EVIDENCE OF PITUITARY AUTONOMY IN HYPERPROLACTINEMIC SECONDARY AMENORROEA: RESULTS OF HYPOTHALAMIC-PITUITARY TESTING

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SHORT TITLE: PRL TESTS IN SECONDARY AMENORRHOEA
ABSTRACT. Twenty-seven women with secondary amenorrhoea of greater than six months duration were subjected to multiple testing of hypothalamo-pituitary function. They were divided into normo-prolactinemic (Group 1 mean serum prolactin (PRL) 9.9 ± 0.5 SE ng/ml, n = 9) and hyperprolactinemic (Group 2 mean 35.0 ± 4.7 SE ng/ml, n = 18) groups on the basis of 4 weekly baseline determinations of pituitary, ovarian and thyroid hormones. Group 2 had significantly (P <.05) lower serum FSH, LH and urinary pregnanediol levels than did Group 1; there was no statistical difference between the groups in serum T4, T3 or urinary estrogen measurements. Two women in Group 2 were found to have a pituitary chromophobe adenoma.

Group 2 women showed no significant rises in serum PRL following stimulation tests with thyrotropin releasing hormone (TRH, 200 µg iv) and metoclopramide (10 mg orally), which caused significant responses in Group 1. Group 2 also had a significantly higher (P <.05) basal serum TSH (mean 1.82 ± 0.25 SE µU/ml) than did Group 1 (mean 1.13 ± 0.13 SE µU/ml) with preservation of the TSH response to TRH. This response was subnormal in Group 1 subjects. Both groups showed similar FSH and LH responses to luteinizing hormone-releasing hormone (LH-RH , 25 µg iv). No significant suppression of serum PRL was seen in Group 2 patients given L-Dopa (500 mg orally), which produced a significant response (P <.05) in Group 1 subjects, while all patients showed marked reduction in serum PRL values following 2-bromo-α-ergocriptine (CB-154, 2.5 mg orally). When compared with other Group 2 members, the 2 cases with proven pituitary adenomata gave similar responses to the stimulation-inhibition tests and were not clearly distinguished on this basis.

We conclude: 1. The pattern of PRL responses to dynamic
testing is consistent with the presence of an autonomous pituitary lesion in patients with hyperprolactinemic secondary amenorrhea. 2. Such dynamic tests, although of pathophysiological interest, provide no clinical information additional to that provided by the mean basal serum PRL value. 3. In clinical practice, such dynamic tests should be confined to patients with mean serum PRL levels at around the upper limit of the normal range.
Hyperprolactinemia is a common finding in women with secondary amenorrhea (1-3). However, with the exception of those patients who have clinical and/or radiological evidence of a pituitary tumor, the nature of the lesion causing hyperprolactinemia is unclear. In order to define the functional characteristics of this disorder, a number of dynamic tests of the anterior pituitary and hypothalamus have been described. Stimulation tests used have included the giving of luteinizing hormone-releasing hormone (LH-RH or GnRH) (2, 4, 5) and thyrotropin-releasing hormone (TRH) (6, 7) which both act directly on the pituitary to release FSH and LH, and TSH and PRL respectively. Other stimulation tests described have included the administration of chlorpromazine (4), which is thought to act via the hypothalamus to increase PRL secretion and metoclopramide (8), a dopamine receptor antagonist (9), that appears to act at both the pituitary and hypothalamus to increase PRL secretion. Inhibition tests have included the giving of L-Dopa (4, 10, 11) which acts via the hypothalamus and 2-bromo-α-ergocriptine (CB-154) (11, 12), a dopamine receptor agonist, which appears to act mainly at the pituitary to suppress prolactin secretion.

Despite their availability, such stimulatory and inhibitory tests have not been used together in a single group of women with disorders of ovulation. We have applied these tests to such patients with the following aims:

1. To determine basal pituitary, ovarian and thyroid hormone secretion in women with disorders of ovulation, with and without hyperprolactinemia.
2. To assess the diagnostic and prognostic value to be gained from
hypothalamic-pituitary tests of prolactin secretion.

