

# Effect of Thyrotropin-Releasing Factor on Serum Thyroid-Stimulating Hormone

## AN APPROACH TO DISTINGUISHING HYPOTHALAMIC FROM PITUITARY FORMS OF IDIOPATHIC HYPOPITUITARY DWARFISM

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**ABSTRACT** To test the hypothesis that the primary defect in some patients with idiopathic hypopituitary dwarfism is failure to secrete hypothalamic hypophysiotropic-releasing factors, synthetic thyrotropin-releasing factor (TRF), 500  $\mu$ g, was given intravenously, and timed venous samples obtained for determination of the concentration of plasma TSH by radioimmunoassay in three groups of subjects: (a) 11 patients without evidence of endocrine or systemic disease, (group I) (b) 8 with isolated growth hormone deficiency and normal thyroid function, (group II) and (c) 9 patients with idiopathic hypopituitary dwarfism and thyroid-stimulating hormone (TSH) deficiency (group III). The mean fasting plasma TSH value was 4.1  $\mu$ U/ml in group I, and 3.9  $\mu$ U/ml in group II; in both groups there was a brisk rise in plasma TSH to peak levels of 12–45  $\mu$ U/ml at 30–45 min, and a fall toward base line levels at 120 min. All children in group III had basal TSH levels of < 1.5  $\mu$ U/ml; one failed to respond to TRF; eight exhibited a rise in plasma TSH with peak values comparable with those in groups I and II. In four of eight children in group III who responded to TRF, the TSH response was delayed and the initial rise in plasma TSH was not detectable until 10–60 min. In these four patients, plasma TSH levels continued to rise at 120 min.

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The mean fasting concentration of plasma thyroxine iodide ( $T_4$ ) in subjects with normal thyroid function (groups I and II) was 5.6  $\mu$ g/100 ml, and the mean plasma  $T_4$  level at 120 min was 6.6  $\mu$ g/100 ml. This difference between fasting and postTRF plasma  $T_4$  was significant ( $P < 0.001$ ) by paired analysis. Mean fasting plasma  $T_4$  concentration in group III patients was 1.3  $\mu$ g/100 ml; after TRF a significant rise in  $T_4$  concentration was not detected in this group.

The results indicate that TRF test is useful in distinguishing between primary hypothalamic and pituitary forms of TSH deficiency. In light of the evidence of TRF deficiency in eight of nine patients with idiopathic hypopituitary dwarfism, it seems likely that in these patients, other pituitary hormone deficiencies may be attributable to deficiency of their respective releasing factors.

## INTRODUCTION

A large body of experimental data supports the concept of the hypothalamic neurohumoral control of pituitary hormone secretion (see reviews by Harris [1], Reichlin [2], McCann and Porter [3], Everett [4], Burgus and Guillemin [5], and Meites [6]). Axons arising in the basal hypothalamus pass to the median eminence, where hypophysiotropic neurosecretions are released to the primary capillary plexus of the hypophyseal portal vessels.

Thyrotropin-releasing factor (TRF)<sup>1</sup> is the first of the hypothalamic-releasing factors whose structure is known. Burgus and associates (7–9) have reported that

<sup>1</sup> Also known as thyrotropin-releasing hormone (TRH).

TABLE I  
*Patients with Idiopathic Hypopituitary Dwarfism and TSH Deficiency (Group III)*

Patient	Age	Height deficit (sd*) before therapy	Fasting plasma T <sub>4</sub>	Fasting plasma TSH	HGH deficiency	ACTH deficiency
	yr		μg/100 ml	μU/ml		
L. R.	19-11/12	— 5.0	0.3	<1.5	+	+
J. B.	17-6/12	— 5.7	1.0	<1.5	+	+
J. O.	15-5/12	— 6.8	2.0	<1.5	+	+
L. N.	9-6/12	— 5.5	2.4	<1.5	+	+
J. M.	19-11/12	— 4.6	0.5	<1.5	+	+
J. T.	12-0/12	— 6.9	1.1	<1.5	+	—
D. W.	24-0/12	—12.0	1.5	<1.5	+	—
P. D.	13-10/12	— 6.0	1.4	<1.5	+	+
J. R.	9-1/12	—11.0	1.3	<1.5	+	—
Mean	15-8/12	—7.1	1.3			

