Evidence for dopamine-related and TRH-related pituitary TSH and PRL pools in patients with prolactinoma

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Abstract. The sources of TSH, which was excessively released by sulpiride (dopamine D2 receptor antagonist), were studied in 15 female patients with PRL-secreting adenoma (18-43 years). Sequential 3-day administration of sulpiride (100 mg, im) was given to 12 patients with prolactinoma and 6 normal female subjects (19-24 years). Patients with prolactinoma showed much greater TSH responses than normal subjects on the first day. However, TSH responses to sulpiride disappeared on the 2nd and 3rd day in both groups. In contrast, plasma PRL responses to the 1st sulpiride administration were smaller in patients with prolactinoma than in normal subjects, and the response disappeared following the 2nd administration in both groups. When TRH (500 μg, iv) was administered 120 min after the 3rd sulpiride injection, TSH and PRL increments were not different from those before the sulpiride injection in both patients with prolactinoma (N=6) and normal subjects (N=6). Further, combined administration of sulpiride and TRH in 5 patients with prolactinoma clearly enhanced the TSH and PRL responses compared with the single administration of each agent. These results suggest that there may be two readily releasable pituitary TSH and PRL pools, i.e. one dopamine-related and the other TRH-related, in patients with prolactinoma and normal female subjects.

It is well known that patients with prolactin-secreting adenoma (prolactinoma) often show rapid and exaggerated TSH responses to administration of dopamine antagonists (1-3). In humans, administration of exogenous dopamine agonists inhibits TSH secretion and administration of dopamine antagonists stimulates TSH secretion in normal subjects and primary hypothyroid patients (3,5-7). Therefore, it is possible that hypothalamic dopaminergic pool exerts an inhibitory effect on TSH secretion. The degree of TSH response to dopamine antagonists would reflect the degree of hypothalamic dopamine inhibition on thyrotropes (1,8). For these reasons, pituitary portal dopamine concentration in patients with prolactinoma is considered to be elevated by the positive feedback effect of PRL on hypothalamic dopamine neurons (1,2,8,9), although reduced hypothalamic dopaminergic tone was previously inferred (10,11).

At present, it is not clear whether the TSH sources released by dopamine antagonists from thyrotropes are solely dependent on dopamine or also dependent on TRH. To clarify this, we have examined the TSH responses to sequential 3-day administration of sulpiride (dopamine D2 receptor antagonist), to TRH before and after the sulpiride administration, and to combined administration of sulpiride and TRH in patients with micro- and macro-prolactinomas. Similarly, the PRL sources released by sulpiride and TRH were also examined. Our results seem to indicate that dopamine-related and TRH-related pituitary TSH and PRL pools are independently present in both patients with prolactinoma and normal subjects.

Patients and Methods

We studied 15 patients with untreated prolactinoma, all females, aged 18-43 years, and 6 normal female subjects in the early follicular phase (19-24 years). Eleven of these patients had microadenomas and the remaining 4 had macroadenomas, proven by CT-scan. All patients had
manifest hyperprolactinemia and amenorrhea (Table 1). Pituitary-adrenal [plasma cortisol responses to 250 μg of ACTH (1-24)], and pituitary-thyroid functions were normal in all patients. Informed consent was obtained from all patients and normal female subjects.

Twelve patients with prolactinoma (Nos. 1, 3, 4, 6-10, 12-15) and 6 normal controls received sulpiride (Fujisawa, Osaka, 100 mg, im) once a day for 3 consecutive days and TRH (Tanabe, Tokyo, 500 μg, iv) before and 120 min after the sulpiride injection on the third day. Five patients with prolactinoma (Nos. 2, 4, 5, 8, 11) received a combined administration of sulpiride and TRH in addition to single administration of sulpiride and TRH. The examinations were carried out after an overnight fast at an interval of 3-7 days. Blood specimens were collected at 30, immediately before and 15, 30, 45, 60, 90 and 120 min after administration of the agents.

