RADIOIMMUNOASSAY OF THYROTROPHIN-RELEASING HORMONE (TRH) IN NORMAL SUBJECTS, IN ABNORMAL THYROID STATES AND UNDER CATECHOLAMINERGIC INFLUENCES

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ABSTRACT

Plasma TRH was determined by radioimmunoassay in normal and abnormal thyroid state. In euthyroid adult subjects, the plasma TRH level were 31.9 ± 11 pg/ml (mean ± sp). No significant differences were observed with regard to sex, pregnancy or age, except for the acute increase in newborn infants. The plasma TRH levels evaluated in abnormal thyroid states were found significantly ($P < 0.001$) increased in hyperthyroidism (49.1 ± 14 pg/ml) and decreased in hypothyroidism (22.1 ± 6 pg/ml). Nor were any significant differences observed in simple goitre, pituitary adenoma and empty sella. Lastly, plasma TRH and TSH levels were also evaluated after acute administration of L-dopa, 2-Br-$\alpha$-ergocriptine and chlorpromazine in normal and hypothyroid subjects. Contemporaneous modifications of plasma TRH and TSH levels (decreased after L-dopa and 2-Br-$\alpha$-ergocriptine, increased after chlorpromazine) were observed in hypothyroidism.

The results indicate that plasma TSH modifications at birth as well as under catecholaminergic influences are at least partially mediated by variations of TRH secretion. Moreover, the inverse plasma TRH and TSH modifications in abnormal thyroid states agree with different feedback effects at the hypothalamic (positive) and pituitary (negative) level. It is possible that the significance of the positive feedback at the hypothalamic levels can be interpreted when taking into account the extra-pituitary effects of TRH.
Immunoreactive TRH has been determined in hypothalamic and extra-hypothalamic areas of the brain (Bassiri & Utiger 1974; Brownstein et al. 1974; Jackson & Reichlin 1974a,b; Oliver et al. 1974c,d, 1975a,b; Winokur & Utiger 1974; Jeffcoate et al. 1975; Montoya et al. 1975), in the cerebrospinal fluid of man (Oliver et al. 1974d, 1975a; Shanbough et al. 1975), in portal blood of the rat (Eskay et al. 1975), in systemic blood and urine of man and rat (Oliver et al. 1974a,d, 1975a,b; Emerson & Utiger 1975; Eskay et al. 1975; Montoya et al. 1975; Mitsuma et al. 1976). When considering the significance of TRH radioimmunoassay, it must be mentioned that tissue plasma and urine TRH show, as compared to synthetic TRH, the same immunoreactivity, elution pattern during gel filtration on Sephadex G-10 and electrophoretic migration on Whatman 3 MM (Oliver et al. 1974a). However, plasma and urine TRH is not destroyed during incubation in plasma at 37°C (Oliver et al. 1975b), in contrast to enzymatic degradation of immunoreactive synthetic TRH by enzymes present in blood (Jeffcoate et al. 1975; Eskay et al. 1976). The reason for this discrepancy is not clear. These findings, however, suggest caution in interpreting the results of urine or plasma TRH immunoassay and the significance of the TRH-like immunoreactivity found in plasma and urine samples.

The present study carried out in man indicates that plasma TRH, plasma TSH and thyroidal function states show some physiological and pathological correlations. These relationships are reliable evidence of the biological significance of plasma TRH radioimmunoassay.

**MATERIALS AND METHODS**

**Clinical studies**

Plasma TRH was estimated by radioimmunoassay in 30 normal subjects (16 males and 14 females) aged 20–40 years, and in 10 normal pre-puberal subjects (6 males and 4 females) aged 6–9 years. Moreover, studies of plasma were performed on 16 pregnant women and 3 normal infants at birth and after 1/2, 1, 2, 6, 24 and 48 h. Plasma TRH was evaluated in hyperthyroidism (15 cases), hypothyroidism (10 cases), simple goitre (13 cases), pituitary adenoma (4 cases of chromophobe and 1 case of acidophil adenoma) and empty sella (2 cases). The thyroid function was evaluated by determining the total plasma thyroxine and triiodothyronine using the T₄ and T₃ Kit set RIA (Richter, Milan, Italy) and the plasma TSH using TSH Kit set RIA (Biodata, Milan, Italy). In 5 normal and 3 hypothyroid subjects, the catecholaminergic effects on TRH and TSH secretion were evaluated by acute administration of L-dopa, 2-Br-α-ergocriptine and chlorpromazine. L-dopa was given in one oral dose (0.5 g), 2-Br-α-ergocriptine in one oral dose (5 mg), chlorpromazine in one im dose (0.7 mg/kg body weight). Blood samples were taken at −15, 0, 30, 60, 120, 180, 240, 300 and 360 min by means of an indwelling venous catheter.
**TRH radioimmunoassay**

