Catecholamines and pituitary function.
IV. Effects of low-dose dopamine infusion and long-term bromocriptine treatment on the abnormal thyrotroph (TSH) dynamics in patients with pathological hyperprolactinaemia

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Abstract. In order to gain further insight into the role of dopamine (DA) in the control of TSH release and to investigate whether an increased or defective DA inhibition on pituitary thyrotrhops may be considered responsible for the abnormal TSH dynamics in pathological hyperprolactinaemia, we examined the effect of low-dose DA infusion on TRH stimulated TSH secretion in normally cycling women and in patients with pathological hyperprolactinaemia. The effect of long-term bromocriptine therapy on TSH dynamics was also evaluated in a selected group of hyperprolactinaemic women.

Fifty-two hyperprolactinaemic patients with no other signs of pituitary or thyroid dysfunction had significantly higher mean TSH serum concentrations and mean TSH peak values after TRH administration than 75 healthy controls. Furthermore, the TSH rises induced by the DA-synthesis inhibitor α-methyl-p-tyrosine (AMPT, 500 mg orally) were enhanced in both prolactinoma and "idiopathic hyperprolactinaemia" patients as compared with controls. There was a positive correlation between the TRH- and AMPT-induced TSH rises in the hyperprolactinaemic group.

Low-dose DA infusion (0.1 µg/kg·min) reduced TSH response to TRH in both regularly cycling women and patients with hyperprolactinaemic amenorrhoea. Long-term bromocriptine therapy (2.5 mg tid over 60–150 days) not only normalized serum Prl levels, but also reduced the TSH response to TRH in 7 hyperprolactinaemic women who had presented exaggerated TSH responses to the basal TRH test.

These findings confirm that DA plays a physiological role in the inhibition of TSH release, probably at the level of the anterior pituitary. The fact that both low-dose DA infusion and long-term bromocriptine treatment effectively reduced TSH release in hyperprolactinaemic patients seems to indicate that endogenous DA inhibition of pituitary thyrotrhops is reduced rather than enhanced in pathological hyperprolactinaemia.

Although the hypothalamus exerts a dominant stimulatory effect on TSH synthesis and release through TRH secretion in the pituitary portal blood (Jackson 1982), there is now very convincing evidence that hypothalamic dopamine (DA) is a physiological inhibitor of TSH release in both animal models and man (Scanlon et al. 1977, 1980; Krulich 1982). High affinity DA receptors, which are functionally related with the inhibition...
of TSH secretion, have been identified in rat anterior pituitary cells monolayer cultures (Foord et al. 1983). In addition, DA infusion reduces both basal TSH levels and TSH response to TRH administration in man (Massara et al. 1978; Burrow et al. 1977; Bessis et al. 1975), whereas DA-antagonists as well as DA-synthesis inhibitors enhance serum TSH concentrations (Scanlon et al. 1979; Massara et al. 1981).

The exaggerated TSH response to DA-antagonist drugs such as metoclopramide or domperidone, reported in patients with Prl-secreting pituitary microadenomas (Quigley & Yen 1980; Scanlon et al. 1981), classically has been attributed to the short-loop feedback effect of raised circulating Prl levels on tubero-infundibular dopaminergic neurons and to a consequent increase in DA concentrations in hypothalamic portal blood (Cramer et al. 1979; Moore et al. 1980). However, both raised basal TSH serum levels and enhanced TSH responses to TRH (Thorner et al. 1977; Erfurth et al. 1983; Rodriguez-Arnao et al. 1983), which have been ascribed to a defective DA inhibition on pituitary thyrotrophs (Erfurth et al. 1983), have been observed in hyperprolactinaemic subjects.

The aim of this study was to gain further insight into the role of DA in the control of TSH release and to investigate whether defective or increased DA inhibition of TSH-producing pituitary cells might be responsible for the abnormal TSH dynamics in patients with pathological hyperprolactinaemia.