Each sample obtained was kept frozen at −20°C until assay. Plasma TSH was measured using an ultrasensitive commercial immunofluorometric assay kit (Pharmacia, Uppsala, Sweden). Plasma PRL was measured by an immunoradiometric assay kit (Daichi, Tokyo), and T4 and T3 were measured using a RIA kit (Dainabot, Tokyo). All samples from an individual subject were analysed in duplicate in the same assay. The intra- and inter-assay coefficients of variance were 4.0 and 5.2% for TSH, 3.2 and 5.6% for PRL, 4.0 and 4.6% for T4, and 5.2 and 8.5% for T3, and the sensitivities of the TSH, PRL, T4 and T3 assays were 0.02 mU/l, 0.3 μg/l, and 3.1 nmol/l and 0.14 nmol/l, respectively. The normal range of TSH, PRL, T4 and T3 in our institute is 0.3 to 3.0 mU/l, 1.5 to 15.0 μg/l, 49.9 to 193.1 nmol/l, and 0.9 to 2.77 nmol/l, respectively. The pituitary adenoma size was estimated by CT-scan (Siemens, Erlangen) with 2 mm slice width. Statistical analyses were carried out by Wilcoxon's non-parametric analysis or Student's t-test, and all values were expressed as the mean ± SEM.

**Table 1.**
Laboratory data in 15 patients with prolactinoma.

<table>
<thead>
<tr>
<th>Subjects No.</th>
<th>Age (years)</th>
<th>Plasma T4 (nmol/l)</th>
<th>Plasma T3 (nmol/l)</th>
<th>Plasma TSH (mU/l)</th>
<th>Plasma PRL (μg/l)</th>
<th>Tumour size* (mm)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>87.5</td>
<td>1.42</td>
<td>1.1</td>
<td>40.9</td>
<td>5.6</td>
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<tr>
<td>2</td>
<td>26</td>
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<td>1.56</td>
<td>0.7</td>
<td>42.6</td>
<td>8.0</td>
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<tr>
<td>3</td>
<td>22</td>
<td>95.2</td>
<td>1.85</td>
<td>0.5</td>
<td>50.7</td>
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<tr>
<td>4</td>
<td>24</td>
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<tr>
<td>5</td>
<td>25</td>
<td>84.9</td>
<td>1.77</td>
<td>0.8</td>
<td>63.7</td>
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<tr>
<td>6</td>
<td>18</td>
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<td>0.3</td>
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<tr>
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<td>2.19</td>
<td>0.2</td>
<td>81.0</td>
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<td>25</td>
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<td>1.40</td>
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<td>2.05</td>
<td>1.4</td>
<td>103.0</td>
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<tr>
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<td>20</td>
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<td>1.56</td>
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<td>106.0</td>
<td>3.5</td>
</tr>
<tr>
<td>11</td>
<td>18</td>
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<tr>
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<td>41</td>
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<tr>
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<td>23</td>
<td>110.7</td>
<td>1.51</td>
<td>2.4</td>
<td>192.0</td>
<td>4.0</td>
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<tr>
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<td>22</td>
<td>69.5</td>
<td>1.06</td>
<td>1.8</td>
<td>228.0</td>
<td>15.0</td>
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</tbody>
</table>

*: Maximum diameter estimated by CT-scan.

**Results**

Plasma TSH and PRL responses to sequential 3-day administration of sulpiride in patients with prolactinoma and normal subjects

On the first day, 12 patients with prolactinoma showed a significant elevation of plasma TSH (mean peak 6.6±1.0 mU/l) compared with basal value (1.3±0.2 mU/l, p<0.001). In contrast, normal female subjects showed a slight but significant elevation of TSH from the basal level (mean peak 2.1±0.7; basal value 1.0±0.2 mU/l, p<0.05). The mean peak value in patients with prolactinoma was significantly greater than that in normal subjects (p<0.01) and the mean TSH increment at each time point in these patients was also much greater than in normal subjects from 30 to 120 min (p<0.05-0.01) (Fig. 1). However, plasma TSH responses disappeared completely in both groups after the second administration of sulpiride. The basal plasma TSH levels did not change throughout the study in both groups.

Plasma PRL response to the first administration of sulpiride in patients with prolactinoma was subtle (mean peak 158.4±20.0 μg/l) but was slightly higher than basal value (112.5±17.9 μg/l; p<0.05). On the contrary, normal controls showed a rapid and marked PRL increase in response to the first administration (mean peak 197.5±15.8; basal value 4.7±0.5 μg/l, p<0.001), and the maximal PRL increment (194.6±16.0) was far greater than in patients with prolactinoma (45.9±7.8 μg/l, p<0.01). Again, the mean PRL increment at each time point in these subjects was significantly greater than in patients with prolactinoma from 15 to 120 min (p<0.01) (Fig. 2).