3. To try to define the etiology of the patients' failure to ovulate.

Materials and Methods

Patients

Twenty seven women, aged 20 to 39 years, attended the Endocrine Clinics of the authors' hospitals, with the complaint of secondary amenorrhoea of greater than 6 months duration with or without associated infertility. The initial assessment of each patient included history and examination with particular attention to past obstetric complications, previous use of oral contraceptives, past response to Clomiphene and the presence of galactorrhea. All patients were clinically euthyroid.

Baseline evaluation

All women had skull x-rays including coned views of the pituitary fossa and visual field charting when indicated. In order to establish adequate baseline data each woman was tested weekly for 4 weeks. A morning blood sample was taken between 0900 and 1100 h with the patient supine and after 15 min rest for PRL, FSH, LH and thyroid hormone (T₃, T₄) estimates and a 24 hour urine sample collected for determination of urinary estrogen and pregnanediol as described previously (13).

Hormone stimulation tests and assay methods

Following this period, 3 stimulation tests were given: 200 µg of TRH and 25 µg of LH-RH were injected simultaneously iv and blood samples taken via an indwelling venous cannula at -20, 0, 10, 20, 30, 60, 90 and 120 min for determination of PRL, TSH, T₃, T₄, FSH and LH values.
Next morning a 10 mg tablet of metoclopramide was given and blood samples taken for PRL assay at 0, 60, 120, 180, 240 and 300 min.

All pituitary and thyroid hormones were measured in duplicate in double antibody homologous radioimmunoassays. Serum PRL was determined using reagents supplied by the National Pituitary Agency (VLS 2 antiserum and VLS 2 hPRL for radioiodination and standards). Purified hPRL (2.5 μg) was labelled with $^{125}$I by the chloramine T method (14), initially purified using Sephadex G50 filtration and further purified using Sephadex G100 filtration on the day the hormone was added to the assay. The second antibody was supplied commercially by Wellcome (U.K.). The sensitivity of the assay was 1.6 ng/ml, the intra-assay variation 5.8% and the inter-assay variation 11.2%. The normal range, given by 95% confidence limits, for premenopausal women, not on oral contraceptives, was 4.6 to 16.6 (mean 10.6) ng/ml.

Serum TSH levels were measured as described previously (15), the normal range for women not on oral contraceptives being <0.2 to 3.6 μU/ml. Similarly, gonadotropins were assayed using methods previously described (16), the normal female range being indicated in Table 3.

Hormone inhibition tests

At least 1 day after the metoclopramide stimulation test, a 500 mg tablet of L-Dopa (L-3,4-Dihydroxyphenyl-alanine) was given and blood samples taken for PRL assay at 0, 60, 120, 180, 240 and 300 min. On the next morning, a further inhibition test using a 2.5 mg tablet of 2-bromo-α-ergocriptine (CB-154) was performed and blood samples taken as above over a 5 hour period.
Statistical analyses

Student's "t" test was used in the comparison of data.

Results

Basal evaluation

Weekly blood sampling for 1 month showed that the patients could be divided into groups by the average or transverse mean of their basal serum prolactin concentration. Two groups were defined: Group 1 patients (n = 9) had a mean basal serum prolactin value within the normal range (mean 9.9 ± 0.5 SE ng/ml) while Group 2 patients (n = 18) had an elevated mean serum prolactin value (mean 35.0 ± 4.7 SE ng/ml). Of the patients in Group 1 (Table 1), 6 had previously used oral contraceptives, no patient had any abnormality detected on radiology of the skull and only 1 woman (patient 8) had demonstrable galactorrhoea. This patient was the only one to ovulate, as estimated by urinary pregnanediol estimations, during the tracking period. Of the Group 2 subjects, 14 had previously used oral contraceptives and galactorrhoea was evident in 15 cases. In 2 patients (patients 12 and 20), an intrasellar mass was evident radiologically and transpalatal removal of a pituitary chromophobe adenoma was performed. A visual field defect was detected clinically in only 1 patient (patient 12) and this was confirmed by perimetry and Bjerrum screen charting. No patient in Group 2 ovulated during the basal tracking period and they had statistically lower (P < .05) basal pregnanediol and gonadotropin values than did the subjects in Group 1 (Table 3).
PRL and TSH Response to TRH

