Cyproheptadine-mediated inhibition of growth hormone and prolactin release from pituitary adenoma cells of acromegaly and gigantism in culture

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Abstract. The effect of cyproheptadine on growth hormone (GH) and prolactin (Prl) secretion from cultured pituitary adenoma cells of acromegaly and pituitary gigantism was studied. When varying doses of cyproheptadine ranging from 0.01 to 1 μM were added to the incubation media, GH secretion was consistently inhibited and a dose-response relationship was observed between the cyproheptadine concentrations and the amounts of GH released into the media. In pituitary adenomas which concurrently produced and secreted Prl, cyproheptadine likewise suppressed Prl release in a dose-related manner. This effect of cyproheptadine was not blocked by coincubation with serotonin. Similarly, coincubation with a dopaminergic antagonist, haloperidol, failed to reverse the inhibitory action produced by cyproheptadine. When coincubated with dopamine, cyproheptadine further inhibited GH and Prl secretion. These results suggest that cyproheptadine possesses a direct action on human somatotroph adenoma cells to inhibit GH and Prl secretion by an unknown mechanism that is different from serotonergic and dopaminergic systems.

Evidence for the serotonergic control of growth hormone (GH) secretion has accumulated in men as well as in experimental animals. Serotonergic blockade by cyproheptadine or methysergide was reported to inhibit GH release induced by insulin hypoglycaemia (Bivens et al. 1973; Smythe & Lazarus 1974; Mendelson et al. 1975) or by other pharmacological stimuli (Nakai et al. 1974), and to suppress the circulating GH levels during the sleep period in healthy subjects (Chihara et al. 1976). The effect of serotonergic antagonists on GH secretion in acromegalic patients, on the other hand, has not been extensively studied and is controversial. Methergoline was shown to decrease plasma GH contentations in patients with acromegaly (Chiodini et al. 1976; Delitala et al. 1976), although this effect of methergoline may have been occasioned via its intrinsic dopamine agonist activity (Chiodini et al. 1976). In a limited number of acromegalic patients, the inhibitory effect of cyproheptadine on GH secretion was demonstrated (Feldman et al. 1976; Kato et al. 1983), however, the site or the mechanism of action of cyproheptadine remains unknown. The present study was undertaken to answer this question by examining the effect of cyproheptadine on GH release from cultured pituitary adenoma cells of acromegaly.

Materials and Methods

Subjects

Seven patients with acromegaly and 1 patient with pituitary gigantism, 4 men and 4 women, were studied (Table 1). The age of the patients ranged from 12 to 61 years. They exhibited characteristic physical features of acromegaly or gigantism, elevated baseline serum GH

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concentrations and failure of serum GH levels to suppress during an oral glucose tolerance test. Light microscopy of the pituitary adenomas removed from these patients at surgery revealed that 5 (Nos. 2–5 and 8) of them were eosinophilic, while the remaining 3 were chromophobic.

Monolayer culture of pituitary adenoma cells

Pituitary adenoma tissues obtained at surgery were cultured in monolayer. The method for the monolayer culture of pituitary cells has been previously described in detail (Ishibashi & Yamaji 1981, 1984). In brief, pituitary adenomas were cut into small pieces and dispersed by incubation with trypsin-collagenase solution at 37°C. An aliquot containing 2–8 × 10⁵ dissociated cells was planted in plastic Petri dishes (35 × 10 mm) and incubated at 37°C in a humidified atmosphere of 95% air-5% CO₂. The culture medium consisted of Eagle’s Minimum Essential Medium in Earle’s solution including 10% foetal calf serum, 100 U/ml penicillin and 10 μg/ml streptomycin sulphate.

When pituitary adenoma cells of acromegaly cultured in monolayer were incubated with labelled leucine and harvested after increasing periods of time, incorporation of radioactivity into immunoprecipitable GH in cell extracts proceeded linearly for the entire 48 h incubation (Ishibashi & Yamaji 1984). Secretion of GH and prolactin (Prl) from adenoma cells in culture was well maintained as long as 1 month by changing culture medium at 2–4 day intervals, although a gradual decline in hormone release was seen when the culture was continued (Ishibashi & Yamaji 1984). The results suggest that de novo synthesis as well as secretion of hormones is actively taking place in monolayer culture under the present experimental conditions.