Following the second administration of sulpiride, plasma PRL responses were markedly reduced in both groups. On the second day, the PRL response was not different between the two groups. However, the PRL response on the third day was slightly higher in patients with prolactinoma than in normal controls at 15 and 60 min (15 min 18.0±5.0 vs 5.4±2.7 μg/l, p<0.01; 60 min 21.6±5.5
Plasma TSH responses (Δ TSH) to sequential 3-day administration of sulpiride in patients with prolactinoma (○—○, N=12) and normal subjects (●—●, N=6). The TSH responses are shown as the increment from basal value. 1st, 2nd, 3rd day of the sulpiride injection. *p<0.05, **p<0.01.

The basal plasma PRL levels increased gradually in normal controls (1st day 7.0±2.0; 2nd 27.0±2.8, p<0.01; 3rd 38.0±5.2 μg/l, p<0.001 compared with basal level of the first day). On the other hand, the basal levels in patients with prolactinoma were similar throughout the study (1st day 112.5±17.9; 2nd 107.8±15.4; 3rd, 113.3±16.4 μg/l, not significant).

Plasma PRL responses (Δ PRL) to sequential 3-day administration of sulpiride in patients with prolactinoma (○—○, N=12) and normal subjects (●—●, N=6). * p<0.05, ** p<0.01.
**Plasma TSH and PRL responses to TRH in patients with prolactinoma and normal controls before and after the sulpiride administration**

Plasma TSH increment ($\Delta$) in response to TRH before and 120 min after the third sulpiride injection was not different either in patients with prolactinoma (mean $\Delta$ max. $\text{TSH} 8.5 \pm 2.8$ vs $9.2 \pm 3.1$ mU/l) or in normal controls ($10.9 \pm 2.0$ vs $7.8 \pm 1.6$ mU/l), and the response patterns were almost similar in both groups (Fig. 3). Again, plasma PRL response to TRH before and after the sulpiride injection was also not different in patients with prolactinoma (mean $\Delta$ max. $\text{PRL} 21.0 \pm 5.8$ vs $28.7 \pm 8.3$ µg/l) or in normal controls ($32.7 \pm 4.1$ vs $31.4 \pm 4.0$ µg/l), although there were slight differences in the patterns and the magnitude of the responses between the groups (Fig. 4).

Plasma T₄ and T₃ values did not change significantly throughout the study ($\text{T₄} 0$-min value at 1st, 2nd, and 3rd sulpiride injection: patients with prolactinoma $103.0 \pm 5.1, 115.8 \pm 6.4, 110.7 \pm 3.1$; normal controls $103.0 \pm 10.3, 121.0 \pm 6.4, 118.4 \pm 12.9$ nmol/l; $\text{T₃}$: patients with prolactinoma $1.59 \pm 0.1, 1.90 \pm 0.13, 1.77 \pm 0.13$; normal controls $1.75 \pm 0.15, 2.10 \pm 0.14, 1.78 \pm 0.16$ nmol/l, respectively).

**Plasma TSH and PRL responses to single administration of sulpiride or TRH and to combined administration of sulpiride and TRH in 5 patients with prolactinoma**

The combined and simultaneous administration of sulpiride and TRH brought a greater TSH response (mean $\Delta$ max. $\text{TSH} 21.0 \pm 2.7$) compared with the single administration of sulpiride.
Plasma TSH responses (Δ TSH) to single administration of TRH (O—O) or sulpiride (●), and to the combined administration of TRH and sulpiride (△—△) in 5 patients with prolactinoma. Δ vs O and ●: * 1: p<0.05; * 2: p<0.02; * 3: p<0.01; * 4: p<0.05; * 5: p<0.001.

(4.1±1.0, p<0.005) or TRH (11.4±3.7 mU/l, p<0.02) (Fig. 5). Again, the combined administration caused a greater PRL response (Δ max. PRL 27.0±4.5) than the administration of sulpiride (13.9±2.5, p<0.02) or TRH (10.9±2.4 μg/l, p<0.01) (Fig. 6). However, TSH or PRL increments after the combined administration did not significantly exceed the sum of the increments after the single sulpiride and TRH test at any time points.