Serum PRL responses following the intravenous administration of 200 μg of TRH to both groups are shown in Figure 1. In Group 1 patients, a maximal rise in PRL had occurred 15 min after the injection (mean 26.9 ng/ml; range 16.3 to 49.2 ng/ml), persisted until 30 min and then declined slowly to reach basal values 180 min after the injection. In contrast to this response, all Group 2 patients were unresponsive to TRH, their serum PRL failing to rise significantly from the basal level of 34.4 ng/ml (range 19.7 - 93.2 ng/ml).

Figure 2 depicts the serum TSH values following intravenous injection of 200 μg of TRH. All patients had basal TSH values within the normal range although the Group 2 value (mean 1.82 ± 0.25 SE μU/ml) was higher (p <0.05) than for Group 1 (mean 1.13 ± 0.13 SE μU/ml). The response pattern of Group 2 subjects to the TRH injection was also (15), within the normal female range/ peak serum values occurring at 30 min (mean 17.1 μU/ml; range 5.3 to 35.6 μU/ml). Five women in this group (patients 15, 20, 23, 25 and 26) had subnormal TSH responses. As a group, the TSH response to the TRH injection in Group 1 patients was subnormal, where only 2 women (patients 3 and 4) had TSH responses within the normal female range. Moreover, Group 1 subjects had statistically lower TSH values at 0, 30, 60 and 90 min after the TRH injection compared with patients in Group 2.

Thyroid hormone values

Basal T3 and T4 levels are shown in Table 3. All patients were clinically and biochemically euthyroid and there was no difference between the 2 groups for any test of thyroid function. At 120 mins after the TRH test, both groups showed significant increases in serum T3 values
(Group 1: mean 1.8 ± 0.1 SE nmol/l, Group 2: mean 1.9 ± 0.1 nmol/l).

However, there was no difference between the groups in this T₃ increase and no correlation between the T₃ increases and the differing TSH responses to TRH shown in Figure 2.

**PRL response to metoclopramide**

The serum PRL response of both groups to a 10 mg tablet of metoclopramide is shown in Figure 3. In Group 1, a marked increase in serum PRL occurred by 1 hour after ingestion which peaked at 2 hours (mean 34.5 ng/ml; range 20.5 to 42.9 ng/ml) and was still significantly elevated above the basal value 5 hours after oral administration. As for their response to TRH, all Group 2 subjects showed no increase in serum PRL at any time after the metoclopramide tablet, regardless of their initial PRL value.

**Gonadotropin response to Gn-RH**

Basal FSH values for both groups were in the range seen at the early follicular stage of the menstrual cycle. Despite lower basal gonadotropin values for Group 2 patients, both groups gave near identical FSH and LH response patterns after intravenous administration of 25 µg of Gn-RH (Figure 4). The FSH response was at the upper limit of, or slightly above, the FSH values seen following LH-RH in the early follicular phase of the menstrual cycle. There was an increase to peak values 30 min after the injection and then a broad plateau which persisted until at least 120 min following LH-RH.

The LH response pattern also showed an increase to peak values at 30 min with levels then declining steadily towards basal over the next 90 min.
PRL suppression by L-Dopa

Figure 5 depicts the results from oral administration of 500 mg of L-Dopa. Both groups of subjects behaved similarly. In Group 1, there was a modest fall in serum PRL at 1 hour after ingestion which reached its nadir at 2 and 3 hours \( (P < 0.05) \), and then returned towards the basal value. In Group 2, a similar pattern was observed although at no time did the decrease in serum PRL reach statistical significance.

