MINI REVIEW

Variable biological activity of thyroid-stimulating hormone

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Thyroid-stimulating hormone (TSH), like the other pituitary glycoprotein hormones, is produced and secreted as a mixture of isoforms, the majority of which represent differences in oligosaccharide structure and possess different bioactivity. When samples are quantified simultaneously by immunometric assay and bioassay, the ratio between bioactivity (B) and immunoreactivity (I) may serve as an index of the overall potency of TSH. Variations of the TSH B/I ratio have been documented in both physiological and pathological conditions associated with alteration of the two most important mechanisms controlling TSH synthesis and secretion, i.e., TRH release and the thyroid hormone feedback system. Major examples of this assumption are the low TSH bioactivity found in samples from patients lacking TRH and thus bearing a hypothalamic hypothyroidism, and the enhanced bioactivity that is invariably found in TSH from patients with thyroid hormone resistance. Moreover, variations of TSH bioactivity have been recorded in normal subjects during the nocturnal TSH surge, in normal fetuses during the last trimester of pregnancy, in patients with primary hypothyroidism and in patients with TSH-secreting pituitary adenoma and non-thyroidal illness. In conclusion, the secretion of TSH molecules with altered bioactivity plays an important pathogenetic role in various thyroid disorders, while in some particular physiological conditions the bioactivity of TSH may vary in order to adjust thyroid hormone secretion to temporary needs.

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Thyroid-stimulating hormone (TSH), along with luteinizing hormone (LH) and follicle-stimulating hormone (FSH), belongs to the pituitary glycoprotein hormone family (1, 2). These hormones are heterodimers constituted by two non-covalently linked subunits, α and β. The α-subunit is common to all glycoprotein hormones, while the β-subunit is unique and confers biological and immunological specificity to the heterodimer (3, 4). Each pituitary glycoprotein hormone exists as a mixture of isoforms that represents differences in oligosaccharide structure and not in the peptide backbone (2, 5-8). As many studies have documented that the various isoforms possess different biological activity, it clearly appears that glycosylation plays a fundamental role in modulating the expression of hormone bioactivity (4-6, 8). Thyroid-stimulating hormone has three asparagine N-linked oligosaccharide chains, two on the α-SU and one on TSH-β, which represents approximately 15% of the hormone’s molecular weight (1). The variability in the quality and extent of carbohydrate chains, as well as their different degree of sialylation and sulfation, accounts for the heterogeneity of circulating TSH when separated on the basis of overall charge (5, 6, 8-10). Immunometric assays measure the total amount of TSH in the samples independently from the intrinsic bioactivity of each isoform, whereas bioassays represent the sum of the biopotency of each individual TSH isoform. When TSH-containing samples are quantified simultaneously by immunometric assay and bioassay, the ratio between bioactivity (B) and immunoreactivity (I) may serve as an index of the overall potency of TSH. Variation of the B/I ratio will result from changes in the amount of biological activity per unit of immunological activity, provided that the immunoassay does not discriminate between isoforms (11-13). Therefore, the possible presence of interfering factors in both measurements should be considered carefully before attaching any particular pathophysiological meaning to the results.

In the present work, we will describe briefly the current methods for the measurement of TSH immunological and biological activities, and summarize all the clinical situations associated with altered B/I ratios. Emphasis will be given to those clinical and biochemical findings that may suggest the possible presence of biologically altered TSH.

Methods for the assessment of TSH immunoreactivity

The recent availability of "non-competitive" immunometric assays (IMA) based on the use of monoclonal
antibodies directed against different epitopes on TSH α- and β-subunits makes it possible to measure TSH immunoreactivity with a sensitivity much higher than that of previous "competitive" radioimmunoassays (RIA) (14–16). Moreover, the IMAs are, in principle, highly specific, because only compounds containing both of the epitopes recognized by the monoclonals are able to bridge the antibodies specifically. Nevertheless, some factors, such as heterophilic antibodies and non-analyte antibody-binding substances (16, 17), anti-TSH antibodies or antibodies cross-reacting with human TSH (18), may interfere in the IMAs, resulting in a loss of assay specificity. Although such interferences are very rare using the most recent IMAs, such as the third-generation IFMA (Delfia®, Pharmacia) employed in our studies, they have to be ruled out by appropriate dilution and recovery tests. Furthermore, in the presence of circulating or immunopurified TSH isoforms with altered oligosaccharide structures or bioactivity, the identity of these molecules and TSH international reference preparations (IRPs) has to be documented clearly (Fig. 1). It is worth noting that, by using appropriate monoclonal antibodies, circulating TSH molecules with abnormal immunoreactivity have never been reported. Therefore, the variations of circulating TSH B/I ratios described up to now result from the secretion of molecules with intrinsically altered biological activity.

