Serum levels of prolactin, TSH and gonadotrophins following LRH/TRH – double stimulation tests during the menstrual cycle

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Abstract. The objective of this study was to re-investigate the capacity of pituitary prolactin (Prl) and thyrotrophin (TSH) secretion throughout the normal menstrual cycle to respond to repeated thyrotrophin releasing hormone (TRH) stimulation analogous to the double luteinizing hormone-releasing hormone (LRH) stimulation test. This test has been shown to be a sensitive parameter for oestrogenic effects on the gonadotrophins. In addition, the volunteers were selected carefully on the basis of ovulatory cycles and otherwise normal endocrine function.

In 9 women a combined LRH/TRH double stimulation test was performed during the early follicular, periovulatory and mid-luteal phases. TRH (200 µg) and LRH (25 µg) each were given iv twice, 2 h apart.

Basal and LRH stimulated luteinizing hormone (LH) and follicle stimulating hormone (FSH) were found to follow characteristic cyclic response patterns. The LH responses after both LRH stimulations were greatest in the periovulatory phase; \( \Delta_1 \) and \( \Delta_2 \) were higher in the mid-luteal phase than in the follicular phase. Maximum FSH response to LRH was found during the periovulatory phase, but the FSH response in the early follicular phase was greater than that found in the mid-luteal phase. In contrast, basal and TRH stimulated serum concentrations of TSH and Prl remained constant throughout the cycle.

The gonadotrophin ratios \( \Delta_2/\Delta_1 \) were generally greater than 1. They increased from 1.4 in the early follicular phase to 3.0 in the late follicular phase, concomitant with the rise in oestrogens. The \( \Delta_2/\Delta_1 \) ratios for TSH and Prl were less than 1, ranging from 0.66 to 0.98 for TSH and from 0.26 to 0.99 for Prl. They did not show any cyclic changes.

Thus, this study shows that after LRH/TRH double stimulation, the gonadotrophin but not the Prl and TSH responses vary with the physiological changes in oestrogens during the menstrual cycle. The supposed mechanism of oestrogen effects on pituitary hormone secretion and their possible clinical significance are discussed.

The cyclic pattern of serum LH and FSH throughout the menstrual cycle is mainly caused by the modulating effects of peripheral oestrogens at the hypophyseal level (Knobil et al. 1980; Leyendecker et al. 1980; Römmler 1980). Previous studies have shown that after two LRH stimulations with 25 µg LRH at a 2 h interval the second LH and FSH increase (\( \Delta_2 \)) is the more sensitive parameter for the influence of both physiological and pharmacological oestrogens on hypophyseal gonadotrophin secretion than the first increase (\( \Delta_1 \)) or the basal value (Römmler 1980). Thus, with increasing oestrogen blood levels in the course of the mid- and late-follicular phase, \( \Delta_2 \) increases more than \( \Delta_1 \), and only after the dramatic rise of oestrogens in the periovulatory phase the basal gonadotrophin values also show a sharp elevation (positive feedback). The same behaviour can be imitated by rising doses of exogenous oestrogens (Römmler 1980).

In contrast to the gonadotrophins, the cyclic behaviour of basal and TRH-stimulated Prl and TSH has given rise to some controversy. Several investigators (Sanchez-Franco et al. 1973; Franchimont et al. 1976; Reymond & Lemarchand-Béraud 1976; Boyd & Sanchez-Franco 1977; Vekemans &
Robyn 1975; Bohnet et al. 1979) have shown varying basal and/or TRH stimulated levels of Prl and TSH while others (Hwang et al. 1971; Tyson & Friesen 1972; Weeke 1974; McNeilly & Chard 1974; Reymond & Lemarchand-Béraud 1976; Sawin et al. 1978) have found no differences.

If it is true that physiological levels of oestrogens are not involved in the pituitary TSH and Prl secretion it would be expected that neither the peripheral levels of these hormones nor \( \Delta_1 \) or \( \Delta_2 \) after TRH double stimulation would change throughout the cycle. If, however, oestrogens also possess modulating properties upon the hypophyseal secretion of Prl and TSH during the menstrual cycle, then a TRH double stimulation test should disclose this in analogy to the LRH double stimulation test.

It has been the objective of this study to assess which of these alternatives is the correct one.

Methods

Of 15 clinically healthy volunteers, 9 were selected on the basis of the criteria listed in Table 1.

The double stimulation tests were performed on each volunteer during the follicular phase (day 3 or 4), in the periovulatory phase (1–3 days prior to the rise in basal body temperature) and in the mid-luteal phase. LRH (25 \( \mu \)g) and TRH (200 \( \mu \)g) were given iv as a bolus injection at time zero and time 120 min. Blood samples were taken at 15 min intervals from 30 min prior to the first injection until 120 min after second injection. The tests were always started at the same time in the morning ensuring that the volunteers had been awake for at least 3 h.