PRL suppression by CB-154

The response to oral administration of 2.5 mg of CB-154 is shown in Figure 6. All patients showed a decreased serum PRL level after this test. In Group 1, serum PRL decreased significantly by 2 hours and continued to decline until at least 5 hours after ingestion. A similar response pattern was seen in Group 2. When compared with their basal value, the Group 2 subjects showed more marked suppression of serum PRL than did the women in Group 1 and at 5 hours, 11 of the 18 patients had serum PRL within the normal range.

Responses in patients with proven pituitary adenomata

Two patients (patients 12 and 20) had transpalatal removal of a pituitary chromophobe adenoma. Immunofluorescent localization of PRL cells within the tumor was confirmed in 1 patient (patient 12). The responses of these 2 patients, prior to surgery, to the various tests of the pituitary and hypothalamus is described in Figure 7. Although the average basal PRL value of patient 12 was higher \( (57.5 \text{ ng/ml}) \) than for patient 20 \( (27.9 \text{ ng/ml}) \), both women responded in a similar manner to those other patients in Group 2, who did not have obvious pituitary neoplasms, when tested with TRH, metoclopramide, L-Dopa and CB-154. The
presence of a pituitary neoplasm was not suggested by their response to these tests, when compared with other women with hyperprolactinemia and secondary amenorrhea. Serum TSH determinations after TRH testing showed that patient 12 responded normally and that patient 20 had a subnormal response. However, 4 other patients in Group 2 also responded subnormally to this stimulation test and did not have evidence of a pituitary adenoma. Following LH-RH, patient 20 gave the smallest peak FSH and LH response of any subject with hyperprolactinemia. Although the peak gonadotropin values after LH-RH for patient 12 were also below the mean for Group 2, similar values and patterns of response were also found in other patients in this group who did not have evidence of a pituitary neoplasm.

Discussion

In patients with secondary amenorrhea, it is important to identify those with persistently elevated serum PRL values, as specific medical therapy with ergot alkaloid derivatives such as CB-154 (11, 12), ergocornine (17) and lergotrile mesylate (18) is now available and as some have pituitary neoplasms amenable to surgery. In such women, incorrect clinical decisions can follow when elevated serum PRL values are due, not to persistent hypersecretion, but to transient elevations following recent breast examination for the presence of galactorrhea (19, 20), slight increases in plasma osmolality (21) or the stress and pain associated with attending hospital, exercise, minor surgery and repeated venepuncture (22). In this study, 4 blood samples were taken at weekly intervals under standardized conditions in an attempt to minimize
this source of error. The resultant mean serum PRL level for each patient seemed to divide subjects reliably into those with normal and elevated PRL secretion. Hyperprolactinemic women with secondary amenorrhea had lower serum FSH and LH values and lower urinary pregnanediol values on basal tracking than did similar women with normal PRL values. Low basal LH, but not FSH, has been reported by others (2) in patients with this menstrual disorder and it has been suggested that this arises from PRL inhibition of LH-RH release from the hypothalamus (10). Our data would be consistent with this view. Reduced pregnanediol excretion by patients with hyperprolactinemia is consistent with in vitro data of decreased progesterone secretion by cultured human granulosa cells in the presence of high PRL concentrations (23).

In this study the group of women with hyperprolactinemia and secondary amenorrhea were refractory to further increases in serum PRL following TRH and metoclopramide testing. This phenomenon was uniform throughout all patients in the group regardless of their basal PRL value. Other studies have found blunted or absent responses after TRH in such patients (12, 24, 25) although this is in contrast to another report (7). Metoclopramide stimulates PRL release from the pituitary in normal volunteers (8, 26), acting centrally to increase the turnover of cerebral dopamine (9), where it behaves as a dopamine receptor antagonist. Its effect in women with secondary amenorrhea has not been previously described. A hypothalamic site of action is proposed for this drug (26) which has recently also been shown to release PRL directly from cultured rat pituitary glands (27). If the hyperprolactinemia of the Group 2 patients was due to a prolactin-secreting microadenoma in the pituitary
gland, as has been suggested (28), with subsequent de-differentiation or loss of receptors in such cells to TRH and metoclopramide, the failure of these cells to be stimulated would be explained. However, this would not explain the failure of the remaining PRL-secreting cells in the adjacent, normal pituitary (29) to be stimulated by these tests. Consistent with our data is the hypothesis that autonomous PRL-secreting cells exist in these patients which suppress the secretion of any remaining pituitary PRL cells. Such an 'ultrashort' feedback loop for PRL has recently been proposed (25).

