal tissue but had no effect on the carcinoma of Patient J. H. In contrast to this tumor, neoplastic tissue from Patient J. R. exhibited adenylate cyclase sensitivity to PGE₁, comparable to that seen in normal tissue. Since thyroid tumors may also respond to other polypeptide hormones (14), the effects of lutropin, follitropin, and adrenocorticotropin were assayed. None of these hormones had any effect on adenylate cyclase activity.

**DISCUSSION**

Previous studies on human (7, 9, 12, 14, 15, 20, 36) and rat (21–23) thyroid tumors have raised the possibility that well-differentiated thyroid tumors which do not regress with TSH suppression may lack functional TSH receptors. Other investigators (10, 27) have suggested that defects in adenylate cyclase activity may be responsible for lack of clinical response. In this study, we have tested these hypotheses in human thyroid by comparing the functional status of TSH receptors in plasma membranes prepared from normal and neoplastic tissue. In 11 of 12 thyroid tumors studied, the binding of ¹²⁵I-labeled TSH to plasma membranes was detected. In each of these specimens, high-affinity low capacity TSH receptors were identified by Scatchard analysis of equilibrium binding data. However, these experiments revealed significantly diminished low-affinity binding in tumors, when compared to adjacent normal tissue. In 3 samples, computer analysis of the data detected no low-affinity binding. In all of these cases, however, the high-affinity site was intact, exhibiting $K_d$’s essentially equivalent to that of normal thyroid.

The differences observed in Scatchard plots of normal and neoplastic were not accompanied by differences in other parameters of hormone binding. NaCl was equally potent in inhibiting TSH binding to normal and diseased membranes. Dissociation of ¹²⁵I-labeled TSH was biphasic and was enhanced by the addition of excess unlabeled hormone in both normal tissue and thyroid carcinoma, even when equilibrium binding parameters differed significantly. As has been suggested by previous studies on normal human thyroid (31, 32) and thyroid adenoma (32), the presence of dissociation enhancement by excess unlabeled hormone is not related to equilibrium binding profile and therefore not valid proof of the negative cooperativity model of hormone-receptor interaction, as proposed by DeMeyts et al. (11). Thus, these results provide further evidence for the heterogeneous, noncooperative nature of TSH binding.

To determine whether the decreased low-affinity TSH binding observed in thyroid tumors had any significance with respect to the second stage of hormone action, we examined the adenylate cyclase responsiveness to TSH in these tumors. Although basal activity was higher in most tumors, a detailed evaluation of the effects of nucleotides and TSH on the enzyme revealed that in all 11 specimens tumors were no less responsive than normal adjacent tissue. Interestingly, both normal and neoplastic human thyroid exhibited a limited response to TSH in the absence of Gpp(NH)p. Furthermore, the addition of GTP had no effect on adenylate cyclase (Chart 3) at low ATP concentrations (0.2 mm). These results are in sharp contrast to observations in porcine thyroid (35), in which (a) the TSH response is detected in the absence of Gpp(NH)p and (b) the addition of GTP results in deactivation of the enzyme (due to conversion to GDP). It is likely that these contrasting observations reflect a species-related difference in the nature of intracellular metabolism. GTP appears to be inherently more stable in human thyroid, possibly due to a less active membrane-bound guanosine triphosphatase.

Of the 12 patients studied, one specimen was found to be nonresponsive to TSH in vitro. This tumor also failed to respond to other polypeptide hormones (27). In none of the other specimens were the effects of nucleotides and TSH on adenylate cyclase activity consistent with a normal response profile. This is in contrast to observations on normal human thyroid (31, 32) and rat thyroid (33, 34), in which adenylate cyclase activity is enhanced by the addition of excess unlabeled hormone in both normal tissue and thyroid carcinoma, even when equilibrium binding parameters differed significantly. As has been suggested by previous studies on normal human thyroid (31, 32) and thyroid adenoma (32), the presence of dissociation enhancement by excess unlabeled hormone is not related to equilibrium binding profile and therefore not valid proof of the negative cooperativity model of hormone-receptor interaction, as proposed by DeMeyts et al. (11). Thus, these results provide further evidence for the heterogeneous, noncooperative nature of TSH binding.