Plasma TRH was measured by radioimmunoassay according to Oliver et al. (1974a). Synthetic TRH was used for iodination and as standard. The antibody (courteously supplied by Dr. Oliver, Laboratoire de Médecine Expérimentale, Marseilles, France) was produced in rabbits by immunization with synthetic TRH combined with bovine serum albumin (BSA) using bis-diazotized benzidine, as described by Bassiri & Utiger (1972). Immunization was performed by injecting 75–150 μg TRH once to each rabbit using the multisite injection of Vaitukaitis et al. (1971). The antibody was collected 95 days after injecting the TRH-albumin complex. The antiserum was usable at a titre of 1/250. Five μg of synthetic TRH were labelled with 1 mCi of 125I according to Greenwood et al. (1963). The calculated specific activity of iodination was 120 μCi/μg. Purification of the labelled TRH was carried out by gel filtration using Sephadex G-10. The assay was performed by incubation at 4°C for 48 h, and by separation of free and bound labelled TRH with a charcoal suspension. Specificity of the antiserum was evaluated by determining its cross-reactivity with 31 TRH analogues, 5 amino acids, LH-releasing hormone, GH-inhibiting factor and MSH release inhibiting factor. Significant cross-reactivity was observed only with L-pGlu-L-His-L-Hyp-NH₂, LDL-TRH and L-pGlu-L-His-L azotidine carboxamide (considering TRH reactivity as 1, the relative potency was 0.4, 0.25 and 0.24, respectively). Minimum sensitivity of this assay was 1 pg. The intra-assay coefficient was 12.7 %, 9.6 % and 5.9 % for the lower, middle and upper portions of the standard curve, respectively. The inter-assay coefficient was 12.6 %, 11.4 % and 10.7 %, respectively, for the same portions of the standard curve. Blood was collected on heparin and BAL and kept in chilled ice. After centrifugation at 2°C the plasma was separated and quickly frozen at −20°C. TRH was then extracted on charcoal and eluted with 75 % ethanol. The recovery was 59.7 ± 0.5 %. All the determinations were carried out in triplicate in the same radioimmunoassay.

**RESULTS**

**Normal subjects**

In euthyroid adult subjects the plasma TRH was 31.9 ± 11 pg/ml (mean ± sd). There was no significant difference with regard to sex: 33.2 ± 11 pg/ml in females vs. 30.8 ± 10 pg/ml in males. Moreover, no significant difference was noted between pre-puberal and adult euthyroid subjects (31.7 ± 9 pg/ml vs. 31.9 ± 11 pg/ml) nor between non-pregnant (33.2 ± 11 pg/ml) and pregnant women (30 ± 14 pg/ml, 32 ± 13 pg/ml, 35.8 ± 12 pg/ml in the first, second and third trimester of pregnancy, respectively).

In normal newborn infants a marked increase in plasma TRH levels was observed (Fig. 1). Plasma TRH values, still moderately high at birth with a mean of 46 pg/ml (range 34–57) reached rapidly a peak of 78 pg/ml (range 60–93) 30 min after delivery. Plasma TRH levels decreased rapidly between 30 min and 2 h post-partum, then fell gradually to the normal range at 24 h. A comparison between the plasma TRH and TSH levels measured simultaneously showed similar modifications in both hormones after birth. Changes in plasma T₃ and T₄ concentration followed the plasma TSH increase (Fig. 1).
Fig. 1.
Plasma TRH, TSH, T₄ and T₃ concentrations in newborn infants (mean values ± sd).

Fig. 2.
Plasma TRH, TSH, T₄ and T₃ concentrations in abnormal thyroid states. Mean values (height of the columns) ± sd (vertical bars). Results are analyzed by Student's t-test and the statistical significance is indicated.
Plasma T₃ levels, significantly lower at birth than the mean for maternal values, increased gradually with a peak at 24 h. Plasma T₄ levels in the range of maternal values at birth, showed similar modifications of T₃, although less pronounced.