\* sd below the mean value for height for chronological age.

ovine TRF<sup>a</sup> is a modified tripeptide with the structure (pyro) GLU-HIS-PRO (NH<sub>2</sub>). Similar and independent studies by Nair, Barrett, Bowers, and Schally (10); Folkers, Enzmann, Boler, Bowers, and Schally (11); and Schally, Redding, Bowers, and Barrett (12) indicated an identical structure for porcine TRF. Synthetic TRF and its analogues have been prepared, and structure-activity relationships studied by both of the above groups (9, 12–14) and by other workers (15–17). Natural and synthetic TRF is active in the mouse (14, 16, 18, 19), rat (20), and man (16, 17, 21–24) and, hence, seems to lack species specificity. It is effective whether administered intravenously, intramuscularly, intraperitoneally, subcutaneously, or orally. The most rapid increase in plasma thyroid-stimulating hormone (TSH) levels in man occurs after intravenous administration. No specific effect on other pituitary hormones has been found. In normal adult man, the concentration of plasma TSH rises within 5–10 min of intravenous TRF administration, reaches peak levels at 20–40 min, and then declines gradually toward fasting values by 120 min. Maximal responses to intravenous synthetic TRF occur when about 500 μg are administered, with peak plasma TSH levels reaching 3–15 times fasting levels.

The response of plasma TSH to the administration of synthetic TRF provides an approach to the assessment of TSH reserve and a possible means of distinguishing between hypothalamic and pituitary forms of TSH deficiency. Space-occupying lesions of either the hy-

pothalamus or pituitary gland can result in pituitary insufficiency; however, the site of the defect in idiopathic hypopituitarism is uncertain. Previously (25), we suggested that in some patients with idiopathic hypopituitary dwarfism, the primary endocrine defect may be a deficiency of hypothalamic hypophysiotropic-releasing factors rather than a pituitary abnormality. The availability of synthetic TRF, active in man, provides a means of testing this hypothesis in patients with idiopathic hypopituitary dwarfism who have TSH deficiency.

## METHODS

Tests were performed in the morning after an overnight fast in three groups of patients, all from the Pediatric Endocrinology Clinic, University of California San Francisco.

Group I, 11 boys with no endocrine or systemic disease, of which 9 had constitutional short stature, and 2 primordial dwarfism (25); mean age 7 yr 7 months; group II, 8 patients (6 females, 2 males) with idiopathic isolated growth hormone deficiency (25); mean age 13 yr 7 months; group III, 9 patients (8 males, 1 female) with multiple anterior pituitary hormone deficiencies, including deficient TSH, and no demonstrable intracranial mass; mean age 15 yr 8 months.

The response of plasma growth hormone to provocative stimulation with arginine and insulin was evaluated as previously described (25, 26). All children in group I had clearly normal responses (peak concentration of plasma HGH greater than 7.0 ng/ml in all cases), whereas all those in groups II and III were shown to be deficient in growth hormone (highest peak HGH 2.9 ng/ml and 2.0 ng/ml, respectively).

Pituitary ACTH-adrenocortical function was assessed by determination of plasma cortisol at 0 and 60 min of the insulin tolerance tests, as well as by testing with metyrapone and adrenocorticotropin (ACTH) (25, 27–29). All children in groups I and II had normal responses, whereas six of nine children in group III were found to be deficient in ACTH (Table I). All six of these patients had 24 hr excretion of urinary 17-hydroxycorticosteroids of less than

<sup>a</sup> *Abbreviations used in this paper:* HGH, human growth hormone; HTSH, human thyroid-stimulating hormone; PIF, prolactin inhibitory factor; T<sub>4</sub>, plasma thyroxine iodide; TRF, thyrotropin-releasing factor; TSH, thyroid-stimulating hormone.