Materials and Methods

**TSH response to TRH in normal subjects and patients with pathological hyperprolactinaemia**

We carried out a retrospective analysis of the TSH response to TRH in pathological hyperprolactinaemia patients examined in our institution between January 1978 and December 1983. Subjects with goitre, low-normal T₄ and T₃ serum levels, anti-thyroglobulin or thyroid-microsomal autoantibodies, and hypopituitarism were excluded. No patient had initiated therapy and none had taken any drugs for 1 month prior to the study. Fifty-two patients (40 females, 12 males) fulfilled these criteria. Pituitary tumour was proved by hypocyidual tomography or CT scan in all the men and 24 of the women. The Prl levels in this group ranged from 30 to 13 000 ng/ml (mean 918.8 ± 435.4 S). The remaining 16 women, who were considered to have 'idiopathic hyperprolactinaemia', had Prl levels between 30 and 204 ng/ml (mean 93.8 ± 12.5, P < 0.05 vs pituitary tumours). Seventy-five healthy subjects (54 females, 21 males) whose thyroid hormone and Prl values were within the normal reference limits of our laboratory acted as controls.

TRH, 200 µg iv (Relactif TRH, Hoechst AG, Frankfurt am Main, West Germany), was administered in the morning with the subjects at rest, after an overnight fast. Blood samples were drawn through a forearm iv cannula. Basal samples were collected at −50, −15 and 0 min, thereafter sampling was performed at 10, 20, 30, 40, 60 and 120 min.

**TSH response to catecholamine synthesis inhibition in normally cycling and hyperprolactinaemic women**

Eight euthyroid normally cycling women received 500 mg of the tyrosine-hydroxylase inhibitor α-methyl-p-tyrosine (MK 781 from Merck Sharp and Dohme, Hoddesdon, England) by mouth, after informed consent. The test was performed in the morning after an overnight fast, during the early follicular phase of the cycle (days 2–6). Nineteen women with hyperprolactinaemic amenorrhoea, 11 with proved pituitary tumour and 8 with presumed 'idiopathic hyperprolactinaemia', received the same α-methyl-p-tyrosine (AMPT) dose. Blood was drawn from both normal and hyperprolactinaemic women through an indwelling venous catheter at −60, −40, −20 and 0 min for the basal TSH assay and then at 30-min intervals during the 6 h following AMPT administration.

**Effect of low-dose DA infusion (0.1 µg/kg·min) on TSH response to TRH in normally cycling and hyperprolactinaemic women**

Eight normally cycling women and 13 patients with hyperprolactinaemic amenorrhoea, 9 with CT evidence of pituitary tumour and 4 with 'idiopathic hyperprolactinaemia', were studied. All subjects underwent a basal TRH test and a second TRH test at time 240 min of a 6 h low-dose (0.1 µg/kg·min) DA infusion. The tests were performed in the early follicular phase of two consecutive cycles in the normal women and with an interval of at least two weeks in the hyperprolactinaemic patients. For the basal test TRH (200 µg iv) was administered at 13.00 h, after four blood samples had been obtained at −60, −40, −20 and 0 min. Samples were then collected after 10, 20, 30, 40, 60, 80, 100 and 120 min. When the TRH test was repeated during an 0.1 µg/kg·min DA infusion, DA (Revivan, Simes, Milan, Italy) in 5% dextrose solution was started at 09.00 h, after four basal blood samples had been obtained at −60, −40, −20 and 0 min. Blood was collected at 20-min intervals during the 6 h DA infusion. TRH (200 µg iv) was administered at 13.00 h and thereafter samples were obtained at 10, 20, 30, 40, 60, 80, 100 and 120 min.
Effect of long-term bromocriptine treatment on TSH response to TRH in women with hyperprolactinaemic amenorrhoea

TSH response to TRH was re-examined during long-term bromocriptine (Parlodel, Sandoz, Basel, Switzerland) treatment (2.5 mg tid) in 7 hyperprolactinaemic patients who had presented exaggerated TSH responses to the basal TRH challenge. The treatment period ranged from 60 to 150 days and the patients had their morning bromocriptine dose before TRH administration when the test was repeated during the course of treatment. After four basal blood samples had been drawn for the Prl and TSH assays, TRH (200 µg iv) was administered and thereafter sampling was performed at 10, 20, 30, 40, 60 and 120 min.

