Prolactin secretion from human prolactinomas perifused in vitro: effect of TRH, prostaglandin E₁, theophylline, dopamine and dopamine receptor blockers

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Abstract. To clarify the functional characteristics of prolactin (Prl)-producing adenoma cells, the effect of TRH, prostaglandin E₁ (PGE₁), theophylline, dopamine and dopaminergic antagonists on Prl secretion was examined in vitro in perifused pituitary adenoma tissues obtained at surgery from 8 patients with prolactinoma. Perfusion with TRH at a concentration of 10⁻⁶ to 10⁻⁵ M resulted in a significant increase in effluent Prl levels in 3 of the 8 adenoma tissues. In the remaining 5 adenomas, TRH produced no effect on Prl release in vitro. On the other hand, PGE₁ (10⁻⁵ M) stimulated Prl secretion in 2 of the 4 adenomas examined. Addition of theophylline (5.5 mM) caused a marked increase of effluent Prl levels in all 8 prolactinomas regardless of the reactivity to TRH or PGE₁.

Dopamine (5 × 10⁻⁷ M) suppressed Prl secretion from adenoma tissue in 5 of 7 patients tested but had no effect in the remaining two adenomas. When perifused simultaneously with dopamine, sulpiride (D₂-selective dopamine receptor blocker, 5 × 10⁻⁷ M) blocked the inhibitory effect of dopamine on Prl release in 3 of the 4 dopamine-sensitive prolactinomas. In one adenoma responsive to dopamine but resistant to sulpiride, YM-09151-2 (relatively specific D₁-dopamine receptor blocker, 5 × 10⁻⁷ M) antagonized the dopaminergic inhibition of Prl release. When perifused alone, neither sulpiride nor YM-09151-2 affected Prl release from any of the adenoma tissues tested. These findings suggest that a direct action of TRH on the adenoma cells in stimulating Prl release may be lacking in some prolactinoma cells, and that quantitative and qualitative changes in the dopaminergic inhibition of Prl release may occur in some adenomatous lactotrophs.

Numerous studies have shown that serum prolactin (Prl) responses to iv injection of TRH, a reliable means of assessing the functional reserve of pituitary lactotrophs, are blunted in the majority of patients with a Prl-producing pituitary tumour (Kleinberg et al. 1977; Malarkey & Johnson 1976; Snyder et al. 1974). It has also been reported that anti-dopaminergic agents like chlorpromazine and sulpiride fail to increase plasma Prl levels in these patients, although these drugs cause a doubling of baseline Prl values after injection into healthy subjects (Friesen et al. 1972; Jacobs et al. 1973; Kleinberg et al. 1971). However, little is known about the mechanism of the impaired Prl responses to TRH and anti-dopaminergic agents in patients with a Prl-producing adenoma.

In order to clarify the mechanism of the abnormal Prl response to TRH and anti-dopaminergic agents in patients with prolactinoma, we examined the effect of these substances and their related compounds on Prl secretion from tumour tissues using an in vitro perifusion technique.
Table 1.

Clinical features and in vivo plasma Prl responses to various test substances in 8 patients with prolactinoma.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Clinical manifestations</th>
<th>Basal</th>
<th>Plasma Prl levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peak or nadir values after administration of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TRH (500 µg, iv)</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>F</td>
<td>Amenorrhea, galactorrhea</td>
<td>4800</td>
<td>4980</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>F</td>
<td>Amenorrhea, galactorrhea</td>
<td>1970</td>
<td>1850</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>F</td>
<td>Amenorrhea, galactorrhea</td>
<td>1718</td>
<td>1984</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>F</td>
<td>Amenorrhea, galactorrhea</td>
<td>269</td>
<td>302</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>M</td>
<td>Visual difficulty, galactorrhea</td>
<td>10391</td>
<td>10468</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>M</td>
<td>Visual difficulty</td>
<td>832</td>
<td>935</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>M</td>
<td>Visual difficulty, headache</td>
<td>443</td>
<td>478</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>M</td>
<td>Impotence</td>
<td>189</td>
<td>160</td>
</tr>
</tbody>
</table>

* Suppression of Prl to less than 50% of basal values. ** Antagonization by sulpiride of the dopamine action.