TABLE II

Mean Plasma TSH Concentration ( $\mu\text{U/ml}$ ), before and after Administration of TRF

Time <i>min</i>	Group I	Group II	Group III	Significance§ groups I and II vs. group III
	(11 normal children)	8 children with isolated growth hormone deficiency	8 hypopituitary dwarfs with TSH deficiency who responded to TRF	
0	4.1 ( $\pm 1.0$ )*	3.9 ( $\pm 1.3$ )	<1.5†	<0.005
5	11.5 ( $\pm 3.0$ )	13.3 ( $\pm 3.3$ )	5.2 ( $\pm 1.8$ )	<0.025
10	16.6 ( $\pm 3.6$ )	16.7 ( $\pm 3.1$ )	9.6 ( $\pm 3.0$ )	>0.05
20	19.6 ( $\pm 3.5$ )	22.1 ( $\pm 3.7$ )	11.2 ( $\pm 3.2$ )	>0.1
30	21.4 ( $\pm 3.5$ )	20.6 ( $\pm 3.1$ )	16.0 ( $\pm 3.5$ )	>0.2
45	19.1 ( $\pm 2.8$ )	17.6 ( $\pm 2.6$ )	19.0 ( $\pm 4.6$ )	>0.5
60	17.1 ( $\pm 2.8$ )	12.3 ( $\pm 1.4$ )	19.2 ( $\pm 4.0$ )	>0.2
120	11.5 ( $\pm 2.7$ )	8.0 ( $\pm 0.4$ )	21.2 ( $\pm 4.3$ )	<0.025

\* Figures in brackets are SEM.

† For the purpose of statistical analysis, all undetectable levels of plasma TSH were recorded as 1.5  $\mu\text{U/ml}$ , which is the lowest concentration of plasma TSH which can be measured with our assay.§ Analysis by the unpaired *t* test revealed no significant differences in plasma TSH values of groups I and II at any time during the TRF test. Therefore, the results in groups I and II were pooled and compared with the results of group III.

1.0 mg/m<sup>2</sup> and failed to exhibit a rise after administration of metyrapone (25); or when challenged with insulin, an increase in plasma cortisol (27, 29).

Plasma thyroxine iodide ( $T_4$ ) was measured by the method of Murphy and Pattee (30). Mean concentration of fasting  $T_4$  was 6.1  $\mu\text{g}/100\text{ ml}$  in group I and 5.0  $\mu\text{g}/100\text{ ml}$  in group II. In group III, all children had a fasting  $T_4$  concentration of less than 2.5  $\mu\text{g}/100\text{ ml}$  before the institution of replacement therapy with thyroid hormone. Thyroid and growth hormone therapy were discontinued 3–4 wk before admission, but small replacement doses of cortisone acetate were maintained. The mean fasting concentration of plasma  $T_4$  in group III patients on the morning of TRF administration was 1.3  $\mu\text{g}/100\text{ ml}$ .

Crystalline TRF,<sup>3</sup> dissolved in distilled water which contained 1% human serum albumin, to yield a solution of 500  $\mu\text{g}/\text{ml}$ , was passed through two Millipore filters (Millipore Corp, Bedford, Mass.) (pore size 0.45  $\mu$ ) in a Swinny Adaptor. 1 ml was injected rapidly through an indwelling venous catheter and venous samples collected for determinations of plasma TSH at –15, 0, 5, 10, 20, 30, 45, 60, and 120 min, and plasma  $T_4$  at 0 and 120 min.