In conclusion, no significant differences were observed in plasma TRH levels with regard to sex, pregnancy or age, except for a marked increase in newborn infants.

Abnormal thyroid states

The results are shown in Fig. 2. The plasma TRH levels (mean ± s.d) in hyperthyroidism were 49.1 ± 14 pg/ml and in primary hypothyroidism 22.1 ± 6 pg/ml vs. 31.9 ± 11 pg/ml in normal subjects. The difference is clearly significant in both cases (P < 0.001). In hyperthyroid patients the plasma levels were more markedly (53.7 ± 13 pg/ml) and constantly higher in Graves’ disease than in toxic adenoma (42.1 ± 14 pg/ml). In compensated toxic adenoma, normal or high TRH levels were observed. In simple goitre, plasma TRH levels were 34.1 ± 12 pg/ml, without any significant differences as compared to normal subjects. Lastly, in pituitary adenoma and empty sella, plasma TRH levels were in normal range (Fig. 3). Laboratory and clinical signs of secondary hypothyroidism were present only in one case of pituitary adenoma. In conclusion, plasma TRH levels were significantly increased in hyperthyroidism and decreased in hypothyroidism, showing modifications in inverse direction with regard to TSH levels, in the same direction with regard to T₄ and T₃ levels.

Plasma TRH, TSH, T₄ and T₃ concentrations in pituitary adenoma and empty sella. Mean values (solid line) ± s.d (interrupted line).
Plasma TRH and TSH concentrations after acute administration of L-dopa (left side) and 2-Br-α-ergocriptine (right side) in normal subject (upper part) and in hypothyroidism (lower part). Mean values are indicated by continuous line.

Fig. 4.

Plasma TRH and TSH concentrations after acute administration of chlorpromazine in normal subject (left side) and in hypothyroidism (right side). Mean values are indicated by continuous line and individual values by interrupted line.

Fig. 5.
**Catecholaminergic influences**

The results are shown in Figs. 4 and 5. A comparison of plasma TRH and TSH levels measured simultaneously after acute administration of L-dopa and 2-Br-a-ergocriptine, showed similar modifications of both hormones. A decrease in TRH and TSH levels, reaching in a nadir 1–2 h after L-dopa and 4–5 h after 2-Br-a-ergocriptine (CB-154) was clearly observed in hypothyroid patients (Fig. 4). In contrast, acute administration of chlorpromazine to hypothyroid patients showed an increase of TSH and TRH with a peak at 120 min (Fig. 5). No significant modifications were observed in normal subjects.

In conclusion, contemporary modifications of plasma TRH and TSH levels were observed in hypothyroidism under catecholaminergic influences.

**DISCUSSION**

In the present study the euthyroid plasma TRH levels of 31.9 ± 11 pg/ml agree with the values previously observed in a smaller number of cases by Oliver et al. (1975a). No significant differences were observed in plasma TRH levels with regard to sex, pregnancy or age, except for a marked increase in newborn infants. The absence of differences in plasma TRH between pregnant and non-pregnant women is not in contrast to the well-known increase of T₄ and, to a lesser extent, of T₃ in pregnancy since this increase is due to the increased concentrations of TBG (Burrow 1972) so that the absolute concentration of free thyroid hormones and the consequent feed-back influence, remains essentially normal. Our results showing a marked increase of plasma TSH, T₃ and T₄ modifications after birth are in agreement with the results of Fisher & Odell (1969) and Abuid et al. (1973). The finding of plasma TRH values higher at birth is in agreement with precedent observations (Oliver et al. 1975b). In addition, the rapidly increased concentration of plasma TRH after birth demonstrates that the acute TSH surge in newborn infants may at least be partially due to a primitive neurohormonal event modifying TRH secretion. Considering the hypothesis that the increase in the thyroid function at birth is at least partially a response to cold (Fisher & Odell 1969), it is interesting to note the increase of plasma TRH and TSH in the rat exposure to cold (Eskay et al. 1975; Montoya et al. 1975), even if such a plasma TRH increase is not reported by Emerson & Utiger (1975).

