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PITUITARY TSH RESPONSE TO TRH: INTERRELATIONSHIPS WITH GONADAL ACTIVITY

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INTRODUCTION

It is generally accepted today that the basal plasma concentrations of immunoreactive thyroid-stimulating hormone (TSH) show no significant variations in relation to sex [8, 12].

Analysis of the medical literature, however, shows that there are contradictions in the reported behaviour of the TSH response to the injection of thyrotropin-releasing hormone (TRH). As a matter of fact, some authors [6, 7, 10, 13] report a greater response in adult females, while others [5] affirm that the difference is only evident with low doses of TRH (it disappears, or is completely inverted in favour of males, with higher doses); others [15, 16] find a significant decrease in males, but only in old age.

The relationships between the TSH response to TRH and the two phases of the menstrual cycle also seem to be uncertain. SANCHEZ-FRANCO et al. [14] report an increased TSH pituitary response in a group of females in the follicular phase, as well as in a group of females in the follicular or luteal phase when compared with the response of males of the same age. No increase was found in a group of females in the luteal phase. According to these authors the difference between the response obtained in the follicular phase and that in the luteal phase is significant. On the other hand JENSEN and WEEKE [9] did not find any significant difference in the TSH pituitary response, either when a group of males was compared with a group of females, or when a group of young women in the follicular phase was compared with another in the luteal phase.

In spite of the disagreement in the results reported in the literature, the possibility that oestrogens can in some way modulate the activity of the TSH secretory

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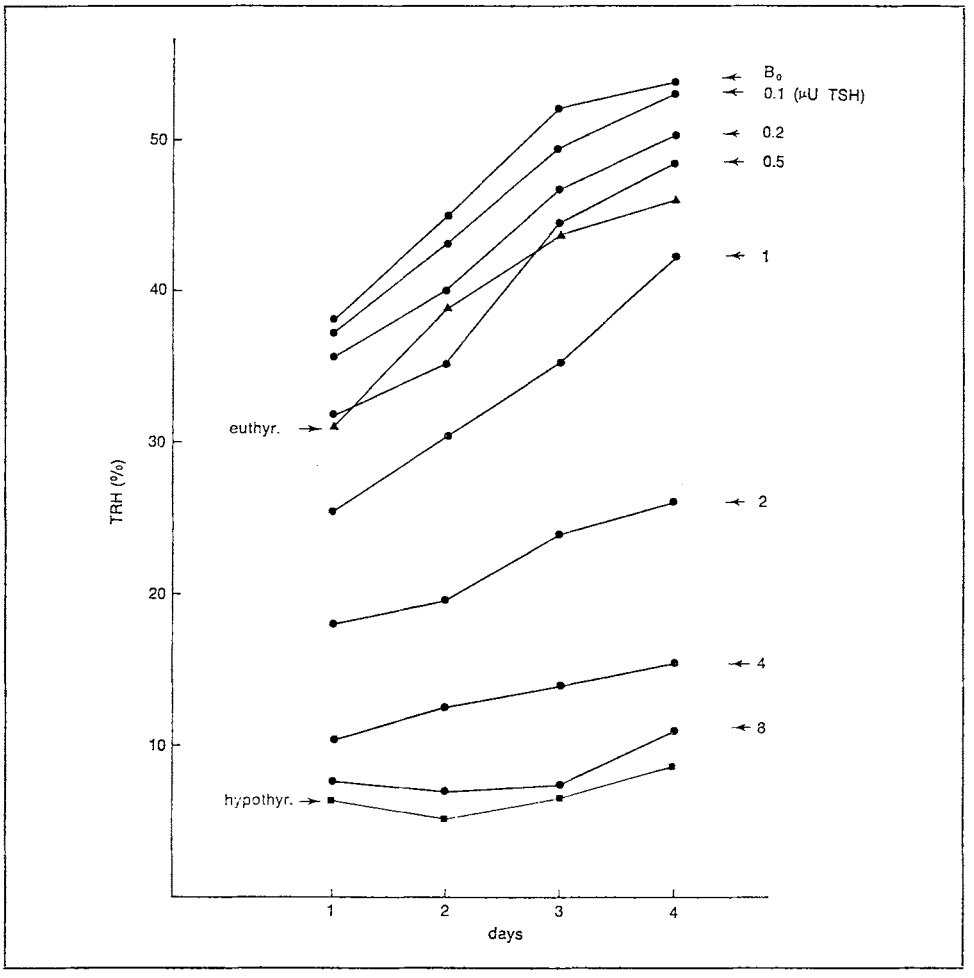

Fig. 1 - Effect of incubation time on the capacity to distinguish the individual points of the standard curve.

system has been suggested by some authors who found different responses in the two sexes and/or in the different phases of the menstrual cycle.

