Effect of GnRH-associated peptide on prolactin secretion from human lactotrope adenoma cells in culture

Miyuki Ishibashi¹, Tohru Yamaji³, Fumimaro Takaku¹, Akira Teramoto³, Takanori Fukushima³, Makoto Toyama⁴ and Kyuzi Kamoi⁴

Third Department of Internal Medicine¹ and Department of Neurosurgery², Faculty of Medicine, University of Tokyo, Hongo, Tokyo,
Department of Neurosurgery³, Mitsui Memorial Hospital, Kanda, Tokyo, and Departments of Neurosurgery and Medicine⁴, Nagaoka Red Cross Hospital, Nagaoka, Japan

Abstract. The effect of GnRH-associated peptide on PRL secretion by human pituitary lactotropes in culture was studied. Pituitary adenomas obtained at selective transphenoidal adenomectomy from a patient with prolactinoma, and two patients with mixed GH- and PRL-secreting pituitary adenomas were cultured in monolayer. When cells were incubated with dopamine (10 nmol/l), a significant inhibition in PRL secretion was observed in all the experiments, which was blocked by co-incubation with haloperidol. In mixed GH- and PRL-secreting adenoma cells, dopamine likewise decreased GH secretion. Incubation of cells with synthetic GnRH-associated peptide at concentrations up to 100 nmol/l, on the other hand, failed to affect both PRL and GH secretion. These results suggest that synthetic GnRH-associated peptide has no inhibitory effect on PRL secretion in human pituitary lactotropes.

Cloned cDNAs encoding the precursor protein for gonadotropin-releasing hormone (GnRH) have recently been isolated from libraries derived from placental and hypothalamic mRNAs (Semburg & Adelman 1984; Adelman et al. 1986). Nucleotide sequence analyses predict precursor protein of 92 amino acids, which contains an enzymatic processing site that separates the GnRH structure from a C-terminal 56 amino acid sequence termed GnRH-associated peptide. Immunocytochemical studies have demonstrated the presence of GnRH-associated peptide in neuronal cell bodies and exons of the hypothalamus (Phillips et al. 1985). Of potential interest is the fact that GnRH-associated peptide possesses gonadotropin-releasing as well as prolactin (PRL) release-inhibiting activities when tested in vitro using rat pituitary cells in culture (Nicolics et al. 1985). Moreover, active immunization with GnRH-associated peptide has been shown to increase serum PRL levels in rabbits (Nicolics et al. 1985). The present study aimed at determining whether GnRH-associated peptide exerts an inhibitory action on PRL secretion from human PRL-secreting pituitary adenoma cells in culture.

Subjects and Methods

Subjects
One patient with prolactinoma (35 year-old woman) and two patients with acromegaly associated with hyperprolactinaemia (50 year-old man and 61 year-old woman) were studied. Their pre-operative clinical data are summarized in Table 1. Light microscopy of the pituitary adenomas obtained at surgery revealed that the prolactinoma was chromophobic, whereas the pituitary adenomas from the two acromegalic patients were eosinophilic.
Monolayer culture of pituitary tissue

Pituitary adenoma tissues obtained at selective transsphenoidal adenomectomy were cultured in monolayer. The method for the monolayer culture of pituitary cells was previously described in detail (Ishibashi & Yamaji 1984). In brief, pituitary adenoma tissues were cut into small pieces and dispersed by incubation with trypsin-collagenase solution at 37°C with gentle magnetic stirring. The digestion procedure was repeated two or three times until the cells were completely dispersed. The cells were washed with cold culture medium and resuspended in 40–60 ml of culture medium, which consisted of Eagle’s Minimum Essential Medium in Earle’s solution including 10% foetal calf serum, 100 kU/l penicillin and 100 mg/l streptomycin sulphate. A 2-ml aliquot containing 2.4–6.4 × 10^5 dissociated cells was plated in each plastic Petri dish (35 × 10 mm²) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Secretion of GH and PRL from adenoma cells in culture was well maintained for as long as one month by changing culture medium at 2- to 4-day intervals, although a gradual decline in hormone release occurred (Ishibashi & Yamaji 1984).

Incubation of cells with test substances

Incubation studies were performed on the 11th day of culture, when the cells formed a monolayer. Individual cultures were randomly allocated for each experiment. Four or more cultures were used for the control and test substances.

On the day of experiment, the medium was replaced with 2 ml of Eagle’s Minimum Essential Medium in Earle’s solution containing 0.5% human serum albumin. Cells were incubated for 1 h at 37°C in a humidified atmosphere of 95% air and 5% CO₂ (pre-incubation). The medium was then removed, and the cells were further incubated for 2 h in 2 ml of fresh medium with or without test substances (experimental incubation). Synthetic GnRH-associated peptide (Lot 008427, Peninsula Laboratories, Inc, Belmont, CA) was initially dissolved in 8 mol/l acetic acid and serially diluted with incubation medium. Haloperidol was first solubilized in glacial acetic acid, diluted to a concentration of 10 g/l with distilled water and then with 0.9% saline and incubation medium. The final pH was adjusted to 7.4 with 7% NaHCO₃ after gassing with 95% air-5% CO₂. Solution of dopamine hydrochloride was prepared by dissolving it directly into incubation medium before each experiment.

Control dishes received vehicle alone. When the combined effects of two test substances on hormone release were examined, they were added simultaneously to the incubation medium. After incubation, the medium was centrifuged at 150 × g for 10 min, and the supernatant was stored at −20°C until assayed.