Methods for the assessment of TSH bioactivity

In the last 30 years several bioassays have been established and their analytical features greatly improved. The characteristics of the principal TSH bioassays are illustrated in Table 1. The first bioassays, which were carried out with whole or fractionated serum samples, showed a very poor specificity, mainly owing to the presence in human serum of factors other than TSH interfering in the assay response and giving spuriously high or low hormone values (6, 13, 19). These factors include TSH receptor autoantibodies, chorionic gonadotropin and other non-specific substances. Therefore, TSH cannot be bioassayed in plain sera but needs to be purified partially and concentrated by immunofinity chromatography (IAC). Following this procedure, Pekonen et al. (20) could demonstrate a clear improvement of the specificity of the methods for the evaluation of TSH bioactivity. Thyroid-stimulating hormone usually has been separated from serum using both polyclonal (20–23) and monoclonal (24, 25) antibodies. Particular attention has to be paid to the selection of antibodies used in the IAC, because some monoclonal antibodies may recognize only particular isoforms of TSH (26), a fact that also prevented their use in the more recent IMAs. The most critical steps in IAC procedures are the possible leakage of the antibody either coated to plastic tubes or bound to a resin during TSH recovery and the possible dissociation of the heterodimer if too strong reducing substances or very low pH are utilized (20, 25). These initial steps have to be followed by adequate dialysis and concentration of purified TSH samples. At least two or three dilutions of the purified samples should be bioassayed in triplicate, in order to demonstrate both the parallelism with the standard curve and the specificity of the stimulation (Fig. 2). If bioassays sensitivity is less than 2 mU/L, TSH
can be tested directly at serum concentrations below 10% and, therefore, plain sera may be bioassayed reliably only when TSH concentrations are above 50 mU/l (25). Furthermore, owing to the wide heterogeneity of the reference preparations and the differences between intrapituitary and serum TSH (6, 22, 27, 28), the use of the same standard preparation in both biological and immunological assays, as well as the establishment of TSH bioactivity in appropriate groups of normal controls, are mandatory. These precautions will allow the achievement of reliable results that may be compared to those obtained in different experiments and/or with different bioassays (13, 29).

The development of TSH bioassay started in 1958, when McKenzie's method was established. In this bioassay, mice are injected previously with 131I and then treated with thyroid hormones in order to suppress endogenous TSH secretion. The effect of an intravenous injection of standard preparation or serum sample is evaluated by measuring the percentage increase in blood 131I (30). Recently, a non-radioactive in vivo method was established where mice are pretreated with T3, TSH is injected intraperitoneally and the T4 response is measured as the end-point (5). Both of the above in vivo bioassays display low sensitivity and reproducibility. Cytochemical bioassay (CBA) (31) is based on measurement of the labilization of lysosomal membranes in follicular cells of guinea-pig thyroid segments that are cultured in vitro and exposed to graded concentrations of TSH. The change in the functional state of the lysosomal membranes is quantified by microdensitometric measurement of their increased permeability to a chromogenic substrate. Cytochemical bioassay possesses the highest sensitivity, but is technically cumbersome, poorly specific and displays a low index of precision (19). The measurement of cAMP accumulation in human thyroid membrane preparations (32, 33) is highly specific, but the sensitivity is only 20–25 mU/l of TSH and different tissue preparations show variable responsiveness. This system also was the first to provide a reliable radioreceptor assay in order to study the binding activities of different TSH isoforms to the specific thyroid receptor (32). The use of a stable rat thyroid cell line (FRTL-5) greatly improved the analytical characteristics of the measurement of TSH bioactivity. In this system, different end-points, such as cAMP accumulation (22–24, 34), thymidine incorporation (5, 35), iodide uptake (22) and, as documented recently, thyroidal 5'-

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**Table 1. Common methods used for the measurement of TSH bioactivity.**