Consequently, successive experiments were proposed to study the effects of oestrogen treatment on the pituitary metabolism of TSH; as expected, the results of these experiments also did not agree.

ADAMS and MALOOF [1] reported an increase in the basal values of TSH after oestrogen treatment, which was not confirmed by others [6, 7, 11]. Moreover, when the TSH response to TRH is considered in male subjects pretreated with oestrogens, some authors [2, 4] found no changes, while others [3] reported a significant increase.

This paper deals with our investigations on the possible interrelationships between gonadal activity and the TSH pituitary response to TRH. The parameter employed to evaluate this response was the secretory area limited by TSH plasma levels; we consider this parameter is more valid for the interpretation of the effects because it is not influenced by the basal TSH plasma levels and makes the comparison of the findings easier and more reliable.

CASES

We studied 74 subjects; 62 of these, without clinical signs or any laboratory evidence of endocrine or metabolic alterations, were divided into the following groups:

— 10 male subjects of prepubertal age * (aged 5 to 13);
— 10 male subjects of adult age (aged 18 to 44);
— 10 male old subjects (aged 65 to 81);
— 10 female subjects of prepubertal age * (aged 3 to 12);
— 12 female subjects of adult age (aged 18 to 46), all studied in the early follicular phase of the menstrual cycle;
— 10 female old subjects (aged 65 to 79).

Another 7 subjects were girls (aged 10 to 18) with Turner's syndrome. All of these were found on laparoscopy to have ovarian agenesis; the karyotype was 45/XO in 6 and 46/XXp- in the other.
The remaining 5 subjects were males with the following clinical diagnoses:

— 3 with Klinefelter's syndrome (10-, 18- and 25-year-old);
— 1 with the 'Sertoli cells only' syndrome (30-year-old);
— 1 with 'rudimentary testes' (21-year-old).

* These subjects showed no clinical signs of puberty.

