Linear modulation of serum thyrotrphin by thyroid hormone treatment in hypothyroidism

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Abstract. Basal serum TSH and the peak TSH response to a 500 μg TRH bolus were measured in 57 euthyroid and in 29 hypothyroid subjects either receiving graded thyroid hormone replacement or acutely removed from full replacement therapy. Serum TSH, total T₄ and T₃ were determined by sensitive radioimmunoassay methods. The peak versus basal TSH data for hypothyroid patients were linear within individuals. The regression slope of the peak versus basal TSH data for all hypothyroid subjects did not differ significantly from the corresponding slope for all euthyroid subjects. Basal and peak TSH versus T₃ and T₄ data for hypothyroid patients were also linear within each individual. Moreover, the regression of the basal TSH values averaged over the non-replacement to full replacement state against the TSH versus T₃ slope had a significant negative correlation. This trend leads to an array of regression lines which average to the familiar hyperbolic relationship between thyrotrphin and thyroid hormone levels in man.

Pituitary thyrotrphin responsiveness to exogenous thyrotrphin-releasing hormone (TRH) (Hershman 1978) and presumably thyrotrphin circadian rhythmicity (Azukizawa et al. 1976; Weeke 1974) are modulated by the plasma concentration of thyroid hormones in the pituitary (Bowers et al. 1968; Perrone & Hinkle 1978; Vale et al. 1968) and possibly the hypothalamus (Arimura & Schally 1976; Belchetz et al. 1978; Roti et al. 1978). Recent evidence indicates that most of the physiological effects of thyroid hormones are mediated by receptors within cell nuclei of responsive tissues (Oppenheimer 1979). Scatchard plots of the binding of triiodothyronine (T₃) to partially-purified nuclear thyroid hormone receptors are linear (Charles et al. 1975), suggesting an absence of binding cooperativity (Oppenheimer 1979). In this report, we evaluate the relationships between the basal serum thyrotrphin (TSH) and peak TSH secretion following the administration of TRH to normal subjects and hypothyroid patients. The latter either received graded thyroid hormone replacement therapy of increasing dose or were removed abruptly from a regimen of complete thyroid hormone replacement. An initial linear suppression or stimulation of basal and TRH-stimulated pituitary TSH release with either increasing or decreasing thyroid hormone levels along with marked variations in the response slopes within individual hypothyroid subjects were observed. These linear responses may contribute to our understanding of the non-amplified mode of thyroid hormone action (Oppenheimer 1979).

Materials and Methods

Blood was drawn 15 min before, immediately before and at 15, 30, 45, 60, 90, 120 and 240 min after a bolus iv injection of 500 μg of TRH (Abbott) in 57 euthyroid (basal < 5 μU/ml), 20 hypothyroid subjects receiving suboptimal thyroid hormone replacement therapy and 9 other hypothyroid patients who were removed from
complete thyroid hormone replacement. The first group of hypothyroid patients was treated with graded replacement doses of thyroxine (50, 100 or 150 µg of l-thyroxine) or desiccated thyroid (30, 60, 90 or 120 mg) and a TRH-test performed after one month at a given dose level. The hypothyroid subjects on full thyroid hormone replacement received a TRH-test at 3, 7, 10, 14, 17, 21, 28, 35, 42, 49 and 56 days after withdrawal from therapy. All hypothyroid data used for statistical analysis were limited to that corresponding to the 7–240 µIU/ml basal TSH range.

Serum TSH levels were measured by a previously described highly sensitive radioimmunoassay (RIA) method (Pekary et al. 1975). Serum was diluted as necessary to obtain a precise value at the mid-portion of the standard curve. Total serum T3 and T4 were determined by the RIA methods of Chopra (1972) and Chopra et al. (1972) with minor modifications. All sera from a given subject were run in the same assay to avoid inter-assay variation. RIA and statistical computations were carried out on a Hewlett-Packard Model 9830 desk-top computer and Model 9862A X-Y plotter with the aid of the Hewlett-Packard RIA and Statistical Package programmes. The joint 95% confidence region for the bivariate Gaussian distribution (Snedecor & Cochran 1967) was obtained by numerical methods. Parallel line analyses were performed with a bioassay programme by Vivian B. Faden and David Rodbard, National Institute of Child Health and Development. NIH, which has recently been adapted for use on the Hewlett-Packard Model 9830 computer (Pekary 1979).