Hormone assay and analysis of data
Sequential T₃, T₄ and Prl were measured by specific radioimmunoassays as previously described (Nicoletti et al. 1981). Thyroglobulin and thyroid-microsomal antibodies were evaluated by commercial haemagglutination tests (Thymune M and Thymune T, Wellcome, Beckenham, England). Serum TSH was measured by a specific double-antibody radioimmunoassay method using reagents supplied by Becton Dickinson (Orangeburg, NY, USA). The intra- and inter-assay coefficients of variation for this assay in our laboratory were 5.5% and 9% at a value of 5 µU/ml. The sensitivity of the assay was 0.4 µU/ml. The TSH standard was calibrated against the WHO/MCR 68/38.

Results are expressed either in absolute values or as the net change (Δ) from the mean basal concentration, determined in the four basal samples. The values given are mean ± SE. Student's t-test, analysis of variance (ANOVA), and Pearson's regression analysis as appropriate, were used for the statistical evaluation of the results.

Results

Basal TSH serum levels and TSH response to TRH
(Table 1)

There was no significant difference between the T₃ and T₄ serum levels of normal subjects and hyperprolactinaemic patients. However, basal TSH concentrations were significantly (P < 0.01) higher in the hyperprolactinaemic subjects (Table 1). TSH levels were similarly increased in the hyperprolactinaemic patients with (2.70 ± 0.36) and without (2.81 ± 0.70) radiological signs of pituitary tumours. Ten hyperprolactinaemic patients with T₃ and T₄ serum levels in the normal range and no thyroid autoantibodies had basal TSH values above the mean + SD of the controls.

The TSH response to TRH was also enhanced in the hyperprolactinaemic group (Table 1). Irrespective of whether there was radiological proof of tumour or not, hyperprolactinaemic patients as compared with controls displayed similar and significant (F 4.70, P < 0.05 by ANOVA) rises in TSH response to TRH (Δ TSH 10.44 ± 0.53 in normal subjects; 16.87 ± 3.22 in prolactinoma patients: 18.03 ± 4.18 in 'idiopathic hyperprolactinaemias'). Neither basal TSH levels nor the TSH response to TRH correlated with the serum Prl concentrations.

TSH response to catecholamine synthesis inhibition
(Table 2)

AMPT administration was followed by a significant rise in the serum TSH levels in all groups. The AMPT-induced TSH peaks were higher in

<table>
<thead>
<tr>
<th>Serum T₄ nm/l</th>
<th>T₃ nm/l</th>
<th>Serum TSH µU/ml</th>
<th>Peak TSH µU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n = 75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98.89 ± 1.75</td>
<td>1.96 ± 0.07</td>
<td>1.91 ± 0.13</td>
<td>12.38 ± 0.60</td>
</tr>
<tr>
<td>(69.7-121.3)</td>
<td>(0.84-2.47)</td>
<td>(0.40-5.30)</td>
<td>(3.80-25.30)</td>
</tr>
</tbody>
</table>

Hyperprolactinaemic patients n = 52

<table>
<thead>
<tr>
<th>Serum T₄ nm/l</th>
<th>T₃ nm/l</th>
<th>Serum TSH µU/ml</th>
<th>Peak TSH µU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.52 ± 2.31</td>
<td>2.06 ± 0.06</td>
<td>2.73 ± 0.28ᵃ</td>
<td>19.97 ± 2.66ᵃ</td>
</tr>
<tr>
<td>(63.2-118.7)</td>
<td>(1.37-2.49)</td>
<td>(0.40-7.40)</td>
<td>(1.80-111.0)</td>
</tr>
</tbody>
</table>