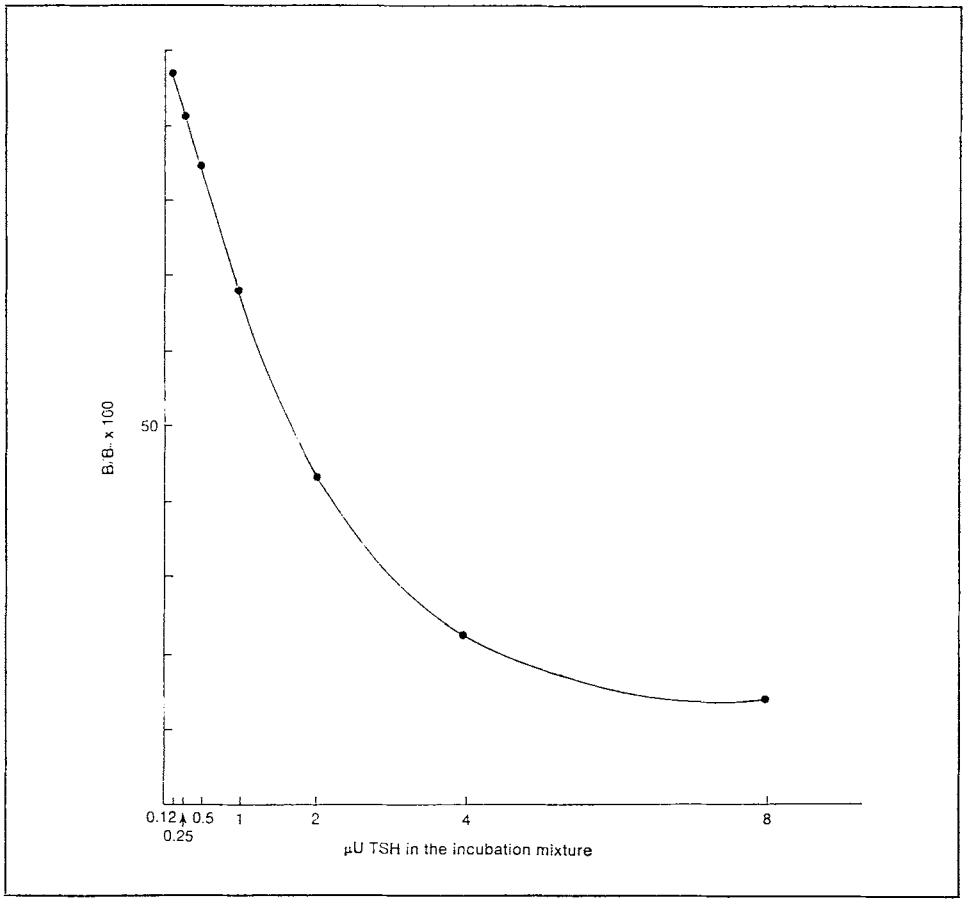

Fig. 2 - Standard curve in a graphic linear system.

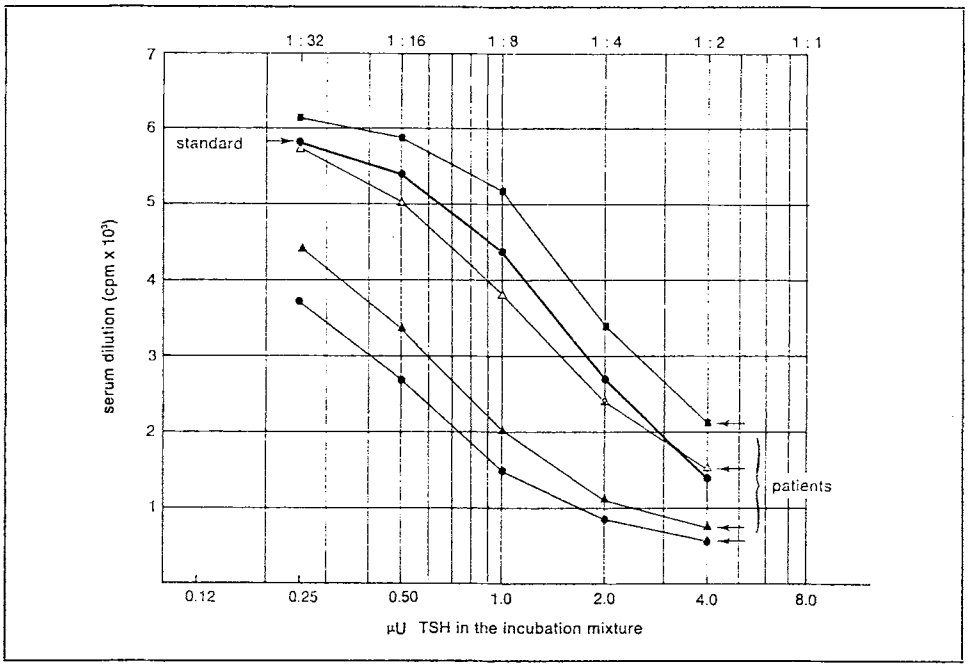

Fig. 3 - Parallelism test. Effect of multiple dilutions of serum obtained from 4 hypothyroid subjects. Comparison with a TSH standard curve.

METHODS

The radioimmunological method to evaluate the plasma levels was that of ODELL et al. [12].

The TSH standard used, supplied by the London National Institute for Biological Standard and Control, was pituitary TSH amp. cod. 68/38 (147 mU TSH/amp. = 46.2 μg).

The TSH antiserum prepared in the rabbit was supplied by the National Pituitary Agency of the NIAMDD, Bethesda, Maryland.

Radioiodinated ^{125}I-TSH (specific activity = 200 mCi/mg).

Precipitating antiserum (goat anti-rabbit gammaglobulin).