Materials and Methods

Subjects

Eight patients with Prl secreting pituitary tumours (4 women and 4 men) age 21–36 years were studied (Table 1). They had a demonstrable pituitary tumour in radiological studies, exhibited a variety of clinical manifestations including visual difficulty, headache, galactorrhea, amenorrhea and impotence, and possessed markedly high basal levels of plasma Prl ranging from 189 to 10391 ng/ml after an overnight fast. The results of pre-operative in vivo tests of Prl secretion are shown in Table 1. An iv injection of 500 µg TRH did not cause a 2-fold increase of basal Prl values in any of the 8 patients. None of 6 patients showed a significant increase in plasma Prl after im administration of 100 mg sulpiride. An iv infusion of dopamine (2.0 µg/kg body weight · min) for 2 h suppressed plasma Prl levels to less than 50% of baseline values in 3 of the 5 subjects examined (cases 1, 3 and 5). When sulpiride (100 mg) was injected im after 2 h of dopamine infusion and the infusion was continued for another hour, the suppression of Prl release by dopamine was significantly antagonized by sulpiride in all of 3 dopamine-responsive prolactinomas. The remaining 2 patients receiving dopamine infusion plus concomitant sulpiride injection (case 7 and 8) showed no significant changes in plasma Prl levels during the experiment.

Perfusion technique

Pituitary adenomas were obtained at the time of surgery from these patients and all were diagnosed as chromophobe adenomas on light microscopical examination. Fragments of pituitary adenomas were cut into small pieces immediately after surgical removal and distributed in culture dishes containing Ham’s F-10 medium with added 2.5% foetal calf serum, 15% horse serum, 100 U/ml penicillin G, and 60 µg/ml streptomycin. No attempt was made to disperse pituitary adenoma cells by use of trypsin or collagenase. Each culture dish was incubated for an overnight period at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

The perfusion study was then performed by our method, described previously (Chihara et al. 1982). The perfusion medium, Krebs-Ringer bicarbonate (KRB) containing 0.2% glucose and 0.25% bovine serum albumin was pumped at a constant flow rate of 0.5 ml/min through a chamber in which 10–20 pieces of pituitary adenomas were packed with Sephadex G-10 gel. The effluent was collected every 5 min on a fraction collector, and stored at −20°C until assayed. By means of a 5-channel peristaltic pump, 4 chambers could be perfused simultaneously with different test materials as required. Test substances were dissolved in the perfusion medium equilibrated with 95% O₂ –5% CO₂ before use, and introduced into the perfusion system by transferring the inlet tube from the reservoir of KRB medium to the test sample, as described previously (Chihara et al. 1982).

Synthetic TRH (Takeda Pharmaceutical Co., Osaka, Japan), prostaglandin E₁ (PGE₁; Ono Co. Ltd., Tokyo, Japan), sulpiride (Fujisawa Co. Ltd., Tokyo, Japan), dopamine hydrochloride and theophylline (Nakarai Chemicals Co. Ltd., Kyoto, Japan) were purchased from the respective companies. The D₁-selective dopamine antago-
nist, cis-N-((1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide (YM-09151-2), was a gift from Dr. Hiroo Maeno, Yamanouchi Pharmaceutical Co., Tokyo, Japan.

PRL RIA and statistical analysis
PRL levels in plasma and perifusate were measured by RIA, as described elsewhere (Chihara et al. 1976). The materials for RIA were kindly supplied by the NIAMDD, Human Pituitary Hormone Program. Intra- and inter-assay variation was 5.2 and 9.4%, respectively. The minimal quantities which could be measured were 1 ng per ml plasma or perifusate. Normal values in our laboratory (mean ± se) for basal plasma PRL levels in 10 male and 10 female normal subjects were 7.8 ± 0.6 and 13.0 ± 0.9 ng/ml, respectively.

Statistical analysis was performed on the basis of the fiducial inference (Steel & Torrie 1960). Since the sample mean (M) is a variable, one hesitates to say that the population mean (µ) is at M. When the standard error (se) is calculated from sample data, the sample quantity $t$ is defined as the following formula:

$$ t = \frac{(M-\mu)}{SE} $$

Then, the lower and upper limits of the confidence interval of $\mu$ with probability 0.05 are shown as a symbolic statement:

$$ M - t_{0.05} \times SE < \mu < M + t_{0.05} \times SE $$

For example, when the sample number is 6 (degree of freedom = 5) the value for $t$ is 2.571 for $P = 0.05$. In the present study, therefore, stimulation and inhibition of PRL release from perifused pituitary adenomas were judged significant ($P < 0.05$) when the peak and nadir values of response were beyond 3 sd of a baseline hormone level (mean of 6 samples preceding the load of test materials).