Results

Fig. 1 summarizes the peak serum TSH versus basal TSH data for 144 TRH response curves obtained from euthyroid subjects and hypothyroid patients either on graded replacement doses of thyroid hormones or withdrawn from complete replacement therapy. Least squares lines have been calculated by first order (linear), second order (quadratic) and third order (cubic) polynomial regression analysis using linear scales for peak and basal TSH values. Only the zero and first degree coefficients in X and the correlation coefficient were significant. The highly significant linear regression for all data and the absence of a significant difference ($P > 0.05$) between the slopes of the corresponding least squares lines for the euthyroid

![Graph](image-url)

Fig. 1.

Plot of all log₁₀ (peak TSH) vs. log₁₀ (basal TSH) data. Log₁₀ (basal TSH) values greater than 0.75 correspond to hypothyroid subjects. The regression lines for Y vs. X and X vs. Y are given by the equations $Y = 0.712X + 0.901$ and $Y = 0.767X + 0.837$, respectively. The correlation coefficient, $r$, equals 0.961, $P < 0.01$.
subjects \((Y = 4.63X + 4.04, r = 0.64)\), compared to that for the hypothyroids \((Y = 1.85X + 37.8, r = 0.88)\), suggest that a linear relationship exists between the basal and peak TSH values for all subjects regardless of the circulating thyroid hormone levels. Regression analysis of log10(peak TSH) versus log10(basal TSH) (Fig. 1), is even more highly correlated. Again, the \(t\)-test comparison of the regression slopes for euthyroid \((Y = 0.57X + 0.93, r = 0.67)\) and hypothyroid \((Y = 0.75X + 0.84, r = 0.88)\) log10(peak TSH) versus log10(basal TSH) data did not differ significant \((P > 0.05)\). Because the hypothyroid data consist of multiple correlated samples from about half the number of individuals as used for the normal range, a bias in the variance of this region may have resulted.

Six representative plots of within-individual log10(peak TSH) versus log10(basal TSH) data for hypothyroid subjects are presented in Fig. 2. The large variability of the log10(peak TSH) versus log10(basal TSH) regression between individuals is apparent from Fig. 2. The slope for log10(peak TSH) versus log10(basal TSH) for 14 hypothyroid subjects (each having 3–8 TRH tests) ranged between 3.04 and 0.03 with a mean of 0.88 ± 0.70 (Sd). The correlation coefficient for the within-individual log10(peak TSH) versus log10(basal TSH) regressions ranged between 1.0 \((P < 0.01)\) and 0.23 \((P > 0.05)\) with a mean correlation coefficient, following the Fisher ‘z’ transformation (Snedecor & Cochran 1967), of 0.94 \((P < 0.01)\) for the 14 patients. It is important to note, again, that the variability of the ‘over-all’ regression analysis in the hypothyroid region of Fig. 1 results from contributions both within individuals and between individuals as evident in Fig. 2.

The over-all relationships between basal TSH and total T4 and total T3 in hypothyroid individuals are illustrated in Fig. 3. Polynomial regression analysis demonstrated significant terms in \(X (P < 0.001)\) and \(X^2 (P < 0.01)\) only. Results for a hyperbolic regression analysis are given in Fig. 3A and 3D. The basal and peak TSH varied in a linear fashion relative to the thyroid hormone levels with a negative correlation observed in most individual hypothyroid subjects as seen in Fig. 3C and 3F. The cases of positive correlation of TSH with serum T3 and T4, though less frequently observed, are entirely compatible with the overall distribution of regression variables as discussed below. A partial linearization of the combined, essentially hyperbolic, basal TSH versus T3 and basal TSH versus T4 plots was obtained by a logarithmic transformation of either the TSH data, Fig. 3B and 3E, or the T3 and T4 results (not shown) without affecting the linearity of the within-individual data significantly. Logarithmic transformation of both the TSH and thyroid hormone concentrations increased the correlation coefficient for the TSH versus T3 and TSH versus T4 regression lines for combined data only slightly above that for logarithmic transformation of the corresponding basal TSH values only. The effect of logarithmically transforming both TSH and thyroid hormone concentration on the correlation coefficients for within-individual data was variable.