Although all basal TSH values were within the normal range, patients with hyperprolactinemia had significantly higher levels than did those with normal serum PRL. This unexpected finding has not been reported previously to our knowledge. It differs from a recent report (24) where no difference in basal TSH values was observed. As the mean sensitivity of the present TSH assay is 0.2 ± 0.05 (S.D.) μU/ml of serum, compared with a minimum detectable level of 2.0 μU/ml for these workers, the increased sensitivity of our assay system may explain this difference in results. The reason for the relatively elevated basal serum TSH is unclear. There is now much data that physiological levels of thyroid hormones determine the amount of PRL and TSH released by TRH (30, 31) but there was no difference between Group 1 and 2 patients in their basal thyroid hormone values. Estrogens, another modulating influence on TSH secretion by the pituitary were also similar between the 2 groups. A third possible agent causing higher basal TSH values in hyperprolactinemic women is higher endogenous TRH secretion. We found that, as a group, these subjects released TSH normally after exogenous TRH stimulation. If endogenous TRH caused the elevated basal TSH value, then one would
expect a reduced TSH response to endogenous TRH stimulation (32). This was not the case. One may speculate that the putative hormone, prolactin releasing factor (PRF), acts on the pituitary to increase TSH as well as PRL secretion in an analogous manner to the known effects of TRH. Women with normal serum PRL values (Group 1) generally had subnormal TSH increases after TRH stimulation. At the time of writing the explanation of this phenomenon is unknown.

In hyperprolactinemic secondary amenorrhea, without the presence of obvious pituitary tumors, LH-RH responses have been reported as excessive (33), normal (12, 34), deficient (2, 4) or completely independent of PRL (35). In some of these studies, the results for normal females are either not stated at all or the stage of the menstrual cycle, against which the comparison is made, has been omitted. We find the pituitary gland in secondary amenorrhea is responsive to LH-RH to a considerably greater degree than during the early follicular phase of the normal menstrual cycle, regardless of the basal PRL secretion of the patient. The ambient serum PRL level does not appear to influence the response of the gonadotroph to LH-RH. We agree with other workers (4, 33) that patients with pituitary adenomata are less responsive to LH-RH than are other women with hyperprolactinemia.

L-Dopa (4, 36) has been reported to suppress serum PRL acutely within 4 hours of ingestion in patients with hyperprolactinemia and menstrual disorders, by increasing hypothalamic dopamine and/or PRL-inhibitory factor (PIF). As an acute suppression test, our patients with hyperprolactinemia were again refractory to this agent. PRL values did not fall significantly although we agree with others (4) that a slight reduction does occur by 2 to 3 hours after ingestion, followed by a
return towards the initial value. By contrast, patients with normal serum PRL values significantly reduce their serum PRL levels but this effect is again transient, lasting less than 5 hours. In terms of hypothalamic-pituitary mechanisms, patients with hyperprolactinemia and secondary amenorrhoea are resistant to the hypothalamic inhibition caused by L-Dopa administration. This may further indicate the autonomy of the anterior pituitary in this condition. In terms of etiologic factors, the frequency with which hyperprolactinemic patients with secondary amenorrhoea gave a history of past use of oral contraceptives (Table 2) suggests that, as in rodents (37), these agents may induce the formation of functioning pituitary adenomata. Complete pituitary autonomy does not exist, however, as shown by suppression of serum PRL with CB-154 in all patients, a finding now well described (1, 5-7, 12, 24).