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**Table 2**

Comparison of adenylate cyclase activity of thyroid tumors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>117 ± 3</td>
<td>119 ± 7</td>
</tr>
<tr>
<td>Gpp(NH)p</td>
<td>289 ± 19</td>
<td>712 ± 62</td>
</tr>
<tr>
<td>NaF</td>
<td>388 ± 32</td>
<td>1618 ± 76</td>
</tr>
<tr>
<td>TSH</td>
<td>310 ± 17</td>
<td>127 ± 9</td>
</tr>
<tr>
<td>Gpp(NH)p + TSH</td>
<td>421 ± 20</td>
<td>717 ± 50</td>
</tr>
<tr>
<td>Gpp(NH)p + FSH</td>
<td>272 ± 24</td>
<td>690 ± 70</td>
</tr>
<tr>
<td>Gpp(NH)p + LH</td>
<td>275 ± 18</td>
<td>620 ± 23</td>
</tr>
<tr>
<td>Gpp(NH)p + ACTH</td>
<td>300 ± 41</td>
<td>595 ± 60</td>
</tr>
<tr>
<td>PGE₁ + ACTH</td>
<td>377 ± 4</td>
<td>634 ± 27</td>
</tr>
</tbody>
</table>

*Mean ± S.D.

*PSH, follitropin; LH, lutropin; ACTH, adrenocorticotropin.

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Regulation of thyroid adenylate cyclase: guanyl nucleotide modulation of thyroid receptor adenylate cyclase function, submitted for publication.
completely lack the ability to bind TSH. Assay of adenylate cyclase activity in this tumor revealed no responsiveness to TSH or other trophic hormones. The enzyme was present, however, as demonstrated by high levels of activity resulting from NaF or Gpp(NH)p addition (Table 2). Interestingly, the addition of PGE, resulted in no stimulation of adenylate cyclase activity, whereas normal adjacent tissue exhibited a 3-fold increase over basal. These results suggest that the defect in this particular tumor is not limited to TSH receptors but appears to reflect a more generalized membrane alteration.

Evaluation of these data with respect to clinical findings showed no correlation between the presence of TSH receptors and growth as measured by failure of regression with TSH deprivation. Nevertheless, TSH binding and response of adenylate cyclase were detected in 11 of 12 tumors. These findings suggest that thyroid cancer possesses certain inherent growth and functional characteristics which may be modulated by but are not necessarily dependent upon TSH-responsive adenylate cyclase system. This would explain the clinical observations that thyroid neoplasms may grow despite TSH deprivation and that increased TSH often accelerates growth of well-differentiated thyroid cancer. Further, it is recognized that some tumors do regress and there is an apparent improved survivorship of some patients with well-differentiated thyroid cancer treated by TSH suppression (8, 24, 38). This concept also could explain that the uptake of radiiodide in vivo could not be correlated with alterations in binding or enzyme responsiveness, and yet in 3 patients there was evidence of uptake in response to increased levels of TSH. Contrary to clinical behavior, there were no apparent differences between “papillary” and “follicular” carcinomas.

It is also possible, however, that changes in TSH-receptor interaction may occur in thyroid cancer which are unrelated to adenylate cyclase stimulation but responsible for altered function and/or response. Recent studies have suggested that there may be as many as 3 distinct effects of TSH on thyroid membranes: (a) stimulation of adenylate cyclase; (b) stimulation of ion flux; and (c) the “phospholipid effect,” an increase in the turnover of PI, which occurs independently of cAMP (5, 13, 29). Takeuchi et al. (37) have proposed that these events are mediated through distinct TSH receptors. Moreover, recent findings of Kishimoto et al. (16) imply an important role of PI in the regulation of Ca2+-dependent protein kinases, suggesting that this acute effect of TSH may in some way constitute a mechanism by which growth is modulated. It is conceivable that tumor cells which are unresponsive to TSH deprivation lack the necessary membrane components for stimulation of PI turnover.

The possibility that there are TSH-regulated processes mediated by messengers other than cAMP necessitates further investigation into the observation that neoplastic membranes exhibit diminished low-affinity binding, clearly not related to regulation of adenylate cyclase. It is possible that these differences may reflect specific changes in membrane composition of malignant tissue. It has been established that in some diseased states thyroid membranes exhibit abnormal ganglioside (18, 21, 26) and phospholipid (1) patterns when compared with normal tissue. Since it has been suggested that membrane fluidity may modulate TSH-receptor interaction (3, 25), it is possible that decreased low-affinity binding may be a direct result of cancer-induced changes in membranes which cause a loss of receptor function other than that ascribed to adenylate cyclase stimulation. The lack of effect of TSH or PGE, on adenylate cyclase of Patient J. H. may illustrate a drastic stage in the morphological alteration of the plasma membrane, in which all receptor function is disturbed. To further probe these questions, it will be necessary to examine each of the sequential intracellular events involved in TSH regulation, as well as those which proceed independently of adenylate cyclase.

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