Buffers

— Phosphate buffer 0.01 M, pH 7.8 (Na_2HPO_4 = 11.4 g; KH_2PO_4 = 0.36 g; NaCl = 8.77 g; merthiolate = 0.20 g; H_2O up to 1 l).

— Phosphate buffer 0.01 M, pH 7.8 with 0.5 % human albumin (*Behring Institut*) *.

— Phosphate buffer 0.01 M (pH 7.8) + 0.1 M EDTA + HCG (1,000 IU %) + 0.2 % normal rabbit serum **.

Evaluation of the method

The antiserum dilution used was 1:200,000 (the final dilution in the incubation mixture) with a maximal binding capacity (B_0) of about 40-50 % of the total radioactivity. The non-specific binding capacity of the precipitating antibodies for ^{125}I-TSH in the absence of antiserum was negligible (0.6-0.8 %).

As regards the incubation we studied the effects of its duration on the capacity to distinguish the individual points on the standard curve. This capacity was satisfactory after 24 hrs, reaching the maximal efficiency in 48-72 hrs (fig. 1), the latter being chosen for our procedure.

The behaviour of the standard curve obtained after 72 hrs of incubation is reproduced in a graphic linear system (fig. 2). The lowest TSH concentration statistically different from B_0 ($P < 0.001$) was 0.125 μU/ml.

* Antigen diluent; ** antiserum diluent.

The reproducibility of the system was shown by repeating 10 times the TSH determinations on the same serum sample with the following results: $B/B_0 = 88.17 \pm 1.55$ SD, coefficient of variability = 1.75.

In the parallelism test the multiple dilutions of hypothyroid serum gave curves substantially similar to those of the TSH standard (fig. 3), thus excluding the presence of any interfering plasma factor in the system.

The recovery test gave good results: the TSH added to serum of known TSH concentration was well detected by our assay, as shown in fig. 4.

In order to avoid cross-reactions with LH and also with FSH (although minimal) in our system we used antiserum diluted in buffer containing HCG (Profasi® 1 U HCG for each tube) thus eliminating the non-specific component in the antiserum. When Pergonal® was employed instead of Profasi® to purify the antiserum also for the FSH component, we found in some batches an unexpected decrease of very high degree in the binding capacity, as if the Pergonal® was contaminated sometimes with TSH. Consequently, in our procedure, we used antiserum matched only against HCG.

To summarize, our trial was carried out as follows:

1) 0.1 ml standard TSH diluted in antigen diluent or 0.1 ml plasma was added to 0.1 ml of rabbit anti-TSH serum diluted 1:50,000 in antiserum diluent and incubated for 24 hrs at 4 °C.

2) 0.1 ml ^{125}I-TSH (0.05 ng) in antigen diluent was added and the mixture was incubated for 48-72 hrs at 4 °C.

3) 0.1 ml of goat anti-rabbit gammaglobulin (dilution 1:8 in phosphate buffer) was added, and the mixture further incubated at 4 °C. for 24 hrs.

4) These first 3 steps were followed by microfiltration and determination of the radioactivity (Packard, Tri-Carb, model 3330).

Pituitary TSH stimulation was produced by rapid i.v. infusion of TRH. The standard dose for adult and old subjects was 200 μg; prepubertal subjects received a body surface-dependent dose of 200 μg/1.73 m².

TSH was assayed in plasma samples taken at — 15, 0, 10, 20, 30, 45, 60, 90 and 120 min after the TRH injection.

The triangulation method was used to calculate the areas circumscribed by the TSH secretion curves and the results expressed in μU/2 hrs.