The multiple linear regression of log10(basal TSH) versus T4 and T3 for all hypothyroid subjects is given by

\[
\log_{10}\text{TSH} = 2.12 - 0.0596 \text{T4} - 0.0040 \text{T3},
\]

The partial regression coefficients for T4 and T3 were highly significant by the F-test, \(P < 0.005\). The following correlation coefficient were also obtained: log10TSH versus T4: \(-0.70, P < 0.01\); log10TSH versus T3: \(-0.73, P < 0.01\); T4 versus T3: \(0.81, P < 0.01\).

The average basal TSH and TSH versus T3 slopes for individual hypothyroid patients were found to be negatively correlated, as shown in Fig. 4. For those individuals with the highest average
basal TSH values, the suppression per increment in measured plasma T4 and T3 was the greatest; i.e. the slope values are large and negative. Conversely, those with relatively low average basal TSH values, despite a severe state of hypothyroidism, were proportionately less responsive to the suppressive effects of thyroid hormone replacement. Stimulation of TSH release with increasing thyroid hormone levels was seen in two hypothyroid patients with very low T3 and T4 levels and relatively low serum TSH values. This is consistent with the observation in Fig. 4 that 35% of the joint 95% confidence ellipse for average basal TSH and TSH versus T3 slope lies in the region corresponding to positive slope values. The average basal TSH rather than the TSH intercept at zero serum T3 concentration was used to avoid the problem of a priori correlation of the least squares slope and intercept. Combining the measurements of pituitary-thyroid status from hypothyroid subjects who are becoming progressively more or less hypothyroid does not bias the slopes of the least squares lines in Fig. 4. Parallel line analysis of the corresponding regressions for the two groups of hypothyroid subjects considered separately showed no significant differences.
Regression of average basal TSH against TSH vs. T₃ slope for 10 hypothyroid subjects. The least square lines are: 
\[ Y = -55.33X + 3.43 \quad (Y \text{ vs. } X) \]
\[ Y = -50.11X + 26.78 \quad (X \text{ vs. } Y) \]
\( r = -0.84, P < 0.01 \). The oval encircles 95% of the volume under the associated bivariate Gaussian distribution. The downward pointing triangles correspond to hypothyroid subjects receiving partial replacement therapy consisting of increasing doses of thyroid hormone. Upward pointing triangles denote hypothyroid subjects maintained on full thyroid hormone replacement and then withdrawn abruptly from all thyroid hormone supplementation.

**Discussion**

Graphic representations of serum TSH versus protein-bound iodine (Reichlin 1971; Reichlin & Utiger 1967) or total T₄ and free T₄ (Cotton et al. 1971; Reichlin & Utiger 1967) obtained with data from several hypothyroid subjects are characteristically hyperbolic in shape. In Fig. 3C and 3F, it is apparent that the effect of thyroid hormone, either stimulatory (Gershengorn 1978; Ridgway 1979; Fischer et al. 1978; Sawin et al. 1978) or inhibitory (Cotton et al. 1971; Reichlin 1971; Reichlin & Utiger 1967), on basal (and peak) TSH levels within the individual hypothyroid patient is linear.

