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Thyroid Function in Fragile-X Syndrome Males

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Twelve males with fragile-X syndrome between the ages of three and 28 years underwent assessment of thyroid function. All 12 subjects demonstrated normal baseline values for thyroid stimulating hormone (TSH), thyroxine, thyroid binding globulin (TBG), and estimated free thyroxine (EFT). Relative to a control group reported in the literature, however, the fragile-X subjects exhibited a blunted TSH response to thyrotropin releasing hormone (TRH). This finding suggests the presence of subtle dysfunction within the hypothalamic-pituitary-thyroid axis. Elevated baseline prolactin levels were also observed among the fragile-X subjects. These results support previous reports of hypothalamic-pituitary abnormalities among fragile-X syndrome males.

INTRODUCTION

Fragile-X syndrome is an X-linked disorder which rivals Down's syndrome as the most common genetic cause of mental retardation. The presence of the Xq27 fragile site has been associated with a characteristic clinical phenotype, which includes a set of unique facial features and macro-orchidism in males [1]. It has been hypothesized that the macro-orchidism of fragile-X males may result from abnormal thyroid metabolism [2]. This hypothesis is consistent with the association of precocious testicular enlargement and primary hypothyroidism described among pre-pubertal boys [3–7].

Several investigators have studied the thyroid functioning of fragile-X syndrome males. The age groups have been diverse, spanning early childhood to middle adulthood. A total of 53 subjects were reported in eight studies and all demonstrated normal thyroxine levels [2,8–14]. In addition, 24 of 28 fragile-X males evaluated by four of these investigators manifested normal, baseline TSH levels [2,9,11,13]. Shapiro et al., however, reported that four of their ten adult subjects had elevated TSH levels despite normal T_3, T_4, and free T_4 values [2]. It is of note that two of these four men also had depressed testosterone levels.

The hypothalamic-pituitary regulation of thyroid metabolism has been studied through the use of TRH stimulation testing. Wilson et al. evaluated 13 two- to 35-year-old fragile-X males and reported that all 13 manifested a significantly blunted TSH response following a TRH dose of 7 μg/kg [13]. Subject data were compared with normative values reported by Foley et al. [15]. O'Hare et al. found that three of

Abbreviations: EFT: estimated free thyroxine PRL: prolactin TBG: thyroid binding globulin TRH: thyrotropin releasing hormone TSH: thyroid stimulating hormone

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TABLE 1
Clinical Data: Fragile-X Syndrome Subjects

S	CA	I.O.	Tanner Stage	Test[a] Volume (ml)	T_4[b]	TBC[b]	EFT[b]	BSLN TSH	Delta TSH	BSLN PRL	Delta PRL
1	3-2	89	1	1.5	9.4	24.7	1.8	2.0	—	15.0	—
2	5-2	80	1	1.0	8.0	19.6	1.9	2.0	18	7.0	19
3	7-6	67	1	2.25	6.7	18.6	1.7	2.0	—	21.0	—
4	11-3	55	2	8.0	7.9	25.8	1.4	1.3	16.5	10	63
5	12-4	45	2	15.0	8.3	23.1	1.7	2.7	7	7.1	12.5
6	15-6	44	3	17.5	7.9	22.9	1.6	3.0	16	14.0	27
7	15-8	42	3	19.5	6.3	22.8	1.3	2.3	15	13.8	65.3
8	16-0	34	4	35	6.1	23.8	1.3	4.5	1	17.3	0.53
9	16-3	38	4	39.0	7.9	24.4	1.5	4.0	—	22.0	—
10	18-0	44	5	24.0	9.5	24.8	1.8	3.5	18.1	7.3	52.9
11	25-2	53	5	50.0	5.9	24.1	1.1	2.4	12.5	20.5	19
12	28-2	45	5	38.5	5.8	21.4	1.3	2.0	9	10.0	55

S, subject
CA, chronological age in years and months
Test Volume, mean testicular volume in ml
BSLN TSH, baseline TSH measurements in μU/ml (mean of several values, which may be slightly different from the pre-TSH value)
Delta TSH, peak TSH value − pre-TRH value
BSLN PRL, baseline prolactin measurements in ng/ml (mean of several values which may be slightly different from the pre-PRL value)
Delta PRL, peak PRL value − pre-PRL value
[a]Normative Data (tenth to ninetieth percentiles) adapted from [17,20]

Tanner Stage	Tenth Percentile	Ninetieth Percentile
Prepubertal (≤11 years)	1	2
Stage 2	3	13
Stage 3	5	17
Stage 4	7	19
Stage 5	12	23

[b]Normative Values
T_4: 4.6–9.2 μg/dl TBC: 16.8–25.7 μg/dl EFT: 1.0–2.1 ng/dl

five fragile-X adults demonstrated virtually no response to parenterally administered TRH [14]. The details of the procedure were not reported, however.

These findings suggest that, although fragile-X syndrome males appear to be clinically euthyroid, subtle abnormalities within the hypothalamic-pituitary-thyroid axis may be present.