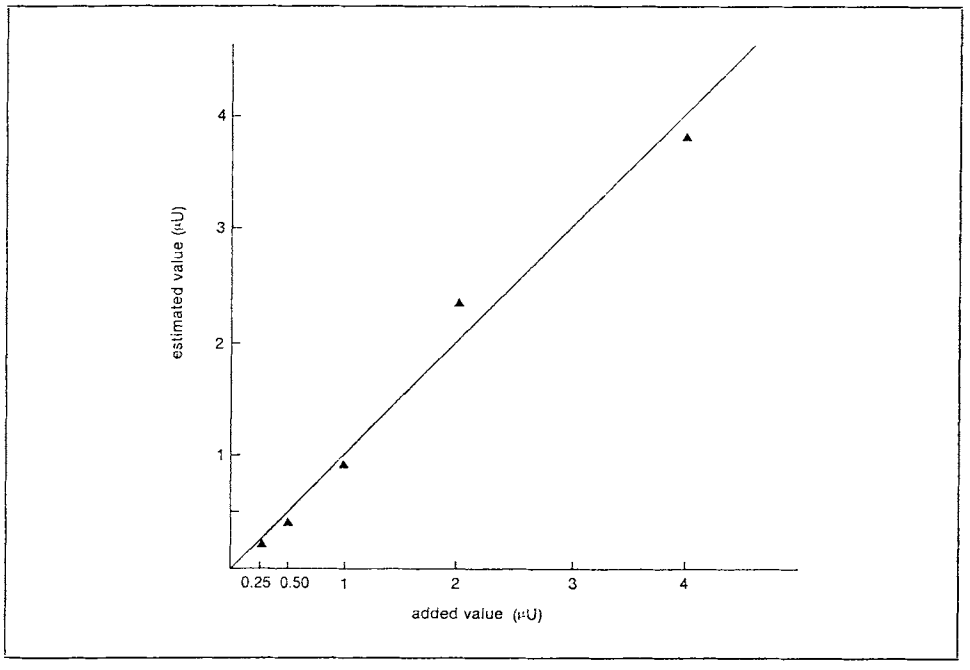

Fig. 4 - Recovery test of TSH added to serum *in vitro*.