**Radioimmunoassay of TSH.** TSH was assayed by a double-antibody radioimmunoassay procedure similar to that described by Odell, Rayford, and Ross (31) and by Utiger (32) with the following modifications: A purified preparation of human TSH<sup>4</sup> was iodinated with <sup>125</sup>I by the method of Greenwood, Hunter, and Glover (33). TSH, 2.5  $\mu\text{g}$ , was added to 0.025 ml of 0.5 M phosphate buffer (pH 7.5) followed by 2 mCi of carrier-free <sup>125</sup>I<sup>5</sup> of high specific activity (300 mCi/ml) then, in rapid order, 0.008 ml chloramine-T (35.2 mg/10 ml), 0.02 ml sodium metabisulfite

(24 mg/10 ml), and 0.05 ml of normal human serum. Labeled hormone was separated from free iodide by gel filtration with a 10 cm  $\times$  1 cm column of Sephadex G-75. Equilibration of the gel and elution from it was carried out with 0.01 M phosphate buffer, pH 7.8. Before the Sephadex column was used, crystalline bovine serum albumin, 2%, was passed through it, followed by a wash with 20 ml of the same buffer. Small portions of the labeled TSH peak were checked for damage, using 10% charcoal coated with 0.5% bovine serum albumin (34). Damage estimated by this procedure was consistently less than 10%.

Each assay was performed within 2 days of iodination. Assays were run in two stages: (a) unlabeled human TSH,

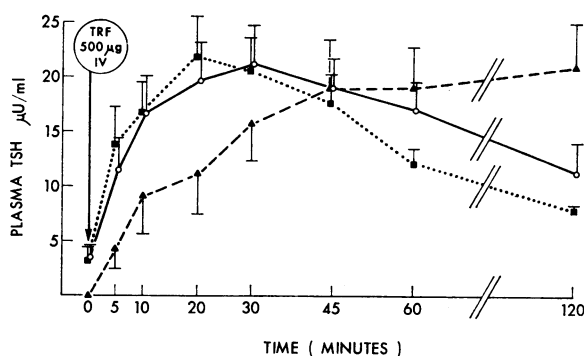
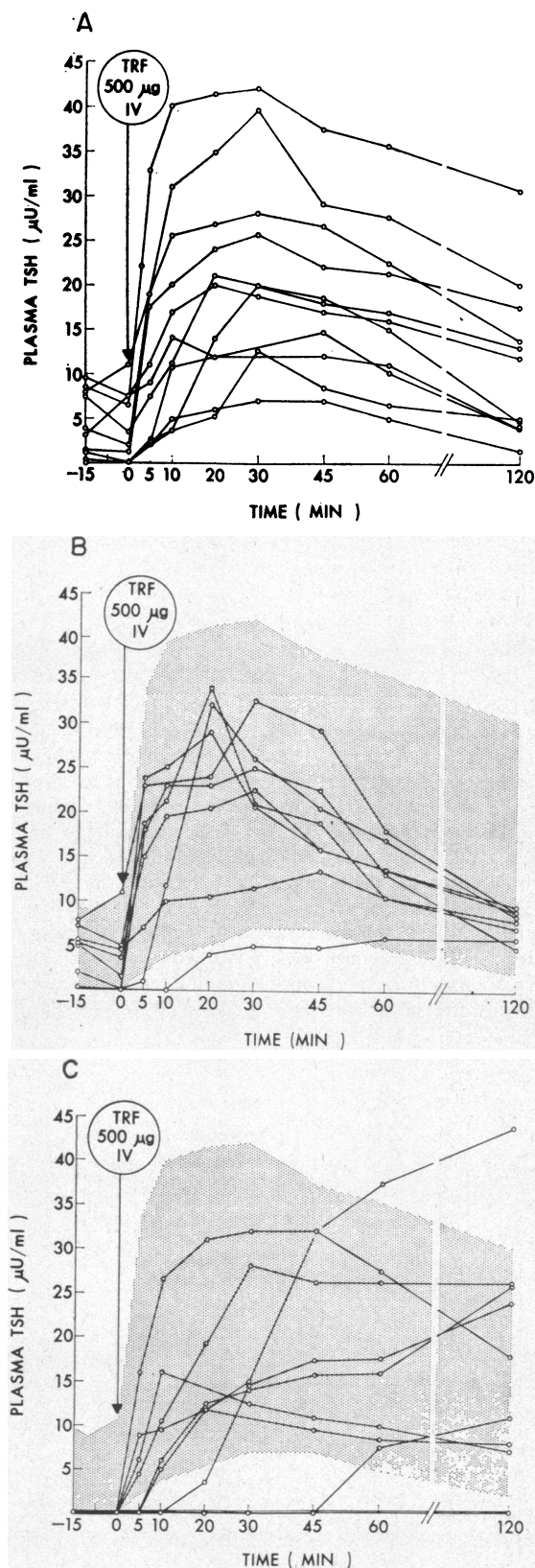