SUBJECTS AND METHODS

Twelve males with fragile-X syndrome between the ages of three and 28 years participated in the study. All subjects were residing in either family or group homes. Cytogenetic assessments were conducted as described by Lubs et al., and involved the examination of a minimum 50 lymphocytes cultured in medium 199 with 2 percent calf serum [16]. Each subject received thorough physical and psychological assessments. Clinical data are presented in Table 1. The degree of intellectual impairment experienced by the subjects ranged from mild to moderate levels of mental retardation, as assessed by performance on the Stanford-Binet. All subjects were in good physical

health and free from general medical or neurological disease. Three subjects were pre-pubertal (Tanner stage 1) and nine were pubertal (Tanner stages 2 through 5). Testicular volume measurements were made using a Prader orchidometer [17] or by direct measurement, using the equation for ellipsoids, $\pi/6 \times \text{length} \times (\text{width})^2$ [18,19]. Macro-orchidism was present in eight of the nine pubertal subjects and in one of the three pre-pubertal subjects, based on available norms [17,20].

Baseline assessments of thyroid status were conducted during outpatient visits and TRH stimulation tests during brief inpatient hospitalizations on the Children's or the Adult Clinical Research Center of Yale–New Haven Hospital. Baseline laboratory studies were obtained for all 12 subjects and included measurement of serum thyroxine, thyroxine binding globulin (TBG), and estimated free thyroxine (EFT), as described by Seligson and Seligson [21,22]. In addition, baseline values also were obtained for TSH—modification of the method of Odell et al. [23] and for prolactin (PRL)—modification of the method of Sinha et al. [24].

Hypothalamic-pituitary-thyroid axis functioning was assessed in nine subjects, utilizing the TRH stimulation test. Following baseline determinations, 7 μg/kg of purified TRH was administered intravenously. Serial serum specimens for TSH and prolactin were collected at baseline, 30 minutes, 60 minutes, 90 minutes, and 120 minutes, in order to identify the highest (peak) value. The standard procedure for assessing pituitary responsiveness was followed, namely, the calculation of the maximum change (delta) in serum TSH following TRH administration (peak TSH value − pre-TRH value) [25]. Statistical analyses were employed for the evaluation of differences between the fragile-X subjects and 20 control subjects reported by Foley et al. [15]. The Foley et al. study and the present one employed the same procedure, using assay techniques developed during the same period of time. The Foley et al. report included data on individual subjects and was used for comparison with fragile-X subjects by Wilson et al. [13]. *T*-tests were used for the comparison of baseline TSH and PRL measures, as well as of delta TSH values determined from the TRH stimulation test.

RESULTS

Thyroid Function

All 12 subjects demonstrated T_4, TBG, and EFT values which were within the normal range (refer to Table 1). Negative correlations were observed between age and both serum T_4 and EFT levels ($r = -0.57$ and -0.74, respectively). These ontogenetic changes are similar to those which occur within the normal population [26,27]. Clinical examination of the subjects also was consistent with the presence of a euthyroid state.

Pituitary Functioning—Tonic State

Tonic pituitary functioning relevant to thyroid status may be inferred from an examination of baseline TSH and PRL levels. All subjects demonstrated TSH levels within the normal range, as reported by our laboratory. When compared with 20 normal children and adolescents studied by Foley et al., however, our fragile-X group exhibited significantly higher baseline levels of prolactin ($t = 3.71$, $p < 0.001$) [15]. This result contrasts with baseline TSH levels which were equivalent for the two groups ($t = 1.23$, $p = 0.23$). Tonic pituitary functioning was equivalent across the ages and Tanner stages represented in the present sample.

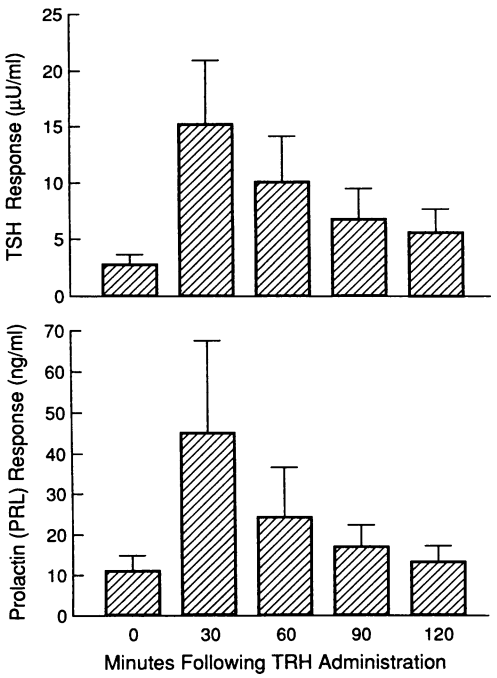

FIG. 1. TRH stimulation test: Serum TSH and prolactin responses of the fragile-X subjects.