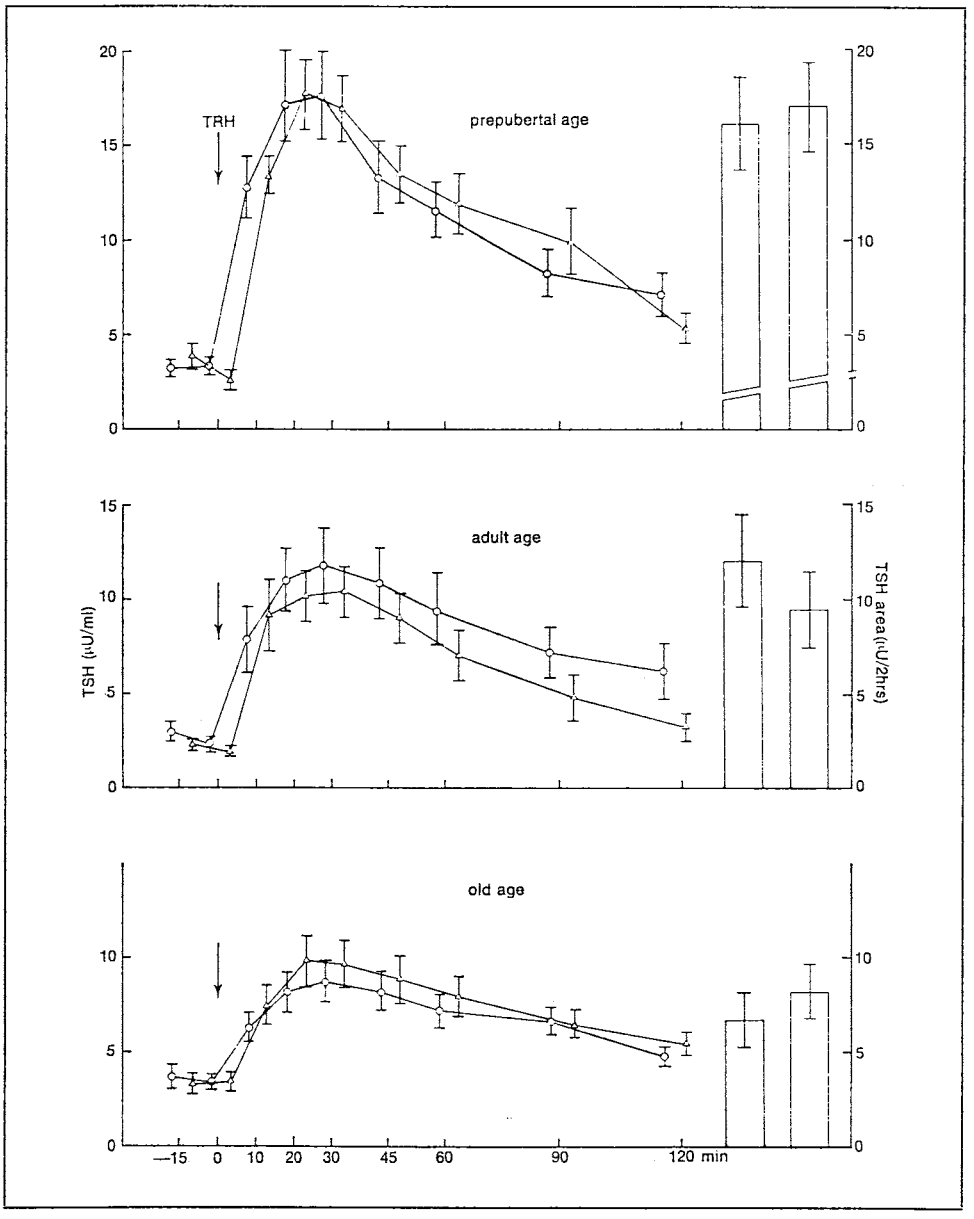

Fig. 5 - TSH pituitary response to TRH in relation to sex; behaviour in prepubertal, adult and old subjects. Red lines = males; blue lines = females.

RESULTS

Our results can be summarized as follows:

1) no significant difference was found between the secretory areas of male and female subjects, whether they were of prepubertal, adult (all female subjects were in the early follicular phase of the menstrual cycle), or old age.

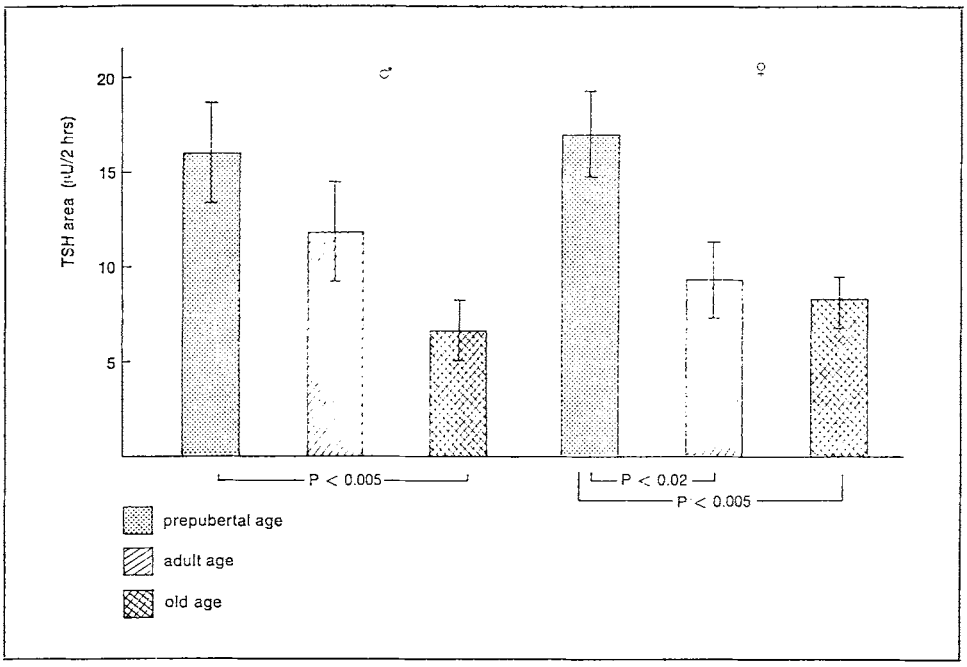

Fig. 6 - TSH pituitary response to TRH in relation to age (males and females are considered separately).

2) There were, however, decreases in the secretory areas with age in both sexes (fig. 6). In the male group the decrease was significant only when comparing prepubertal with old subjects, whereas in the female group it was significant when prepubertals were compared with both adult and old subjects.

3) No significant difference was observed between the TSH response obtained in the early follicular phase of the menstrual cycle and that in the luteal phase (in the 4 subjects studied for this purpose, fig. 7).