Our results on catecholaminergic influences on plasma TSH, showing a depression of this hormone in hypothyroidism after oral L-dopa and 2-Br-a-ergocriptine administration, are in agreement with the results of Rapoport et al. (1973), Refetoff et al. (1974) and Miyai et al. (1974). The plasma TSH increase after chlorpromazine administration agrees with the observations of Ferrari et al. (1976). In addition, our study shows contemporary modifications of
plasma TRH. Although these findings confirm catecholaminergic influences on TSH secretion and dopaminergic inhibitory influence, caution should be exercised in the interpretation of the results, taking into account the complexity of effects of these drugs on CNS and the influences of dose and routes of administration. In fact, a diphasic response of plasma TSH (initial increase followed by depression) has been observed by Minozzi et al. (1975), using different doses and routes of L-dopa administration. In every case, contemporary modifications of plasma TRH and TSH levels seem to indicate that the effects of these drugs are exercised at least partially at hypothalamic level.

Our findings in abnormal thyroid states of a significant increase in plasma TRH levels in hyperthyroidism and of a decrease in hypothyroidism confirm the previous results of Oliver et al. (1975a), whereas they are in contrast with the results of Mitsuma et al. (1976). These investigators have found plasma immunoreactive TRH levels in hyperthyroidism below the limits of detectability and a marked increase in primary hypothyroidism. This discrepancy cannot be easily explained. It must be stressed that there are certain differences in the radioimmunoassay methodology, particularly a difference in the reagents used to prevent TRH inactivation by serum. In our method, BAL (2,3 dimercapto-1-propanol) was used whereas Mitsuma et al. (1976) used a mixture of HQ (8-hydroxyquinoline sulphate) and T (Tween 20). When using urine radioimmunoassay in man, some investigators have not found significant TRH variation between hyper- and hypothyroidism (Oliver et al. 1975a; Gagel et al. 1975; Martino et al. 1976). It is possible that absence of changes in urinary TRH concentration, in contrast to significant changes in plasma, may be justified by major non-specific interference in urine radioimmunoassay. It must be underlined that Vagenakis et al. (1975) reported an interference of urea in urine TRH assay and inactivation by urease of urinary TRH immunoreactivity. Although Martino et al. (1976) have not observed an interference of urea, interference cannot be excluded of other substances greater in urine than in plasma TRH radioimmunoassay. Conflicting results have also been reported in the rat. Eskay et al. (1975) have observed plasma TRH modifications in a rat similar to those observed by us in man, while Emerson & Utiger (1975) and Montoya et al. (1975) reported no changes in euthyroid, hypothyroid and hyperthyroid rats. Lastly, the absence of modifications of hypothalamic TRH concentrations in various thyroid abnormalities in the rat (Bassiri & Utiger 1974; Montoya et al. 1975) may be explained either on the ground of coupling between the TRH secretion and synthesis rates or taking into account the consideration of Bassiri & Utiger (1974) that significant TRH secretion can occur with minimal depletion of hypothalamic TRH stores. Plasma TRH modifications in inverse direction to TSH levels in opposite thyroid function states is in agreement with the positive feedback of thyroid
hormones at the hypothalamic level and with negative feedback at the pituitary level, as hypothesized by Reichlin et al. (1972), in their study of brain TRH synthetase.

Considering the hypophyseotrophic effects of TRH, the interpretation of the positive feedback of the thyroid hormones on TRH secretion is not clear, but it must be outlined that TRH, besides the well-known stimulatory effect on thyrotrophin release, seems to operate as a central neurotransmitter (Jackson & Reichlin 1974b), as has also been suggested for other hypothalamic hormones (Marks 1975), justifying the behavioural changes directly produced by TRH administration. On the other hand, extra-pituitary effects of TRH are also in agreement with the presence of TRH in other areas of CNS besides the hypothalamus. In fact, TRH is also present in the thalamus, brain stem cerebrum and cerebellum (Oliver et al. 1974d). Moreover, these investigators (Oliver et al. 1974b,d) have also hypothesized a brain secretion of TRH into the cerebral spinal fluid and subsequently into the hypophyseal portal systemic blood. Lastly, studies on the subcellular distribution of TRH have demonstrated a very high concentration of the synaptosomal fraction (Winokur & Utiger 1975).

In conclusion, the observed relations between plasma TRH immunoreactivity and TSH levels after birth, under catecholaminergic influences and in abnormal thyroid function states are reliable for the biological significance of plasma TRH radioimmunoassay.

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