Hypothalamic-Pituitary Axis Functioning

The overall *pattern* of the pituitary response to TRH stimulation was normal, with a relatively rapid rise of both TSH and PRL to peak values by 30 minutes and a return to pre-TRH values by 120 minutes following TRH administration (Fig. 1) [28,29]. In addition, the magnitude of both the TSH and PRL response remained essentially constant across the age range of our sample and that of Foley et al. ($r = -0.29$ and -0.11, respectively), consistent with findings for the general child and adult population [25,28,29].

There were no significant group differences with regard to the PRL response (delta PRL) ($t = 0.38$;, DF = 26, $p = 0.70$). The fragile-X group, however, exhibited a significantly lower TSH response (delta TSH) than that demonstrated by the normal controls reported by Foley et al. ($t = -2.39$, DF = 27, $p = 0.02$). Within our sample, there was a tendency for the TSH response to correlate negatively with testicular volume ($r = -0.49$).

DISCUSSION

The present study examined hypothalamic-pituitary-thyroid function within a sample of fragile-X subjects representative of the developmental spectrum. Our fragile-X subjects exhibited normal thyroid function, including normal T_4, TBG, and EFT values, as well as a normal, age-related decrement in the magnitude of T_4 and EFT. In addition, they appeared to exhibit a relatively typical *pattern* of hypothalamic-pituitary response; however, hypothalamic-pituitary regulation may be subtly disordered. The fragile-X subjects demonstrated significantly higher baseline PRL values than the control subjects reported by Foley et al. [15]. No significant group differences

were observed in the PRL response to TRH stimulation. The fragile-X subjects did, however, exhibit decreased *TSH* responsiveness to TRH, suggesting dysfunction at the pituitary level under conditions of maximal stimulation.

Before discussing possible interpretations of these findings, comment should be made regarding the use of literature controls. Because of the potential for several additional sources of experimental variance associated with the use of literature control data (differences in procedures, assays, and the like), findings should be interpreted cautiously and regarded as preliminary. The present study was designed to identify potential abnormalities in the central regulation of thyroid functioning among fragile-X subjects warranting more intensive study (including the addition of a more traditional control group). The Foley et al. study was chosen for comparison with our data because it employed the same procedure (administration of 7 mcg/kg of TRH with similar time measurements for TSH), used a radioimmunoassay developed during the same time period as that used in our study (late 1960s to early 1970s), and had been used for comparison with fragile-X subjects in a previous study [13]. Although assay differences may be present for the two studies, it is likely that they are relatively small (there were no significant group differences in baseline TSH values: $t = 1.23$, DF $= 27$, $p = 0.23$). It is of note that recent reviews of thyroid function reference studies from that same era for discussions of normative values (including the Foley et al. study), reflecting the time period during which the bulk of such studies were performed [25,28,29].

In considering the findings of the present study, several explanations regarding pathophysiology are possible. Hypothyroidism secondary to pituitary insufficiency has been associated with a subnormal TSH response to TRH [30]. In the syndrome of isolated TSH deficiency, for example, TRH stimulation results in a blunted TSH response yet a normal PRL response [31]. Furthermore, the hypothyroid state, itself, has been noted to result in hyperprolactinemia [32,33]. The subjects in the present study, however, demonstrated normal thyroid indices, making such an explanation of the findings unlikely. Thyrotoxicosis is, perhaps, the most frequent cause of a subnormal TSH response to TRH [31]. Signs, symptoms, and laboratory studies suggestive of hyperthyroidism were, however, lacking in the subjects under investigation.

Arnetz et al. have reported that the TRH-stimulated PRL response decreases during the sixth decade (although the TSH response remains stable) [32]. Foley et al. found no age-related changes in either the TSH or the PRL response within an age range of four to 13 years [15]. Similar findings were obtained for the fragile-X subjects, three to 28 years of age. Therefore, the group differences in the TSH response noted in the present study cannot be ascribed to the older age of the fragile-X subjects.

The findings of this study include a tendency for subjects with greater degrees of macro-orchidism to exhibit lower pituitary reserve (i.e., a decreased TSH response to TRH stimulation). This observation is quite interesting, given the association between hypothyroidism and premature testicular enlargement in pre-pubertal boys [3–7]. The majority of the 17 macro-orchid boys reported in these studies had primary hypothyroidism (with secondary elevations of serum TSH and gonadotropins).

The results of the present study suggest that subtle abnormalities may exist within the hypothalamic-pituitary axis of fragile-X syndrome males, leading to elevated baseline PRL levels and subnormal pituitary TSH reserve. Should these findings be confirmed by future studies which include more suitable controls, a search can begin for the factors that underlie both the testicular and the thyroid abnormalities (presum-

ably operating at the hypothalamic-pituitary level). An hereditary form of congenital hypothyroidism has been linked to the short arm of chromosome 8. It would be most interesting, although premature, to speculate that a similar thyroid abnormality may result from aberrant genetic expression either within the fragile-X site itself or within a closely linked locus. Future research may answer these most interesting questions.

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