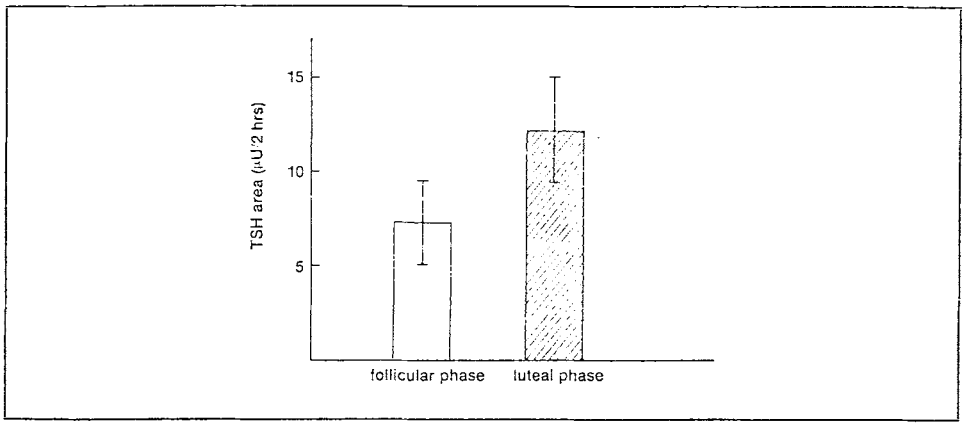

Fig. 7 - TSH pituitary response to TRH in 4 females, considered both in the follicular and in the luteal phases of the menstrual cycle. The difference was not statistically significant.

patients	age (years)	diagnosis	TSH (μU/ml)									TSH area (μU/2 hrs)
B.G.	30	'Sertoli cells only' syndrome	4.5	4.7	5.9	8.6	9.5	6.5	6.8	5.6	5.2	4.42
G.G.P.	10	Klinefelter's syndrome	1.5	<1.2	3.6	8.9	10.0	8.0	6.5	4.6	3.6	9.21
T.A.	18	Klinefelter's syndrome	7.5	5.5	10.5	12.0	15.0	14.0	12.0	12.0	8.0	10.41
B.M.	22	Klinefelter's syndrome	1.6	<1.2	1.8	3.4	1.9	1.6	1.8	<1.2	1.3	0.57
P.S.	21	'rudimentary testes'	4.2	4.7	27.0	42.0	35.0	29.0	24.5	17.0	10.5	37.82
time (min)			—15	0	10	20	30	45	60	90	120	

Table 1 - Behaviour of the TSH pituitary response to TRH in some conditions of primary male hypogonadism.

4) In subjects with Turner's syndrome (fig. 8), no difference was found when comparing their TSH response with that of normal adult female subjects; the slight difference observed in the TSH response of prepubertal subjects was not statistically significant ($t = 1.90$).

5) In the small group with primary male hypogonadism, we found conflicting values for the TSH responses, as was to be expected considering the small number and the clinical heterogeneity of the cases. They showed great diversity in the degree and type of functional impairment, as shown by the histological picture of the biopsy samples and the rebound behaviour of LH and FSH. In fact we sometimes observed selective impairment of the germinal tissue (the 'Sertoli cells only' syndrome), sometimes at the same time the partial involvement of interstitial cells (individual cases of Klinefelter's syndrome) and even the pattern of completely 'rudimentary testes'. In each of these cases the behaviour of the TSH secretory area (tab. 1) was variable