### Table 1.

Laboratory findings of the patients studied and in vitro GH and Prl secretion rates at the initial incubation study.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Basal plasma levels</th>
<th>In vitro secretion rates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH (ng/ml)</td>
<td>Prl (ng/dish)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prl (ng/ml)</td>
<td>GH (ng/dish/h)</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>F</td>
<td>Acromegaly</td>
<td>143</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>M</td>
<td>Acromegaly</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>F</td>
<td>Acromegaly</td>
<td>409</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>F</td>
<td>Acromegaly</td>
<td>129</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>M</td>
<td>Acromegaly</td>
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<td>M</td>
<td>Acromegaly</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>M</td>
<td>Acromegaly</td>
<td>171</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>F</td>
<td>Gigantism</td>
<td>9</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

**Incubation of cells with test substances**

Incubation studies were started after the cells formed a monolayer and performed at an interval of at least 2 days. Individual cultures were randomly allocated for each experiment. Four or more cultures were used for the control and variables, and run simultaneously. On the day of experiment, the medium was replaced by 2 ml of Eagle’s Minimum Essential Medium in Earle’s solution containing 0.5% human serum albumin instead of foetal calf serum. 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 cells were further incubated for 2 h in 2 ml of fresh medium with or without test substances (experimental incubation).

Solutions of cyproheptadine, dopamine hydrochloride and serotonin creatinine sulphate were prepared by dissolving initially in distilled water or 0.9% saline and diluting with the incubation medium to appropriate concentrations prior to each experiment. Haloperidol was first solubilized in glacial acetic acid, which was diluted to a concentration of 10 mg/ml with distilled water. This stock solution was serially diluted to a desired concentration with 0.9% saline and then with incubation medium.

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

**Radioimmunoassays**

GH and Prl concentrations in the medium of both preincubation and experimental incubation were deter-
determined by radioimmunoassays, as previously described in detail (Ishibashi & Yamaji 1984; Yamaji 1974). Immunological materials for the radioimmunoassays were kindly donated by the National Hormone and Pituitary Program and NIADDK, U.S. Public Health Service. Cross-reactivity of GH in Prl radioimmunoassay was 0.13% and that of Prl in GH radioimmunoassay was 0.73%. The coefficients of variation for GH averaged 5.3% for intra-assay error and 9.6% for inter-assay error, while they were estimated to be 7.4% and 8.2%, respectively, for Prl. In order to minimize experimental errors resulting from a variability in hormone secretion from individual cultures, results were expressed as the percentage of hormone secreted in the experimental incubation compared with that secreted during the preincubation for individual cultures. For comparison, the mean values obtained in the control study were designated as 100%.

**Statistical analysis**

Values in the figures and text are given as the mean ± SEM. The significance of differences was calculated using Student’s t-test and analysis of variance.

**Results**

Pituitary adenoma cells of acromegaly in monolayer culture actively secreted GH into the incubation media. The amounts of GH accumulated in the media at the initial incubation study ranged from 41 ± 1 (mean ± SEM, n = 20) (No. 8) to 3504 ± 125 ng/h/dish (n = 25) (No. 1) (Table 1). In 4 out of 8 pituitary adenomas of acromegaly and gigantism, the concomitant secretion of Prl was observed. The secretion rates of Prl varied from tissue to tissue and ranged from 66 ± 1 (mean ± SEM, n = 20) (No. 8) to 1268 ± 52 ng/h/dish (n = 25) (No. 2) at the initial incubation study, corresponding to 3.1% (No. 1)-161% (No. 8) of GH secretion rates (Table 1). In view of the degree of the cross-reaction of GH in Prl radioimmunoassay, Prl secretion by the remaining 4 adenomas was judged to be only minimal.

Fig. 1 shows the effect of varying doses of cyproheptadine on GH and Prl secretion by cultured
pituitary adenoma cells obtained from 3 acromegalic patients and 1 patient with pituitary gigantism. In all of the adenoma cells, cyproheptadine significantly inhibited GH secretion, and a dose-response relationship was observed between the cyproheptadine concentrations and the amounts of GH released into the incubation media. The minimum effective dose of cyproheptadine required for a significant decrease in GH secretion was highly variable. Cyproheptadine as low as 10 nM decreased GH secretion in one adenoma (No. 8), while 1 µM cyproheptadine was necessary for a significant inhibition of GH release in others (Nos. 1 and 3 in Fig. 1). In three pituitary adenomas which concurrently secreted PRL into the incubation media (Nos. 1, 2 and 8 in Fig. 1), cyproheptadine significantly reduced PRL secretion in a dose-dependent manner. The magnitude of suppression of PRL secretion by cyproheptadine was similar to that of GH in two adenomas, although PRL release was inhibited to a greater extent in the remainder (No. 2 in Fig. 1).