In our hands, stimulation and suppression tests of PRL secretion gave no more clinically useful information than repeated single serum PRL values taken under standardised conditions. Diagnostically, the tests did not differentiate patients with proven pituitary adenomata from other women with hyperprolactinemia (Figure 7). Prognostically, the role of these tests is unclear. A possible clinical use is in the patient with a truly borderline basal PRL. In this case, the response pattern on dynamic testing of absent stimulation of serum PRL by TRH and metoclopramide, preservation of the TSH rise after TRH, and poor gonadotropin elevation after LH-RH, may suggest an increased risk of developing a pituitary adenoma.
**TABLE 1**

Clinical data in 9 women with secondary amenorrhoea and normal serum PRL values (Group 1)*.

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* All patients had normal skull radiology
+ AB Spontaneous abortion
* HPG Human pituitary gonadotropin
* AO Anovulatory
Δ Mean urinary pregnanediol 2.0 mg/24 h; range 0.9 - 3.8 mg/24 h.
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* N - Normal.  + CB-154 - 2-bromo-oergocriptine
TABLE 3

Basal pituitary, ovarian and thyroid hormone values in secondary amenorrhoea following weekly sampling for 1 month

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<th>Group 2 (n = 18)</th>
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<td>LH</td>
<td>3.8-11.2 mIU/ml</td>
<td>11.3 ± 3.0</td>
<td>5.4 ± 1.4</td>
</tr>
<tr>
<td>Estrogens</td>
<td>5.1-34 μg/24 h</td>
<td>12.4 ± 3.3</td>
<td>9.0 ± 3.2</td>
</tr>
<tr>
<td>Pregnanediol</td>
<td>0.2-1.0 mg/24 h</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>T₄</td>
<td>52-166 nmol/l</td>
<td>82.0 ± 4.9</td>
<td>76.8 ± 3.6</td>
</tr>
<tr>
<td>T₃</td>
<td>1.1-2.7 nmol/l</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

* All values expressed as mean ± SE

+ Significantly different on comparing Group 2 with Group 1 values, P < .05.

x Normal early follicular range values

# The normal range differs from that in ref (16) as the majority of the present FSH and LH determinations were made in the laboratories of one of the authors (RJP) using different reagents from those of (16). Results from the other laboratory (HGB) have been transformed on the basis of a group of sera assayed in common.
Legends to Figures

FIGURE 1: Effect of TRH (200 µg iv) on serum PRL in secondary amenorrhoea. ‡P < .01 compared with the basal value.

FIGURE 2: Effect of TRH (200 µg iv) on serum TSH in secondary amenorrhoea. The stippled area represents the range of values (± SE) for normal women. ‡P < .01 compared with the basal value. *P < .05 comparing Group 1 with Group 2.

FIGURE 3: Effect of metoclopramide (10 mg orally) on serum PRL in secondary amenorrhoea, ‡P < .01 compared with the basal value. *P < .001 comparing Group 1 with Group 2.

FIGURE 4: Effect of LH-RH (25 µg iv) on serum FSH and LH in secondary amenorrhoea. The stippled area represents the range of values (± SE) for normal women during the early follicular phase. *P < .05 comparing Group 1 with Group 2.

FIGURE 5: Effect of L-Dopa (500 mg orally) on serum PRL in secondary amenorrhoea. *P < .05 compared with the basal value.

FIGURE 6: Effect of CB-154 (2.5 mg orally) on serum PRL in secondary amenorrhoea. ‡P < .05 compared with the basal value; *P < .05 comparing the Group 2 response with that in Group 1.

FIGURE 7: Results of various hypothalamic-pituitary tests in 2 patients with proven pituitary adenomas (patients no. 12 and 20) compared with other hyperprolactinemic subjects with secondary
amenorrhoea. The stippled area represents the range of values (± SE) seen in other Group 2 patients.
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