![Fig. 1.](image)
PRL responses to 30 min pulses of TRH, prostaglandin E$_2$ (PGE$_2$), and theophylline in perifused prolactinoma tissues of cases 1, 3, 6 and 8. The experiment was started at zero time. The PRL values (●) during the perifusion of test substances were judged significant ($P < 0.05$), since they were beyond 3 sd of a mean of 6 samples preceding loading with the test material.
Results

Effect of TRH, PGE₁ and theophylline on Prl secretion from perifused prolactinoma explants

Collection of the perifusate was begun 60 min after the start of a perifusion, when the secretion rate of Prl from the adenoma tissues became stable.

When TRH was perifused for 30 min at a concentration of 10⁻⁶ to 10⁻⁵ M, Prl secretion was significantly stimulated in 3 of the 8 adenoma tissues examined (cases 1, 3 and 6; Fig. 1). In the remaining 5 (cases 2, 4, 5, 7 and 8), TRH failed to affect Prl release (Figs. 1 and 2).

PGE₁ perifused at a concentration of 10⁻⁵ M resulted in a significant increase of effluent Prl levels in 2 of the 4 adenoma explants examined (cases 3 and 8; Figs. 1 and 2). Responsiveness to TRH and PGE₁ was not correlated in these pituitary adenoma tissues (Table 2).

On the other hand, theophylline at 5.5 mM significantly stimulated Prl release from perifused adenoma tissues in all 8 experiments (Figs. 1 and 2). The response of effluent Prl to theophylline was prominent and consistent irrespective of the nature of the adenoma studied.

Effect of dopamine, sulpiride and YM-09151-2 on Prl secretion from perifused prolactinoma explants

In 5 of the 7 prolactinomas examined, dopamine perifused at a concentration of 5 × 10⁻⁷ M gradually but significantly suppressed effluent Prl levels during the 120 min of the test (Fig. 3; Table 2). Dopamine failed to lower Prl secretion in the remaining 2 adenoma of cases 7 and 8 (Table 2), in whom the decrease of plasma Prl by dopamine infusion was less marked in the pre-operative in vivo examination (Table 1).

![Fig. 2.](image-url)
Prl responses to 30 min pulses of TRH, PGE₁, and theophylline in perifused adenoma tissues of cases 2, 4, 5 and 7. Statistical analysis is the same as described in Fig. 1.

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Table 2.
Summary of in vitro Prl responses to various test substances in the perifused prolactinoma tissues of 8 patients.

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Doses (M)</th>
<th>Case No.</th>
<th></th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
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<tbody>
<tr>
<td>TRH</td>
<td>10^{-6}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10^{-5}</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PGE1</td>
<td>10^{-5}</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Theophylline</td>
<td>5.5 × 10^{-3}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine</td>
<td>5 × 10^{-7}</td>
<td>−</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>5 × 10^{-6}</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine + sulpiride</td>
<td>A</td>
<td>NT</td>
<td>A</td>
<td>−</td>
<td>A</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>YM-09151-2</td>
<td>5 × 10^{-6}</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine + YM-09151-2</td>
<td>A</td>
<td>NT</td>
<td>A</td>
<td>A</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Stimulation; −: inhibition; +: no effect; A: antagonization of the dopamine action; NT: not tested.