<table>
<thead>
<tr>
<th>Bioassay</th>
<th>System</th>
<th>End-point (Ref. no.)</th>
<th>Sensitivity (mU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radioactive</td>
<td>Entire mice (endogenous</td>
<td>Percentage increase</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>TSH is suppressed by T3</td>
<td>in 131I release (30)</td>
<td></td>
</tr>
<tr>
<td>Non-radioactive</td>
<td>As above</td>
<td>Increase in serum T4</td>
<td>500</td>
</tr>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBA</td>
<td>Guinea-pig thyroid segments</td>
<td>Increase of lysosome</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>permeability</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>in thyrocytes (31)</td>
<td></td>
</tr>
<tr>
<td>hTM</td>
<td>Membranes of human thyrocytes</td>
<td>cAMP accumulation</td>
<td>20–25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33)</td>
<td></td>
</tr>
<tr>
<td>FRTL-5</td>
<td>Stable rat thyroid cell line</td>
<td>cAMP accumulation</td>
<td>1.5–5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22–24)</td>
<td></td>
</tr>
<tr>
<td>CHO-R</td>
<td>CHO cells transfected with hTSH-R</td>
<td>[3H]Thymidine</td>
<td>40–50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>incorporation (5, 35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-Deiodinase activity</td>
<td>40–50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cAMP accumulation</td>
<td>1.0–3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25)</td>
<td></td>
</tr>
</tbody>
</table>

*a CBA: cytochemical bioassay.

*b hTM: preparations of human thyroid membranes.

*c FRTL-5: Fisher rat thyroid cell line.

*d CHO-R: Chinese hamster ovary cells transfected with recombinant human TSH receptor (hTSH-R).
deiodinase activity (5, 36), may be evaluated. The FRTL-5 cells require particular culture conditions because their growth is TSH-dependent and optimal sensitivity of the bioassay is reached by maintaining the cells in the culture medium without TSH for 5–8 days. Moreover, they are available to any laboratory, thus allowing better standardization of TSH bioassays. Finally, recent studies succeeded in cloning the human TSH receptor (hTSH-R) (37–39) and in setting up a strain of Chinese hamster ovary cells transfected with recombinant hTSH-R (CHO-R) coupled with cAMP pathways (40, 41). A new TSH bioassay based on the measurement of cAMP accumulation in CHO-R cells has been validated subsequently by us (25). This bioassay displays analytical characteristics similar to those of FRTL-5 cells, but provides a slightly higher sensitivity. Moreover, it is a species-specific method and requires less cumbersome culture conditions. The FRTL-5 (42) and CHO-R (43) cell membranes also have been shown to be useful for radioreceptor assay, giving an additional tool for studying structure/function relationships of TSH molecules.

How to suspect variations of circulating TSH bioactivity

Alteration of two most important mechanisms controlling TSH synthesis and secretion, i.e. TRH release and the thyroid hormone feedback system, may lead to the secretion of TSH molecules with altered bioactivity. Several studies in fact have documented the crucial role played by TRH, not only on subunit transcription (with a major effect on the TSH-β gene) (44) but also on maturation of heterodimer carbohydrate branching (9, 45–48), by activating specific endoglycosidases (45, 49) and stimulating the release of bioactive TSH molecules (21, 50). Conversely, thyroid hormones exert a profound inhibitory effect on TSH secretion, acting at both the hypothalamic and pituitary levels (51, 52), and TSH-β transcription is the main site of thyroid hormone feedback regulation (52). Indeed, TSH bioactivity and the maturation process of its carbohydrate branching appear to be altered in primary hypothyroidism (see below), indicating that thyroid hormone action also has specific influences on the post-translational processing of TSH secretion (6, 50, 53). Defects of the feedback mechanisms, such as those occurring in patients with thyroid hormone resistance or in fetuses, may be accompanied by the secretion of TSH molecules with an altered glycosylation and enhanced bioactivity.

In general, the presence of TSH with altered bioactivity may be suspected whenever discrepancies between the clinical features or the biochemical findings and the immunoreactive levels of TSH are found, provided that methodological interferences have been ruled out. In fact, the findings of slightly elevated TSH in the presence of low free T4 values and signs or symptoms of hypothyroidism in patients with hypothalamic–pituitary lesions and normal thyroid response to exogenous TSH strongly suggest the occurrence of hypothalamic hypothyroidism associated with secretion of TSH with reduced bioactivity (21, 54–56). On the contrary, the findings of normal TSH levels in the presence of goiter and thyroid hormone hypersecretion, as in patients with pituitary TSH-secreting adenoma or with thyroid hormone resistance, suggest the release of TSH molecules with enhanced bioactivity (57–59).