ᵃ: P < 0.01 vs controls.
Table 2.
Effect of α-methyl-p-tyrosine administration (500 mg by mouth) on serum TSH concentrations in regularly cycling women and in patients with pathological hyperprolactinaemia. Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Basal TSH µU/ml</th>
<th>Peak TSH µU/ml</th>
<th>Max. diff. µU/ml</th>
<th>Sum incr. µU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n = 8</td>
<td>2.29 ± 0.40</td>
<td>4.05 ± 0.69</td>
<td>1.75 ± 0.43</td>
<td>6.03 ± 2.42</td>
</tr>
<tr>
<td>Prolactinomas n = 11</td>
<td>3.28 ± 0.40a</td>
<td>6.84 ± 0.55b</td>
<td>3.55 ± 0.41a</td>
<td>19.19 ± 3.23b</td>
</tr>
<tr>
<td>Idiopathic hyperprolactinaemia n = 8</td>
<td>3.02 ± 0.47a</td>
<td>7.09 ± 0.81b</td>
<td>4.06 ± 0.64b</td>
<td>20.64 ± 3.60b</td>
</tr>
</tbody>
</table>

a: P < 0.05; b: P < 0.01 vs controls by ANOVA.

patients with prolactinomas and 'idiopathic hyperprolactinaemias' than in normal subjects (F 6.12, P < 0.01 by ANOVA). The TSH response to AMPT was similar in patients with and without radiological proof of pituitary tumour (Table 2). TRH- and AMPT-induced TSH rises were positively correlated in hyperprolactinaemic patients (y = 1.87 + 0.071x, r: 0.68, P < 0.01).

Effect of 0.1 µg/kg · min DA infusion on TSH response to TRH (Fig. 1)
A low-dose infusion of DA induced during its course a fall in mean serum TSH levels which was similar in both controls (−36.0 ± 9.9%) and patients with pathological hyperprolactinaemia (−44.0 ± 8.1%).

Furthermore, 0.1 µg/kg · min DA blunted the
TSH response to TRH in the normally cycling women (Δ TSH 9.83 ± 1.81 in basal TRH test, 6.08 ± 0.86 when TRH was administered during DA infusion, P < 0.001 vs basal test), and reduced the TSH hyperresponsiveness to TRH in hyperprolactinaemic patients (Δ TSH 25.65 ± 7.74 in basal test, 15.92 ± 6.86 when TRH was administered during DA infusion, P < 0.005 vs basal test).

**Effect of long-term bromocriptine treatment on TSH response to TRH in patients with pathological hyperprolactinaemia** (Fig. 2)

A significant reduction in basal Prl levels was observed in hyperprolactinaemic patients during bromocriptine administration (from 143.8 ± 39.3 ng/ml to 11.4 ± 2.7, P < 0.01). Bromocriptine treatment caused an evident, but not statistically significant, reduction in basal TSH levels (from 3.87 ± 0.92 to 2.90 ± 0.37) and a significant decrease in the TSH response to TRH in 7 patients with pathological hyperprolactinaemia who had presented enhanced TSH responses to the basal TRH test (Δ TSH 39.87 ± 6.63 in basal conditions, 17.24 ± 2.11 during bromocriptine, P < 0.005 vs basal test).

**Discussion**

Without any clinical or biochemical evidence of thyroidal dysfunction, a clear abnormality in TSH dynamics was evident in our hyperprolactinaemic patients who had higher mean basal TSH levels and enhanced TSH responses to both TRH and AMPT than the controls.

Since subjects with low-normal T4 and T3 serum levels or anti-thyroglobulin and anti-microsomal antibodies were excluded from the study, the possibility that a subclinical hypothyroidism accounts for the abnormal TSH behaviour can reasonably be excluded. Furthermore, the TSH hyperresponse to both TRH and AMPT and the significant linear correlation between the TRH- and AMPT-induced TSH rises seem to indicate that pituitary reserves of TSH are increased in hyperprolactinaemic patients.

Enhanced TSH responses to DA-antagonist drugs have already been described in patients with prolactinomas and it has been hypothesized that an increased DA inhibition of pituitary thyrotrophs accounts for this phenomenon (Quigley & Yen 1980; Scanlon et al. 1981). Such an assumption is mainly based on animal data which demon-
strate that acute hyperprolactinaemia, provoked by either a transplantable Prl-secreting tumour or the implantation of an ectopic pituitary, leads to a rise in the hypophysial portal blood DA concentrations through the activation of the short-loop feedback between Prl and tuberoinfundibular DA neurons (Cramer et al. 1979). However, as TSH responses to TRH are sometimes increased in hyperprolactinaemic patients (Thornor 1977; Erfurth et al. 1983), it seems unlikely that pituitary thyrotrophs are exposed to raised DA concentrations, since specific DA receptors, which are functionally related with the inhibition of TSH release, have been identified in these cells (Foord et al. 1983) and it is known that DA infusion reduces basal TSH release and also blunts the TSH response to TRH in normal men (Besses et al. 1975; Scanlon et al. 1980).