Patients with a pituitary which is relatively insensitive to thyroid hormone suppression or which actually increases its TSH release during partial thyroid hormone replacement also have relatively low initial basal and peak TSH values, even in the severely hypothyroid state. On the other hand, those with a pituitary which is sensitive to thyroid hormone have the highest average basal plasma TSH levels in severe hypothyroidism (Fig. 4); they secrete the greatest amount of TSH in response to a bolus of TRH (Figs. 1 and 2), and decrease their TSH secretion most readily with exogenous T₄ and T₃ (most negative slope values in Fig. 3). Fig. 4 demonstrates that the negative correlation between the average basal serum TSH at all thyroid hormone replacement levels and the initial rate of thyroid hormone suppression or stimulation (slope value) is significant. This negative correlation is the basis for the hyperbolic pattern usually seen in TSH versus thyroid hormone data averaged between individuals as in Fig. 3A and 3D.

The present results on the plasma TSH-suppressive effects of exogenous T₄ in hypothyroid patients resemble those obtained in rats (Reichlin 1971) for which some mechanistic insights have been gained. The major differences in the negative-feedback regulation of TSH secretion by T₄ and T₃ in humans (Sawin et al. 1977, 1978; Wartofsky et al. 1976; Fischer et al. 1978) versus rats (Reichlin 1971) appears to be kinetic in nature. The TSH suppression observed in man, on the one hand, follows a much slower time course, with maximal decrease observed after 3 days following single intravenous or oral doses of T₃, well after the early elevation of plasma T₃ concentrations has returned to normal (Azizi et al. 1975; Ingbar & Woeber 1968; Wenzel et al. 1975). This inhibition decreases over the ensuing 3 to 4 days, so that by
the 7th day, responsiveness to TRH is near normal (Azizi et al. 1975; Wenzel et al. 1975). A greater delay, necessitated perhaps by the conversion of T₄ to T₃, has been noted with a larger iv dose of T₄ (Wenzel et al. 1975).

In thyroidectomized rats, on the other hand, maximal suppression of plasma TSH follows by about 2 h the injection of T₃ (Silva & Larsen 1977) and by 1 h the maximal occupation of the nuclear T₃ binding sites. Inhibition of protein synthesis by actinomycin D or cycloheximide blocks the TSH-suppressive effects of thyroid hormones in vivo and in vitro (Bowers et al. 1968; Vale et al. 1968). The percentage of the plasma TSH suppression from hypothyroid levels appears to be approximately proportional to the degree of nuclear T₃ receptor site occupation (Silva & Larsen 1977). Moreover, nuclear occupancy is very nearly a linear function of free T₃ concentration throughout the hypothryoid and normal ranges (Oppenheimer 1979) which extend up to 50% receptor occupancy (Silva & Larsen 1977).

If protein synthesis is severely depressed by lack of thyroid hormone (Mathews et al. 1973; Oppenheimer 1979; Wong et al. 1977), then the initial response to thyroid hormone replacement will be an increase in TSH synthesis (D’Angelo et al. 1976) and release (Gershengorn 1978; Sawin et al. 1978; Wartofsky et al. 1976) due, at least in part, to enhanced thyrotriph sensitivity to TRH (Fischer et al. 1978). The protein synthesis-dependent suppression of TSH release will be expressed only after the general level of synthesis of all proteins, including TSH, becomes adequate. On the other hand, individuals who maintain adequate synthesis of proteins even in severe hypothyroidism should be exquisitely sensitive to thyroid hormone therapy because the protein synthesis-requiring negative-feedback does not then require ‘priming’ by thyroid hormone and the high TSH levels may contribute to the inhibitory process itself (Roti et al. 1978). Ultimately, suppression of TSH secretion will be observed in all hypothyroid subjects given sufficient replacement therapy.

The linear regression lines relating TSH and thyroid hormone levels in the serum of hypothyroid subjects receiving increasing partial replacement or undergoing thyroid hormone withdrawal may thus have positive as well as negative slopes and still be arrayed in a pattern which, when averaged, simulate an hyperbola. This pattern is consistent with the mutual dependence of TSH synthesis and negative-feedback inhibition on the same rate-limiting process of protein synthesis (Bowers et al. 1968; Vale et al. 1968) and an extraordinary variability in the sensitivity of protein synthesis to thyroid hormone deficiency within individual hypothyroid patients.

References


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