Plasma samples were stored at \(-20^\circ C\) until assayed for PRL (Serono Kit), TSH (Odell et al. 1967), LH and FSH (Römmler 1978) by radioimmunoassay. The net increase in serum concentrations of the hormones after the two stimulations were expressed by \( \Delta_1 \) and \( \Delta_2 \), respectively. Statistical analyses were done by the Wilcoxon matched pairs signed rank test.

**Results**

The variations in basal and LRH stimulated serum concentrations of LH and FSH during the menstrual cycle are shown in Figs. 1 and 2. Similar basal levels of LH were observed in the mid-luteal and early follicular phase, with the highest levels in the periovulatory phase. The LH responses after both LRH stimulations were greatest in the periovulat-

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**Table 1.**

Criteria of the volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
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<tbody>
<tr>
<td>Age</td>
<td>20–30 years</td>
</tr>
<tr>
<td>Length of cycle</td>
<td>26–32 days</td>
</tr>
<tr>
<td>Biphasic BBT</td>
<td>&gt; 10 days of hyperthermia</td>
</tr>
<tr>
<td>Hormonal parameters in blood</td>
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<tr>
<td>Basal LH and FSH</td>
<td>1–4 ng/ml</td>
</tr>
<tr>
<td>Basal PRL</td>
<td>&lt; 15 ng/ml</td>
</tr>
<tr>
<td>Basal TSH</td>
<td>&gt; 5 ( \mu )U/ml</td>
</tr>
<tr>
<td>Progesterone</td>
<td>&gt; 8 ng/ml in the mid-luteal phase</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt; 600 pg/ml</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt; 250 mg/ml</td>
</tr>
<tr>
<td>DHEA-sulphate</td>
<td>&lt; 2800 ( \mu )g/ml</td>
</tr>
</tbody>
</table>

Fig. 1.

Basal serum LH levels (0) and LH increments after the first and second LRH injection (\( \Delta_1 \), \( \Delta_2 \)) in 9 ovulatory women (\( \bar{x} \pm SE \), ng/ml). * \( P < 0.05 \) \( \Delta_2: \Delta_1 \).

**P < 0.01 \( \Delta_2: \Delta_1 \).
Basal serum FSH levels (0) and FSH increments after the first and second LRH injection (Δ₁, Δ₂) in 9 ovulatory women (X ± SE, ng/ml). * P < 0.05 Δ₂:Δ₁. ** P < 0.01 Δ₂:Δ₁.

Fig. 2.

Basal serum FSH levels (0) and FSH increments after the first and second LRH injection (Δ₁, Δ₂) in 9 ovulatory women (X ± SE, ng/ml). * P < 0.05 Δ₂:Δ₁. ** P < 0.01 Δ₂:Δ₁.

Fig. 3.

Basal serum Prl levels (0) and Prl increments after the first and second LRH injection (Δ₁, Δ₂) in 9 ovulatory women (X ± SE, ng/ml). * P < 0.01 Δ₂:Δ₁.
Fig. 3 shows the corresponding Prl results. There were no significant changes in either basal or TRH-stimulated Prl secretion during the menstrual cycle. Also in contrast to the LH and FSH response to LRH, the Prl response to TRH was lower after the second than after the first stimulation \( (\Delta_2 < \Delta_1: P < 0.01) \). The mean ratio of \( \Delta_2:\Delta_1 \) was 0.6, range 0.26–0.99 (Table 2) and was similar in all phases of the cycle tested.

Again no significant changes in either basal or TRH-induced TSH levels were demonstrable during the menstrual cycle (Fig. 4), and, like Prl the first TSH increase was significantly higher than the second one \( (P < 0.01) \). The mean ratio \( \Delta_2:\Delta_1 \) was 0.78, range 0.66–0.98 (Table 2), which remained similar in the three test periods.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Median and 95% confidence limit</th>
<th>Cyclic variations</th>
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</thead>
<tbody>
<tr>
<td>LH</td>
<td>1.83 (0.73–3.27)</td>
<td>+</td>
</tr>
<tr>
<td>FSH</td>
<td>1.23 (0.5–3.66)</td>
<td>+</td>
</tr>
<tr>
<td>TSH</td>
<td>0.78 (0.66–0.98)</td>
<td>–</td>
</tr>
<tr>
<td>Prl</td>
<td>0.6 (0.26–0.99)</td>
<td>–</td>
</tr>
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</table>

**Discussion**

In accordance with previous findings distinct cyclic variations of basal and LRH double stimulated LH and FSH secretion were found in this study (Wang et al. 1976; Römmler et al. 1978a; Baumgarten 1979). The \( \Delta_2:\Delta_1 \) ratio of the gonadotrophin increments was usually greater than 1 but varied between 0.5 and 3.7 with the phase of the cycle. TSH and Prl secretion, on the other hand, remained constant during the cycle. Their \( \Delta_2:\Delta_1 \) ratio was less than 1 with no observed cyclic changes.