It therefore seems reasonable to hypothesize, as already suggested by Erfurth et al. (1983), that the reduced availability of DA at the pituitary thyrotrophs DA receptor site accounts for both the increased basal TSH concentrations (Rodriguez-Arnao et al. 1983) and the enhanced TSH response to TRH encountered in patients with pathological hyperprolactinaemia. The effectiveness of low-dose DA infusion in reducing the exaggerated TSH responses to TRH in hyperprolactinaemic women seems to confirm that reduced DA inhibition of pituitary thyrotrophs is responsible for the abnormal TSH dynamics in these patients. The DA dose we used has been demonstrated to produce peripheral DA levels similar to those measured in the pituitary portal plasma and to normalize serum Prl concentrations in stalk-transected Rhesus monkeys (Neill et al. 1981). Recent data from our laboratory showed that a similar DA dose both normalizes serum Prl levels after endogenous catecholamine synthesis inhibition in normally cycling women and restores the Prl response to TRH in women with pathological hyperprolactinaemia (Nicoletti et al. 1984). Although we did not measure the peripheral catecholamine levels during DA infusion, Ho et al. (1984) recently reported that a DA infusion rate of 0.5 μg/kg·min, 5-fold greater than ours, gives circulating peripheral DA levels similar to those measured in the pituitary portal plasma of animal models. So, the availability of even small amounts of DA at the DA pituitary receptors apparently both restores the physiological Prl response and reduces the TSH hyperresponsiveness to TRH in patients with pathological hyperprolactinaemia. These findings strongly support the concept that the abnormal Prl and TSH dynamics encountered in hyperprolactinaemia both are due to a relative DA deficiency at the anterior pituitary cells. This view fits in well with the recent demonstration that, in rats, chronic hyperprolactinaemia owing to either spontaneous Prl-secreting pituitary tumours or to prolactinomas induced by prolonged oestrogen treatment, leads to a loss of functional tuberoinfundibular DA neurons and to an accompanying drop in DA concentrations in the median eminence and pituitary portal blood (Sarkar et al. 1982, 1983; Casanueva et al. 1982). Interestingly, in our patients long-term bromocriptine therapy not only normalized serum Prl levels but also considerably reduced the exaggerated TSH responses to TRH. A finding that offers further evidence for a defective DA inhibition at both the pituitary thyrotrophs and lactotrophs in pathological hyperprolactinaemia.

The concurrent increase in TSH responsiveness to TRH and AMPT in our patients and the positive correlation between the TRH- and AMPT-induced TSH rises seem to indicate an expanded pituitary TSH reserve. Similar increases in the pituitary content of gonadotrophins and alphabunits have already been documented in hyperprolactinaemic patients (Monroe et al. 1981) and it is worth noting that in these cases too the gonadotrophin abnormalities occurred independently of changes in gonadal steroids and that the picture reversed when bromocriptine was administered (Klibanski et al. 1983). One can only speculate on the factors involved in the expanding pituitary TSH reserve in hyperprolactinaemia. A possible explanation is that a chronic reduction in the DA supply at the pituitary thyrotrophs leads to a defective inhibition of TSH synthesis and, consequently, to an increase in the TSH stores, as has been demonstrated for Prl (Maurer 1980). If this were so, the enhanced TSH response to AMPT would be the result of an AMPT-induced fall in the DA inhibition of TRH-secreting terminals in the lateral part of the median eminence (Ajika 1980) and to the subsequent action of released TRH on the expanded thyrotroph TSH pool, rather than of a reduction of direct DA inhibition of pituitary TSH-secreting cells. It could also be conjectured that hyperprolactinaemia per se, either through a paracrine effect on pituitary thyrotrophs or modifications of the intrapituitary
negative feedback of thyroid hormones, is responsible for the abnormal TSH dynamics in patients with pathological hyperprolactinaemia.

References


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