Discussion

In this study, patients with prolactinoma showed greater TSH responses than normal female subjects to the first administration of sulpiride, although TSH responses after the second (day 2) sulpiride administration almost disappeared in both groups. On the other hand, plasma PRL responses to the first sulpiride injection were much smaller in patients with prolactinoma than in normal subjects, but the responses in both groups disappeared following the second sulpiride administration.

These findings suggest that hypothalamic dopamine inhibition of the pituitary gland is greater in patients with prolactinoma, since 1. exogenous PRL elevates the pituitary portal concentration of dopamine in rats (9), and 2. TSH responses to dopamine antagonists are considered to be markedly increased when dopamine inhibition on thyrotropes is much greater (1,8).

As for the underlying loss of dopamine inhibition of PRL release, namely the sustained hyperprolactinemia and reduced PRL responsiveness to dopamine antagonism, a hypothalamic dopaminergic defect has been suggested (10,11). However, our data support the possibility that tumour lactotropes in these patients are insensitive to dopaminergic inhibition (12,13). It seems that defective dopaminergic inhibition on tumour lactotropes will further stimulate the tuberoinfundibular dopa-
mine neurons through a negative feedback mechanism.

Plasma PRL levels in normal subjects were significantly elevated even 24 hours after the sulpiride injection, as was reported previously (14,15). This would suggest that the occupation of dopamine receptors by sulpiride persisted over 24 hours. This is thought to be the reason for the refractoriness of TSH and PRL to the second or third sulpiride injection. When TRH was administered under such suppressed state of endogenous dopamine action, TSH and PRL increments were not different from those before the sulpiride injection.

Further, combined administration of sulpiride and TRH to patients with prolactinoma clearly enhanced the TSH and PRL responses compared with the single administration of each agent. It has been reported that plasma PRL response to TRH was significantly enhanced by prior administration (65 to 90 min) of sulpiride (16), and dopamine withdrawal resulted in a marked potentiation of the PRL-releasing action of TRH in rats (17). These results indicate a difference between sulpiride and TRH in the intracellular mechanism of TSH secretion.

There are two hormone pools in endocrine cells, one is readily releasable and the other newly synthesized (18-20). In pituitary cells, it takes about 30 min from stimulus to mRNA production (20,21), and it takes about 60 min for the hormone secretion through gene transcriptional and translational processes (e.g. through new hormone synthesis) (22). The TSH and PRL peaks appeared early (at 30 min) after the single administration of sulpiride and TRH as well as after the combined administration. Therefore, these results indicate the presence of dopamine-related and TRH-related readily releasable TSH pools in normal thyrotropes of normal subjects and patients with prolactinoma, and also indicate the presence of dopamine-related and TRH-related readily releasable PRL pools in normal and tumour lactotropes. Why Bernini et al. (14) could not observe a definite PRL response to sulpiride and TRH 24 hours after sulpiride injection is not clear.

When we consider the known intracellular transduction mechanism modulated by dopamine and TRH, it is remarkable that the combined administration of sulpiride and TRH clearly enhanced the TSH and PRL responses in patients with prolactinoma. Dopamine antagonists might stimulate TSH and PRL secretion through the cAMP-protein kinase A system, since dopamine inhibits TSH and PRL secretion from rat pituitary cells in vitro by stimulating the inhibitory GTP binding protein (G) and inhibiting the subsequent cAMP-protein kinase A system (21,23-25), although multiple transduction mechanisms for the dopamine action must also be considered (26,27). In contrast, TRH mainly stimulates TSH and PRL release through the phosphatidylinositol system (21,22,28). From these observations it seems that sulpiride and TRH stimulate TSH and PRL secretion from the different pools, although we must consider the possibility that the dopamine-antagonist and TRH may act on the same pools.

It is reported that the simultaneous activation of those two second messenger systems can cooperatively stimulate hormone secretion (29,30). Therefore, it is possible that sulpiride and TRH stimulated the cAMP-protein kinase A system and phosphatidylinositol system, respectively, and these second messenger systems cooperatively enhanced the TSH and PRL secretion in patients with prolactinoma.

In conclusion, TSH pools in thyrotropes may be increased in patients with prolactinoma, probably owing to the elevated hypothalamic dopaminergic inhibition, and there may be two readily releasable TSH and PRL pools, i.e. one dopamine-related and the other TRH-related, in patients with prolactinoma and normal subjects.

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