Another interesting finding that suggests TSH bioactivity variation is the dissociation between the amount of immunoreactive TSH released in response to TRH and the thyroid hormone response to this endogenous TRH-stimulated TSH (Fig. 3) (59–61). Moreover, the observation that the nocturnal TSH surge is not followed by the expected increase in thyroid hormone levels (62, 63) points to possible reduced bioactivity of TSH secreted at the zenith of its circadian rhythm (Fig. 4). Finally, it is well known that TSH secretion is reduced consistently in various non-thyroidal illnesses, depending on the severity of the underlying disease. Frequently, this low level of TSH is not accompanied by a reduction of the free T4 concentration, thus suggesting a possible increase of the bioactivity of TSH molecules secreted under these conditions (64, 65).

Variations of circulating TSH bioactivity in physiological conditions

Owing to the well-established heterogeneity of TSH molecules, it is conceivable that circulating TSH bioactivity may vary among different subjects and within the same individual under particular physiological conditions. Indeed, variations of the biopotency of other pituitary glycoprotein hormones, such as circulating gonadotropins, have been described in physiological situations such as puberty, menstrual cycle and menopausal state (12, 29, 66). As far as the variations of TSH bioactivity in physiological situations are concerned, very few data have been reported up to now owing to the difficulties in purifying human TSH from serum in quantities sufficient for functional and/or structural studies. The observation that free thyroid hormones do not increase after the nocturnal TSH surge (Fig. 4) prompted us to evaluate the bioactivity of TSH circulating in different periods of the day. By using an FRTL-5 bioassay, we found that the nocturnal TSH surge is made up of molecules with a reduced B/I ratio as compared to those secreted in the same individual during the day (63) (Table 2).

Another physiological situation where discrepancies between TSH levels and free thyroid hormone concentrations are recorded is the fetal life. In fact, during fetal development the TSH levels are quite stable or even increase from 18 weeks to the term of gestation, although a dramatic increase in circulating free T4 is observed during the same gestational period. These
Fig. 3. Dissociation between the net increment of serum TSH and that of free (F) thyroid hormones (FT$_3$ and FT$_4$) after TRH injection (200 µg, iv) in seven patients with hypothalamic hypothyroidism (HH) and in 11 with resistance to thyroid hormones (RTH), as compared to the responses observed in 15 normal controls. Data are expressed as mean ± SE (* p < 0.01 vs controls). These data indirectly suggest that the bioactivity of secreted TSH is reduced in HH and enhanced in RTH.

Fig. 4. Circadian variations of serum TSH and free thyroxine FT$_4$ in one normal male. Blood samples were collected every 30 min between 08.00–13.00 and 23.00–05.00 h and every hour during the other periods of the day. Cosinor analysis revealed a significant (p < 0.03) TSH acrophase (95% confidence limit) at 03.23 h. As no significant variation in FT$_4$ levels were found, these results suggested possible variation of TSH bioactivity during nocturnal TSH surge.

findings suggest both a defect in the negative feedback mechanisms (67) and the possible secretion of TSH molecules with increased bioactivity (68) during fetal life. The results of CHO-R bioassay indicated that TSH forms secreted by the fetal pituitary during the last

<table>
<thead>
<tr>
<th>Condition</th>
<th>TSH B/I$^a$</th>
</tr>
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<tbody>
<tr>
<td>Physiological</td>
<td></td>
</tr>
<tr>
<td>Nocturnal TSH surge</td>
<td>↓</td>
</tr>
<tr>
<td>Fetuses (&gt; 31 weeks of gestation)</td>
<td>↑</td>
</tr>
<tr>
<td>Pathological</td>
<td></td>
</tr>
<tr>
<td>Hypothalamic hypothyroidism</td>
<td>↓</td>
</tr>
<tr>
<td>Pituitary hypothyroidism secondary to TSH-β gene mutation</td>
<td>↓=↑</td>
</tr>
<tr>
<td>Primary hypothyroidism</td>
<td>↓=↑</td>
</tr>
<tr>
<td>TSH-secreting pituitary adenomas</td>
<td>↑</td>
</tr>
<tr>
<td>Resistance to thyroid hormones</td>
<td>↑</td>
</tr>
<tr>
<td>Non-thyroidal illnesses</td>
<td>=↑</td>
</tr>
</tbody>
</table>

$^a$ B/I: bioactivity/immunoreactivity ratio; ↓: reduced; =: normal; ↑: enhanced.
trimester of gestation actually possess a very high B/I ratio when compared with the forms secreted in the fetal circulation at midgestation (Pesani et al., manuscript in preparation) (Table 2). Interestingly, previous studies showed increased bioactivity of pituitary TSH in aborted fetuses (69). Finally, the above data match with the results of the structural analysis of fetal TSH carbohydrate chains, which showed an altered lectin binding profile and the prevalence of poorly sialylated circulating forms (10).