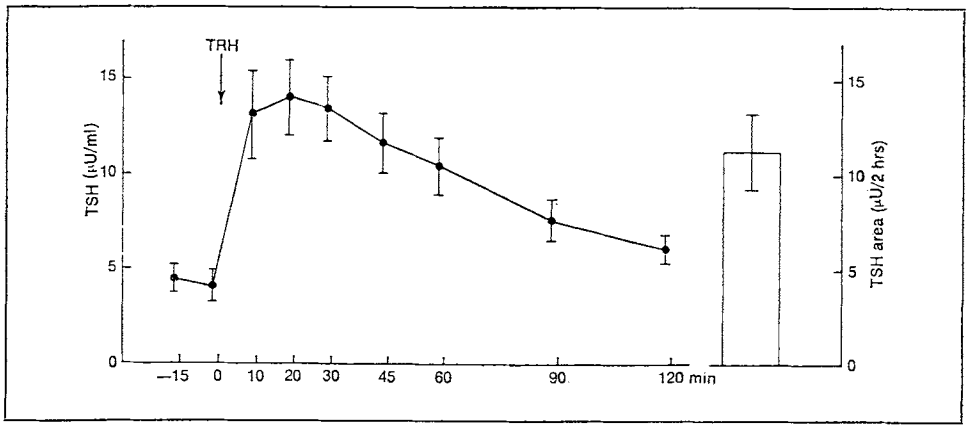

Fig. 8 - Behaviour of TSH pituitary response to TRH in 7 patients with Turner's syndrome.

and did not allow any definite conclusion to be reached; in fact we observed a group with normal responses, along with some that were less definite (in 1 case of Klinefelter's syndrome) and some that were slightly increased (as in the case with 'rudimentary testes').

DISCUSSION AND CONCLUSIONS

As for ovarian secretory activity, our results lead us to agree with those authors who deny that oestrogens — except in pharmacological doses — can influence the TSH pituitary response to TRH. In fact we never found significant differences between the two sexes, even when the differences in the plasma oestrogen levels were greater, as in adult subjects; moreover, the fall in the TSH pituitary response demonstrated in old females was not very much greater than that in old males, although the former are known to have a more complete gonadal failure; also no significant difference was observed in the behaviour in the two phases of the menstrual cycle. Finally, as another proof that ovarian activity in an oestrogenic sense does not influence the dynamics of the TSH secretory system, the TSH pituitary response to TRH in patients with ovarian agenesis was the same as that in normal subjects.

All the aforesaid is in agreement with what is known about the physiological variations in the concentrations of oestrogens in the different phases of the menstrual cycle and at the different ages of women and that these are not capable of interfering in the normal dynamics of the hypothalamic-pituitary system, either directly or indirectly. Conflicting with the hypothesis of an indirect influence mediated by modifications in the peripheral metabolism of the hormones, there are numerous observations of an almost constant free thyroxine index in old subjects [15, 16, 17] demonstrating the stability in the binding capacity of carrier plasma proteins for thyroid hormones with progressing age.

As regards the hypothesis of a direct influence, this could be considered only for large variations in the oestrogen plasma levels; however, the contradictory results reported in literature [2, 3] give rise to reservations also on this point. As for male subjects, our results show no clear interrelationship between testicular activity and the TSH secretory area. Therefore, the physiological variations in gonadal endocrine activity appear incapable of modulating the TSH pituitary response to TRH in males as well as in females. The variable (but not substantially abnormal) behaviour of the TSH secretory area observed in cases of primary hypogonadism seems to agree with a slight interference produced by the testicular hormones in the dynamics of the pituitary TSH secretory system; confirmation of this, however, is required from investigations on a wider and more homogeneous group of patients.

In conclusion, in both sexes the TSH pituitary response to TRH (as measured by the secretory area of immunoreactive TSH) is not influenced by the physiological variations in gonadal hormones, nor by pathologically low levels such as those found in Turner's syndrome and in primary male hypogonadism of various types. This independent behaviour is confirmed by the fact that the widest TSH secretory area is found in prepubertal age, when the endocrine activity of the gonads is far from its maximal efficiency, and the narrowest area in old subjects of both sexes, whose gonads have lost their optimal endocrine efficiency for a long time, due to the inexorable process of involution.

SUMMARY

A TSH radioimmunoassay is described, which was used to investigate the behaviour of the TSH pituitary response (as measured by the secretory area) to TRH in groups of subjects of both sexes and different phases of gonadal activity. It is concluded that the TSH pituitary response

is in no way affected by physiological variations in the plasma gonadal hormones. This independent behaviour of the TSH response, uninfluenced by gonadal activity, was apparently confirmed by the results in some pathological conditions (ovarian agenesis and primary male hypogonadism of various types) characterized by very low plasma levels of gonadal hormones.

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