FIGURE 1 Mean fasting concentration of plasma TSH, and TSH release after TRF administration. (○—○) normal children; (■····■) patients with isolated growth hormone deficiency; (▲---▲) patients with idiopathic hypopituitary dwarfism and TSH deficiency who responded to TRF (the one patient with TSH deficiency who did not respond to TRF is not included).

<sup>3</sup> Kindly supplied by Dr. Roger Guillemin, Salk Institute, La Jolla, Calif.

<sup>4</sup> Supplied by National Institutes of Health, Bethesda, Md.

<sup>5</sup> Obtained from Cambridge Nuclear Corp., Iso/Serv Div., Cambridge, Mass.



0.5–20.0  $\mu\text{U}$  (MRC Standard A),<sup>6</sup> or 0.2–0.4 ml serum to be tested was incubated with 0.1 ml of 1:10,000 rabbit anti-human TSH.<sup>7</sup> The amount of serum in all tubes was equalized by adding 0.4 ml of normal dog serum to the tubes containing the TSH standard, and 0.2 ml to the tubes containing 0.2 ml serum. The diluent (0.01 M phosphate with 0.15 M saline buffer, pH 7.8, 0.01 M EDTA, 1% BSA [bovine serum albumin] and 10 IU HCG/ml) was added to each tube to give a volume of 0.9 ml. Control tubes were run with each assay, omitting unlabeled hormone in one set and anti-TSH and unlabeled hormone in the other. A tube was used without anti-TSH serum to check for damage during incubation.

The tubes were shaken and stored at 4°C for 24 hr, at which time HTSH-<sup>125</sup>I, 0.1 ml (15,000–20,000 cpm) was added, then incubated at 4°C for an additional 96–120 hr to achieve complete equilibrium. At the end of this period, 0.05 ml sheep anti-rabbit gamma globulin and 0.05 ml 1:50 normal rabbit serum were added to all tubes, resulting in a final volume of 1.1 ml; incubation at 4°C was carried out for an additional 24 hr.

Under these assay conditions, the binding of TSH-<sup>125</sup>I tracer ranged from 35 to 50% and significant displacement of TSH-<sup>125</sup>I was obtained with 0.5  $\mu\text{U}$  of unlabeled hormone. The mean difference in duplicates over the range of 20–45% binding was 2%. Sera tested in four consecutive assays varied by less than 10% in all cases.

## RESULTS

Fig. 1 and Table II show the mean resting plasma TSH levels and responses to TRF in the three groups. Fig. 2 illustrates the individual responses in normal children compared with those with isolated growth hormone deficiency and with idiopathic hypopituitarism and TSH deficiency.

Mean fasting level of TSH was 4.1  $\mu\text{U}/\text{ml}$  in the normal children and 3.9  $\mu\text{U}/\text{ml}$  in children with isolated growth hormone deficiency.<sup>8</sup> Children in both groups responded promptly to TRF, with plasma TSH levels reaching a peak of 12–45  $\mu\text{U}/\text{ml}$  at 20–30 min, then declining toward fasting levels at 120 min. Although there is a large variability in individual responses in each group, the children with isolated growth hormone deficiency responded in a manner similar to that of children without endocrine disease. Mean concentration of plasma TSH in group I and II did not differ signifi-

<sup>6</sup> Obtained from Dr. D. R. Bangham, Division of Biological Standards, National Institute for Medical Research, Mill Hill, London, England.