Variations of circulating TSH bioactivity under pathological conditions

Central hypothyroidism

In the early 1970s, the availability of RIA methods for TSH measurement showed the existence of normal or even slightly elevated circulating TSH levels in patients with central hypothyroidism of hypothalamic origin (54–56). The lack of any sign of primary thyroid failure (no antithyroid autoantibodies and normal thyroid response to exogenous TSH injection) suggested that hypothyroidism in these patients could result from the secretion of biologically inactive TSH molecules. Indirect support for this hypothesis was given by Paglia et al. (60), who described that the T3 responses to endogenous TRH-stimulated TSH was absent or largely impaired in spite of exaggerated and prolonged TSH responses to the tripeptide (Fig. 3). Subsequent studies (70, 71) in seven patients with idiopathic hypothalamic hypothyroidism showed undetectable TSH bioactivity, as measured by cytochemical bioassay (Table 2). Furthermore, by using human thyroid membranes it was confirmed that TSH bioactivity in patients with idiopathic hypothalamic hypothyroidism was very low and this observation was extended to patients with hypothalamic hypothyroidism secondary to the presence of pituitary tumors (21). In both circumstances, the lack of bioactivity was due to an impaired binding of circulating TSH to its specific thyroid receptor. Chronic TRH treatment restored both TSH receptor binding and bioactivity, indicating that this hypothalamic factor is necessary for the secretion of TSH molecules with structural features essential for appropriate thyroid stimulation (21, 45–47, 50, 72). Similar results were obtained by using both FRTL-5 and CHO-9 bioassays in patients with central hypothyroidism due to cranial irradiation for nasopharyngeal carcinoma (73). Lectin affinity chromatography showed that the oligosaccharide structure of TSH molecules circulating in hypothalamic hypothyroidism differs from that of circulating molecules from normal and primary hypothyroid subjects and that the secretion of TSH forms with hybrid, high-mannose and biantennary oligosaccharide chains and a minor degree of sialylation is prevalent (9, 10), thus confirming previous data obtained in rats (46, 47).

Finally, a frameshift mutation in the TSH-β gene that alters the apoprotein tertiary structure of the complete hormone has been reported recently (74). This mutation causes a rare form of familial pituitary hypothyroidism characterized by measurable immunoreactive TSH levels that appear to be devoid of biological activity.

Primary hypothyroidism

The high levels of TSH circulating in primary hypothyroid patients allow the TSH bioactivity to be assayed by almost all methods and the results have been used as a reference range in several studies. In fact, the TSH B/I ratios from these patients were shown largely to overlap with those of TSH circulating in normal subjects (21–23, 25, 71, 75). None the less, Dahlberg et al. (76) found a significant inverse correlation between the B/I ratio of immunopurified TSH and thyroid hormone levels, suggesting that circulating TSH bioactivity is correlated inversely with the metabolic status of the subjects (Table 2). On the contrary, by using a more sensitive bioassay the average mean of B/I ratios was found to be significantly lower in patients with primary hypothyroidism than in controls, although a wide overlap of values was recorded (Table 2) (25). Different degree and duration of the disease, as well as different characteristics of the bioassays, may account for the above discrepancy. However, the finding of a slightly reduced bioactivity of TSH molecules from patients with primary hypothyroidism is in agreement with the results of ricin lectin chromatography before and after neuraminidase treatment (9, 10), indicating a marked increase of sialylation that is usually accompanied by a reduction in both metabolic clearance rate and bioactivity of the hormone (5, 6, 77).

Inappropriate secretion of TSH

The syndrome of inappropriate secretion of TSH encompasses TSH-secreting pituitary adenomas and the resistance to thyroid hormones, and is characterized by high thyroid hormone levels in the presence of a measurable TSH concentration. Thus, thyroid hormone hypersecretion and goiter, as well as other signs of thyroid hyperstimulation (e.g. increased radioiodine uptake), are typically TSH-dependent in this syndrome. Nevertheless, the majority of patients with inappropriate TSH secretion have immunoreactive TSH levels within the normal range, thus suggesting the possible secretion of TSH molecules with enhanced bioactivity (61).