Fig. 3.
Prl responses to sulpiride (S 5 × 10^{-6} M), YM:09151-2 (YM 5 × 10^{-6} M), and dopamine (DA 5 × 7 10^{-7} M) in perifused prolactinoma tissues of cases 1, 3, 4 and 5. S and YM were perifused for 30 min, whereas DA was administered alone for 60 min and then for 30 min simultaneously with either S or YM. Statistical analysis is the same as described in Fig. 1.
In the present study, the effects of dopamine receptor blockers were also examined in combination with dopamine perfusion, in order to define the nature of the dopamine receptor in the prolactinomas. When sulpiride, a D₂-selective receptor blocker, was perfused at a concentration of $5 \times 10^{-7} \text{M}$ simultaneously with dopamine $(5 \times 10^{-7} \text{M})$, the inhibition of Prl release by dopamine was significantly antagonized by this agent in 3 (cases 1, 3 and 5) of the four dopamine-sensitive prolactinoma tissues (Fig. 3; Table 2). This prevention by sulpiride of dopamine-induced Prl decrease in vitro was in good agreement with the results of the pre-operative study in vivo (Table 1). In the adenoma tissues of case 4 which were responsive to dopamine but resistant to sulpiride (Fig. 3), YM-09151-2, a relatively selective D₁-receptor blocker, was effective in antagonizing the action of dopamine when used in an equimolar concentration with sulpiride $(5 \times 10^{-6} \text{M})$. YM-09151-2 also significantly blunted the dopamine-induced inhibition of Prl release in the prolactinoma explants of cases 1 and 3, and both were responsive to sulpiride as well. When perfused alone, neither sulpiride nor YM-09151-2 affected Prl secretion from the adenoma tissues of any of the patients examined.

Discussion

In normal human volunteers, a marked increase in plasma Prl is observed after an iv injection of 200–500 μg TRH. In contrast, this dose of TRH fails to raise plasma Prl levels in patients with Prl-producing pituitary adenomas, as found in our present study in vivo. It has so far been unclear whether these abnormal responses are due to a lack of a direct action of TRH on the adenoma cells or whether they are an indirect consequence of hyperprolactinaemia. In the present study, we found that TRH in a dose of $10^{-5}$ to $10^{-6} \text{M}$ did not cause any significant changes in effluent Prl levels in 5 of the 8 prolactinoma tissues perfused in vitro. The dose of TRH in vitro, which might appear to be pharmacological, was determined on the basis of that used in pre-operative studies in vivo. It is unlikely that the dose of TRH was not high enough to stimulate Prl release, since the remaining three adenomas were definitely responsive to the same dose of TRH. The possibility that perfused adenoma tissues were dead or not functioning properly during the experiments is also ruled out, because theophylline added at the end of the perfusion study stimulated Prl release from all 8 prolactinoma tissues examined. These findings suggest that TRH failed to stimulate Prl release because of a lack of direct action on the tumour cells, at least in some of Prl-producing pituitary adenomas.

On the other hand, TRH produced a marked increase of effluent Prl levels in 3 of the 8 prolactinomas examined in the present study. This is consistent with the previous observations by Knazek & Skyler (1976) and by Adams et al. (1979) suggesting an in vitro Prl releasing activity of TRH in prolactinoma cells as well as in normal human pituitary cells. Thus, it seems likely that there are two subclasses of Prl-secreting pituitary adenoma on the basis of the Prl response to TRH. Differentiation of these two types of prolactinomas appears difficult in the pre-operative study in vivo, since iv injection of TRH failed to cause any significant changes in plasma Prl levels in all patients with prolactinoma examined. The impairment of TRH-induced Prl rise in vivo even in cases with prolactinomas responsive to TRH in vitro may be explained by a short-loop feedback operating between circulating Prl and the lactotroph. Circulating Prl, which is not present in experiments in vitro, may inhibit pituitary Prl secretion from the adenomatous as well as from the normal lactotroph.

The reason why the direct stimulating activity of TRH on Prl release at the pituitary level is attenuated in some prolactinoma cells remains unknown. In those prolactinoma cells, TRH receptors might not work normally due to a change in their number of affinity to the ligand, thereby failing to transfer the signal of TRH into the subcellular system. At the present time, however, TRH binding to the cell membranes remains unclarified in human prolactinomas. Also, even if this hypothesis is correct, it is not knwon whether the derangement in TRH receptors is incidental to a neoplastic change in the lactotrophs or is the consequence of down regulation by the hyperprolactinaemic state in vivo.

The role of cAMP in the control of Prl release is still controversial. TRH has been shown to stimulate cAMP and Prl release from the pituitary of a number of species (Labrie et al. 1975). In contrast,
according to the later report by Sundberg et al. (1976) TRH enhances Prl release without inducing an increase in cAMP. Although dibutyryl-cAMP or theophylline added to pituitary cells in vitro increased Prl release (Adams et al. 1979; Sundberg et al. 1976; Nagasawa et al. 1973), cholera toxin augmenting adenylate cyclase in all vertebrate cells does not cause stimulation of Prl release (Sundberg et al. 1976; Thorner et al. 1980). PGE_1 is also known to accumulate intracellular cAMP without accompanying Prl release in normal rat pituitaries (Thorner et al. 1980; Sundberg et al. 1975). In the present study, however, PGE_1 enhanced Prl release in 2 of the 4 human Prl-producing adenomas examined. Furthermore, our observation that theophylline, an inhibitor of phosphodiesterase, always sharply accelerates the release of Prl indicates that a cyclic AMP mechanism is likely to be involved in the control of release of Prl by pituitary adenoma cells.