<sup>7</sup> Obtained from National Institutes of Health, Bethesda, Md.

<sup>8</sup> Mean fasting plasma TSH concentrations at -15 min were 3.9  $\mu\text{U}/\text{ml}$  in group I patients and 3.7  $\mu\text{U}/\text{ml}$  in group II patients.

FIGURE 2 Fasting plasma concentration of TSH and response to TRF in each patient. (A) 11 normal children; (B) 8 patients with isolated growth hormone deficiency; (C) 9 children with idiopathic hypopituitary dwarfism and TSH deficiency. Shaded area in (B) and (C) indicates the range of responses obtained in normal patients (A).

cantly, either at time 0, or at any time after TRF administration, utilizing the unpaired *t* test (35).

All children with idiopathic hypopituitary dwarfism and TSH deficiency had a fasting concentration of plasma TSH of less than 1.5  $\mu$ U/ml (Table II). One patient failed to exhibit an increase in plasma TSH to detectable levels throughout the period of testing. Eight of the nine patients responded to TRF (Fig. 2) with peak plasma TSH levels similar to those in groups I and II; four responded in a manner indistinguishable from normal children. In the other four, the TSH response was delayed and the initial rise in plasma TSH levels did not occur until 10, 10, 20, and 60 min, respectively. In these four patients, plasma TSH levels continued to rise at 120 min. Mean fasting plasma TSH concentration and the TSH level at 5 min after TRF administration in group III were both significantly lower than corresponding TSH levels in groups I and II. Plasma TSH level at 120 min after TRF in group III was significantly higher than the TSH concentration in groups I and II (Table II, Fig. 1) at that time.

The mean fasting concentration of plasma  $T_4$  in the subjects with normal thyroid function (groups I and II) was 5.6  $\mu$ g/100 ml. In 15 of 17 patients in these groups, plasma  $T_4$  rose after TRF; the mean level at 120 min was 6.6  $\mu$ g/100 ml. The difference between the fasting and postTRF plasma  $T_4$  was significant ( $P < 0.001$ ) (Table III).

The mean fasting concentration of plasma  $T_4$  in patients with idiopathic hypopituitary dwarfism and TSH deficiency was 1.3  $\mu$ g/100 ml. The concentration of plasma  $T_4$  did not change in the patient who failed to respond to TRF. Seven of eight patients who responded had levels of plasma  $T_4$  120 min after the administration of TRF slightly higher than fasting levels, but the difference was not significant.

Three patients experienced mild nausea or lightheadedness a few seconds after administration of TRF, but this disappeared spontaneously in less than 1 min. No other side effects were noted.

## DISCUSSION

The effect of synthetic TRF on the release of TSH in adults with organic forms of hypopituitarism (for example, neoplasms arising from the hypophysis), has been described in two recent reports by Fleischer et al. (22) and Hershman and Pittman (24). None of the patients exhibited a rise in the concentration of plasma TSH after the rapid intravenous administration of TRF.

It is now widely recognized that hypopituitary dwarfism not attributable to a mass lesion, is of heterogeneous etiology (25). We recently defined a newly recognized form of hypopituitary dwarfism associated with dysplasia

TABLE III  
Plasma  $T_4$  ( $\mu$ g/100 ml) before and after  
Administration of TRF

Time	Group I*	Group II	Group III
<i>min</i>			
0	6.1	5.0	1.3
120	7.1	6.0	1.8
"p"†	<0.025	<0.025	>0.200

\* When values for children with normal thyroid function (groups I and II) were pooled, the mean fasting concentration of plasma  $T_4$  was 5.6  $\mu$ g/100 ml and the mean  $T_4$  value at 120 min after TRF was 6.6  $\mu$ g/100 ml ( $P < 0.001$ ).