Thyroid-stimulating hormone-secreting pituitary adenomas (TSH-oma). Bioactivity of TSH from patients with TSH-oma were measured initially on material separated by isoelectrofocusing from primary tumor cell cultures (78) or on crude tumor extracts (79). Both studies
McKenzie bioassay, the TSH B/I ratio was shown to be similar to that of TSH from patients with primary hypothyroidism. Recently, the observation that patients with RTH who have normal serum TSH concentrations have an exaggerated thyroid hormone response to endogenous TRH-stimulated TSH (Fig. 3) prompted us to study TSH bioactivity using both CHO-R and FRTL-5 bioassays (59). We showed that TSH circulating in 11 patients with RTH from eight different kindreds with documented mutation in thyroid hormone receptor β gene displays a B/I ratio significantly higher than that of TSH from normal subjects (Table 2). Very recently, this finding was confirmed in two additional patients from different kindreds (Fig. 5). Interestingly, TRH administration did not result in significant changes of circulating TSH B/I ratio, whereas 10 days’ treatment with high doses of T₃ normalized the B/I ratio (Fig. 5). These data suggest that genetic mutations in the T₃-binding domain of thyroid hormone receptor-β lead to the secretion of TSH molecules with enhanced bioactivity and may explain the pathogenesis of goiter and thyroid hyperstimulation despite immunoreactive TSH concentrations within the normal range (59). Moreover, the normalization of TSH bioactivity after the administration of high T₃ doses that are able to overcome the dominant negative effects of mutant receptor clearly indicates the important role of thyroid hormone feedback on the production of TSH with normal bioactivity.

Non-thyroidal illnesses
Thyroid-stimulating hormone secretion is reduced consistently in various non-thyroidal illnesses, depending on the severity of the underlying disease. Frequently, such low levels of TSH are not accompanied by a reduction of the free T₄ concentration, thus suggesting a possible increase in the bioactivity of TSH molecules secreted under these conditions (64, 65). Conversely, increased severity of the disease may be accompanied by the appearance of low T₄ levels and normal/low TSH levels, suggesting the secretion of TSH with reduced bioactivity (82). Kakezono et al. (64) evaluated adenylyl cyclase stimulation in FRTL-5 and porcine thyroid cells incubated with sera from patients with various non-thyroidal illness. These sera were equally or even more potent than those from normal controls in stimulating cAMP accumulation and a significant inverse correlation between TSH bioactivity and thyroid hormone levels was found. On the contrary, immunopurified TSH from patients with chronic renal failure showed the TSH B/I ratios to be similar in patients and in controls (24) (Table 2). Thus, the precise role of variation in TSH bioactivity in patients with non-thyroidal illnesses remains to be established. These studies will contribute to clarify whether these patients have a particular form of central hypothyroidism or their biochemical modifications are secondary to

Resistance to thyroid hormones (RTH). The TSH bioactivity in patients with RTH was measured first in one young girl with very high TSH levels due to previous mistaken thyroidectomy (81). By using a modified

![Diagram](image-url)
physiological mechanisms leading to metabolic adaptation to the underlying disease.

Conclusions

The concomitant measurement of both immunoreactivity and bioactivity of circulating TSH provides a more complete understanding of the complex interactions by which the pituitary and thyroid gland communicate. Alterations of either hypothalamic control or the negative feedback system may cause secreted TSH to be structurally abnormal. These abnormalities are mainly due to alteration of the TSH carbohydrate chains, which affects the biological activity of the hormone in the presence of normal immunoreactivity. Reduced or enhanced bioactivity of circulating TSH plays an important pathogenetic role in several disorders of the hypothalamic–pituitary–thyroid axis, while in some particular physiological conditions it may adjust thyroid hormone secretion to temporary needs.

Acknowledgments.

This work was supported partially by grants from CNR and MURST (Rome). The authors are indebted to Professor G. Faglia for intellectual contributions and continuous support. We wish to thank Dr G. Vassart (Brussels, Belgium) for the generous gift of transfected CHO cells. Dr PB Romelli (Technogenetics, Milan) for the preparation of coated tubes for TSH extraction, Dr M. Moretti (Pharmacia, Milan) for supplying TSH Delfia® kits and Ms V. Giammona for her skilful technical assistance.

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Received April 5th 1994
Accepted May 13th, 1994