While the cyclic variation of the basal and LRH stimulated LH and FSH secretion are well documented, serum concentrations of TSH and Prl throughout the menstrual cycle, as mentioned above, are still giving rise to controversy. No variations of Prl and TSH during the menstrual cycle were observed in this study. The cyclic changes reported by some investigators could be explained by the high inter-individual variations of the hormone levels in the presence of a limited number of volunteers. Therefore, it is necessary for a study like this that the volunteers serve as their own controls. Thus, our results correspond well with those of Sawin et al. (1978) who tested all volunteers three times during two subsequent menstrual cycles.

Furthermore, careful selection of volunteers may
play an important role to exclude even minor endocrine abnormalities which may alter Prl and TSH secretion. For this reason 6 out of 15 'normal' volunteers had to be excluded from our study, because they did not fulfil the criteria listed in Table 1.

In accordance with results of our earlier studies the data reported here are further evidence in favour of the concept that increasing levels of oestrogens during the cycle amplify the LH and FSH responses after LRH double stimulation in a dose-related manner (Baumgarten 1979; Römmler 1980). Thereby $\Delta_2$ is a more sensitive parameter, because it increases more strongly than $\Delta_1$, while the basal gonadotrophins are only elevated after the high pre-ovulatory peak of oestrogen serum concentrations.

The Prl and TSH secretion pattern after TRH double stimulation reveals two major differences from those of the gonadotrophins.

Firstly, $\Delta_2$ is lower than $\Delta_1$ for Prl and TSH which is opposite to the response to LH and FSH.

It has been postulated for the gonadotrophins that this rise in $\Delta_2$ to a great extent is made possible by a rapid and excessive restoration of pituitary gonadotrophin stores by hormone synthesis following the first LRH stimulation under the influence of oestrogens (Römmler et al. 1978a,b; Römmler 1978, 1980). If such a mechanism is also correct for Prl and TSH, the reduced $\Delta_2$ observed for these hormones following TRH double stimulation may indicate a lower or delayed synthesis of Prl and TSH compared with LH and FSH. This relatively low rate of synthesis may therefore only partly replenish the pituitary stores of Prl and TSH during the 120 min interval between the two TRH injections. These observations are comparable with the studies of Bremer et al. (1976), who found clear-cut biphasic LH and FSH secretion during a 4 h infusion of LRH, while a similar infusion of TRH induced only a slight biphasic response of TSH and a monophasic Prl response.

It seems unlikely that it is a negative feedback of rising $T_3$ and/or $T_4$ serum levels which are observable 30 and 120 min, respectively, after a single TRH injection in euthyroid subjects (Ahuja et al. 1980) which cause the diminished TSH and Prl responses to the second TRH stimulation. This is concluded from observations in hypothyroid patients who showed an even lower $\Delta_2:\Delta_1$ ratio of TSH and Prl (Ahuja et al. 1980, and unpublished) than euthyroid volunteers. In these patients there was no response of $T_3$ or $T_4$ after TRH stimulation, as expected.

Secondly, the physiological changes of oestrogens during the menstrual cycle modify the capacity of pituitary LH and FSH secretion as clearly demonstrated by LRH double stimulation. The TRH double stimulation test, however, could not disclose any modulating properties of oestrogens on Prl and TSH secretion during the cycle. Only pharmacological doses of oestrogens may increase hypophyseal Prl and TSH secretion (Carlson et al. 1973; Taglia et al. 1973; Mortimer et al. 1974; Ramey et al. 1975; Reymond & Lemarchand-Béraud 1976; Smyth et al. 1977).

In conclusion, the data presented here indicate no changes of basal TSH and Prl concentrations throughout the normal menstrual cycle. Therefore, minor elevations of serum Prl levels above the upper normal range, even if they occur only in the periovulatory phase, should be considered abnormal. As a consequence, Prl suppression therapy may also be promising in infertile patients with only slightly elevated levels of Prl. Clinical results from our (Römmler et al., unpublished) and other groups (Lenton et al. 1977) support this concept.

Acknowledgments

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References


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