† Utilizing the paired *t* test.

of the optic nerves, malformation of the hypothalamus, and frequently absence of the septum pellucidum (36). Here, the hypopituitarism is ascribable to a congenital abnormality involving the hypothalamus and a deficiency of one or more hypothalamic-releasing factors. However, in other cases of idiopathic hypopituitary dwarfism, whether familial or sporadic in nature, where no gross anatomic abnormality can be found, it has not been possible to distinguish between primary hypothalamic or pituitary deficiency.

The present study supports the view that the TRF test is a useful direct measure of TSH reserve and provides a method for differentiating between primary hypothalamic and pituitary forms of TSH deficiency, especially in patients in whom no gross intracranial lesion is detected. The unexpected finding is the frequency with which TSH deficient patients respond to TRF. In our group, eight of nine responded to TRF, which indicates the presence of functional thyrotrophs in the anterior hypophysis, and suggests that TRF deficiency is the cause of the TSH deficiency and secondary hypothyroidism. Either deficient synthesis of TRF, structurally abnormal TRF, or failure of TRF release could account for the TSH deficiency in these patients. In the one TSH-deficient patient with idiopathic hypopituitarism who failed to respond to TRF, a primary pituitary defect probably accounts for the TSH deficiency. However, it must be appreciated that more prolonged or repeated administration of TRF might evoke a TSH response.

The cause of the delayed and sustained response to TRF in four of eight patients with idiopathic hypopituitary dwarfism and TSH deficiency requires further investigation. The pituitary thyrotroph which has remained chronically unstimulated by releasing factor may require repeated or more prolonged priming with TRF in order to release TSH from one or more labile or stable storage pools. Sustained elevation of plasma TSH may

be related to slower peripheral inactivation of TRF. Alternatively, since thyroxine and triiodothyronine are known to act directly on the pituitary gland to block release of TSH in response to TRF (18, 22), the low concentration of plasma  $T_4$ , both fasting and in response to TRF, in patients with idiopathic hypopituitary dwarfism and TSH deficiency, may permit a more sustained effect of TRF at the thyrotroph cell membrane.

Although patients with normal thyroid function (groups I and II) responded to TRF with significant increases in plasma  $T_4$  concentration, the rise in each individual patient was small. Repeated administration of TRF in multiple doses or by prolonged infusion may lead to a greater increase in the concentration of plasma  $T_4$  in normal patients, or significant increases in the concentration of plasma  $T_4$  in patients with TSH deficiency who responded to TRF. However, with a single rapid injection of TRF, the small increase in plasma  $T_4$  level is not sufficiently discriminatory, so that measurement of plasma TSH is required to evaluate the response to TRF.

In a recent report, Milhaud, Rivaille, Moukhtar, Job, and Binet (17) briefly described two children with hypopituitarism who showed a rise in TSH after intravenous administration of TRF. One of these children had a craniopharyngioma, with fasting concentration of plasma TSH of 1.6  $\mu$ U/ml, the other had idiopathic hypopituitary dwarfism, with fasting plasma TSH level of 5.0  $\mu$ U/ml.

In light of the evidence of deficient TRF in eight of nine patients with idiopathic hypopituitary dwarfism, it seems likely that the other pituitary hormone deficiencies in these patients may be attributable to deficiency of their respective releasing factors. However, since pituitary prolactin release is restrained by a hypothalamic prolactin-inhibitory factor (PIF) (2, 4, 6), those patients who have involvement of hypothalamic neurosecretory neurons which synthesize prolactin-inhibitory factor, would be expected to have elevated levels of serum prolactin. Hence, it may be possible to identify idiopathic hypopituitary dwarfs with primary hypothalamic involvement by detecting elevated levels of serum prolactin in contrast to a deficiency of other pituitary hormones.

We suggest that deficiency of growth hormone releasing factor and a defect at the level of the hypothalamic neurosecretory neurons may be a common cause of idiopathic hypopituitary dwarfism. Growth hormone-releasing factor, like TRF, appears to be a small polypeptide (37, 38). When this becomes available for clinical use it may provide a practical substitute for human growth hormone for the treatment of hypopituitary dwarfs with deficiency of growth hormone-releasing factor.

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