Radioimmunoassays

PRL and GH concentrations in the medium after both pre-incubation and experimental incubation were determined by RIAs, as previously described in detail (Yamaji et al. 1976; Ishibashi et al. 1977). Immunological materials for RIAs were kindly donated by the National Hormone and Pituitary Program, NIADDK. The cross-reactivity of GH in the PRL RIA was 0.13%, and that of PRL in the GH RIA 0.73%. The coefficients of variation for PRL averaged 7.4% for intra-assay and 8.2% for inter-assay error, whereas they were 5.3% and 9.6%, respectively, for GH. To minimize experimental error resulting from variability in hormone secretion in individual cultures, results were expressed as the percentage of hormone secreted during the experimental incubation compared with that secreted during the pre-incubation for individual cultures. For comparison, the mean values obtained in the control incubation were designated as 100%.

---

**Table 1.**
Laboratory findings in the patients.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Basal plasma levels</th>
<th>Response to TRH (0.5 mg iv)</th>
<th>Response to bromocriptine (2.5 or 5 mg po)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH (µg/l)</td>
<td>PRL (µg/l)</td>
<td>GH (%)</td>
</tr>
<tr>
<td>Prolactinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>35</td>
<td>F</td>
<td>–</td>
<td>317</td>
<td>–</td>
</tr>
<tr>
<td>Acromegaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>50</td>
<td>M</td>
<td>28</td>
<td>121</td>
<td>661</td>
</tr>
<tr>
<td>A2</td>
<td>61</td>
<td>F</td>
<td>30</td>
<td>23</td>
<td>2273</td>
</tr>
</tbody>
</table>

* Peak value (percentage of basal). ** Nadir value (percentage of basal).
Statistical analysis
Values in figure and text are given as the mean ± SEM unless otherwise specified. The significance of differences was calculated using Student's t-test.

Results
Prolactinoma cells in culture actively secreted PRL into incubation medium. The amount of PRL accumulated in the medium during pre-incubation was 7.4 ± 0.1 µg/h per dish (mean ± SEM, N = 30). Similarly, cultured adenoma cells of acromegaly released a substantial amount of both GH and PRL into the medium. Secretion rates of GH during pre-incubation were 525 ± 25 (mean ± SEM, N = 20) (case A1) and 839 ± 43 ng/h per dish (N = 25) (case A2), whereas those of PRL were 163 ± 6 (case A1) and 759 ± 36 ng/h per dish (case A2). When cultured prolactinoma cells were incubated with 10 nmol/l of dopamine, PRL secretion was significantly suppressed (Fig. 1). Incubation of pituitary adenoma cells from acromegalic patients with 10 nmol/l of dopamine likewise resulted in a significant decrease in both GH and PRL release. This inhibitory effect of dopamine on PRL and GH secretion was reversed by the addition of 100 nmol/l of haloperidol, a non-selective dopaminergic antagonist. Incubation of cultured prolactinoma cells with synthetic GnRH-associated peptide up to the concentration of 100 nmol/l, on the other hand, failed to affect secretion rates of PRL (Fig. 1). When pituitary adenoma cells of acromegaly were exposed to 100 nmol/l GnRH-associated peptide, no significant change in either GH or PRL secretion was observed (Fig. 1).

Discussion
The present study shows that dopamine at a concentration as low as 10 nmol/l inhibits PRL secretion from cultured prolactinoma cells. In addition, dopamine exerted its direct action by reducing GH and PRL secretion in mixed GH- and PRL-producing pituitary adenoma cells of acromegaly in culture. This inhibitory effect of dopamine on hormone release was blocked by a dopaminergic antagonist, haloperidol, suggesting that the action of dopamine was mediated through dopamine receptor activation. The result is consistent with earlier observations by us and others (Ishibashi & Yamaji 1978, 1984; Adams et al. 1979; Peillon et al. 1979). Synthetic GnRH-associated peptide at concentrations up to 100 nmol/l, on the other hand, failed to affect hormone secretion from both pure and mixed types of prolactinoma cells. In rat pituitary cells in culture, Nocolics et al. (1985) showed that GnRH-associated peptide made by genetic engineering
suppresses PRL secretion at very low concentrations with a half-maximal inhibitory dose value of $2.5 \times 10^{-11}$ mol/l. The maximal degree of inhibition, which was achieved by $10^{-10}$ mol/l GnRH-associated peptide, was comparable with that caused by $10^{-7}$ mol/l dopamine. Furthermore, active immunization with GnRH-associated peptide resulted in increased PRL secretion in rabbits. The discrepancy between their study and ours remains unknown at present. One of the possible explanations is the difference in the PRL responses of adenomatous and non-adenomatous pituitary lactotropes. This possibility seems unlikely, however, since no difference in PRL responses to secretagogues exists between prolactinoma cells and normal human pituitary lactotropes (Ishibashi & Yamaji 1984, 1985). It was concluded from these results that synthetic GnRH-associated peptide has no inhibitory effect on PRL secretion in human pituitary cells.

Very recently, Schally et al. (1986) reported, though in an abstract form, that synthetic GnRH-associated peptide did not inhibit PRL secretion in rats either in vivo or in vitro. Millar et al. (1986) showed that the first 13 amino acids sequence of GnRH-associated peptide does not change PRL secretion in human and baboon anterior pituitary cells in culture, although the peptide stimulated the release of gonadotrophic hormones. Further studies may be required to determine whether GnRH-associated peptide may really possess PRL-release inhibiting activity.

Acknowledgment

This work was supported in part by a research grant from the Ministry of Health and Welfare, Japan.

References


Received January 9th, 1987.
Accepted May 6th, 1987.

Dr M. Ishibashi,
Third Department of Internal Medicine,
Faculty of Medicine, University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.