Dopamine is considered to be the major hypothalamic factor which inhibits Prl secretion in a tonic manner. Dopamine and dopaminergic drugs such as L-Dopa, apomorphine and bromocriptine inhibit Prl release both in normal subjects and in patients with hyperprolactinemia. In contrast, we found that dopamine failed to suppress Prl secretion in 2 of the 5 prolactinoma subjects when infused iv at a rate of 2.0 μg/kg body weight · min. In vitro insensitivity to dopamine was also demonstrated when their adenoma tissues were perfused. These findings are consistent with the recent observations by Bansal et al. (1981) showing a refractoriness of Prl suppression to a graded dopamine infusion in patients with hyperprolactinemia. In patients with elevated Prl levels, a significant decrease in Prl levels was shown to occur at infusion rates of 2 and 4 μg dopamine/kg · min, whereas it already appeared at an infusion rate of 1 μg/kg · min in the control subjects. Diminished lactotroph sensitivity to dopamine resembles the defect previously reported in the GH3 rodent pituitary tumour cell line (Malarkey et al. 1977). However, the results of dopamine binding studies using human prolactinomas are conflicting. Bresson et al. (1980) found that human Prl-secreting adenomas have normal dopaminergic binding affinities with the diminished binding sites. Also, Bethea et al. (1982) reported that human Prl-secreting adenoma cells possess dopamine receptors with high affinity and stereoselectivity. In contrast, Cronin et al. (1980) suggested lowered binding affinities and normal numbers of binding sites in some prolactinomas. Thus, the mechanism by which prolactinoma cells acquire dopamine insensitivity remains to be further investigated.

The present study demonstrates that a D_2-selective dopamine receptor blocker, sulpiride, did not cause stimulation of Prl release in vitro or in vivo. On the other hand, sulpiride could antagonize inhibition of Prl resulting from the infusion of dopamine in all 3 patients with dopamine-sensitive prolactinomas. Similarly, sulpiride was able to block the inhibitory effect of dopamine on Prl secretion from adenoma tissues perfused in vitro. These findings suggest that the release of dopamine into the pituitary portal circulation is decreased in these patients with prolactinomas.

Interestingly, in one of our prolactinomas (case 4) the inhibitory effect of dopamine on Prl release was antagonized by concomitant perfusion of YM-09151-2, but not of sulpiride. YM-09151-2 is known to inhibit the binding of [3H]dopamine to the dopamine D_1-receptor with a Kd value of 4.8 nM and to the dopamine D_2-receptor with a Kd value of 0.98 μM, indicating that YM-09151-2 is a relatively selective D_1-receptor antagonist (Nishikori et al. 1980). In contrast, sulpiride is a highly specific D_2-receptor blocker. Since dopamine receptors on the pituitary lactotroph are classified as D_2-type (Kebabian & Calne 1979), the inhibition by dopamine of Prl release is effectively blocked by sulpiride. In our two prolactinomas, the dopaminergic inhibition was attenuated by YM-09151-2 as well as sulpiride. This may be explained by the D_2-receptor blocking action of YM-09151-2 on the prolactinoma D_2-receptor. In the adenoma tissues of case 4, however, YM-09151-2, but not sulpiride, was effective in blocking the dopaminergic inhibition, suggesting that the dopamine receptors on the prolactinoma cells of case 4 may be of D_1-type. Although these findings suggest qualitative changes of dopamine receptors in Prl-producing adenomas, the existence of D_1-receptors on human prolactinomas or normal lactotrophs remains to be confirmed.

In conclusion, we have examined effects of various agents on Prl secretion from human Prl-secreting adenomas using an in vitro perfusion method and found heterogeneity in Prl responses between prolactinomas. Whether this heterogeneity reflects differences in the aetiology of tumour generation or occurs secondarily to the development of the neoplasm is not yet clear.
Acknowledgments

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