In Fig. 2 is shown the effect of cyproheptadine alone or in combination with serotonin on GH and PRL secretion from cultured pituitary adenoma cells removed from 4 patients. Cyproheptadine at a concentration of 1 µM again inhibited GH release in others in a reproducible manner. Serotonin, on the other hand, did not significantly influence both the basal secretion rates of GH and the cyproheptadine-mediated reduction in GH release. The result was essentially the same when serotonin at a concentration 10
times higher than cyproheptadine was employed (Nos. 4 and 5 in Fig. 2). Similarly, cyproheptadine inhibited Prl secretion, which was not reversed by coincubation with equimolar serotonin in one experiment (No. 8 in Fig. 2).

We showed previously that dopaminergic agonists effectively suppress GH and Prl secretion from pituitary adenoma cells of acromegaly in vitro (Ishibashi & Yamaji 1978, 1984), which can be blocked by equimolar haloperidol (Ishibashi & Yamaji 1984). In order to exclude the possibility that the observed effect of cyproheptadine on GH and Prl secretion was mediated by the dopaminergic mechanism, the cells were coincubated with cyproheptadine and haloperidol (Fig. 3). In all of the experiments conducted using pituitary adenoma cells taken from 3 patients, GH and Prl secretion were significantly inhibited by 0.1 or 1 µM cyproheptadine. Coincubation of the cells with haloperidol, a non-selective dopaminergic antagonist, was without effect of the inhibitory action produced by cyproheptadine.

Fig. 4 illustrates the effects of cyproheptadine and dopamine alone or in combination on GH secretion from cultured pituitary adenoma cells of acromegaly. In both experiments, the addition of cyproheptadine or dopamine to the incubation media resulted in a significant decrease in GH secretion. When coincubated with dopamine, cyproheptadine further decreased GH secretion rates.

**Discussion**

The foregoing results clearly show that cyproheptadine directly acts on pituitary adenoma cells of acromegaly to suppress GH secretion. The effect

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**Fig. 3.**

Effect of cyproheptadine alone or in combination with equimolar haloperidol on GH and Prl secretion by cultured pituitary adenoma cells from 3 patients with acromegaly. Results are the mean ± SEM. *P < 0.05, **P < 0.01 vs control. See text in detail.
of cyproheptadine administration on the circulating levels of GH in acromegalic patients has not been fully studied. Prior administration of cyproheptadine for 2 days resulted in a decrease in plasma GH levels during oral glucose tolerance test in 4 of 6 acromegalic patients (Feldman et al. 1976), while a single oral dose of the drug failed to influence basal plasma GH concentrations during the period of observation for 3 h in 4 such patients (Chiodini et al. 1976). More prolonged treatment with cyproheptadine, on the other hand, was reported to suppress serum GH concentrations with a resultant clinical improvement in 3 out of 4 patient (Kato et al. 1983). Inconsistencies of the effect of cyproheptadine on the circulating GH levels of acromegalic patients may be due to a difference in the dose of the drug administered and, more importantly, to a marked difference in the sensitivities to cyproheptadine of individual adenomas of acromegaly demonstrated in the present study. Of additional interest is the fact that cyproheptadine inhibited Prl secretion from cultured pituitary adenoma cells of acromegaly. Whether this may be characteristic of neoplastic lactotrophs associated with somatotroph adenomas or common to normal human lactotrophs remains obscure. In the rat, cyproheptadine was demonstrated to inhibit Prl secretion by a direct action on the pituitary both in vivo and in vitro (Lamberts & MacLeod 1978; Krulich et al. 1981).

Since cyproheptadine is classified as a serotonin receptor antagonist, the observed inhibition of GH and Prl secretion may be interpreted by blockade of serotonin receptors on cellular membranes of pituitary adenomas. Coincubation of the cells with serotonin, however, failed to block the inhibitory action produced by cyproheptadine. Similarly, the effect was not reversed by coincubation with a dopaminergic antagonist, haloperidol. These results suggest that cyproheptadine suppresses GH and Prl secretion from pituitary adenoma cells of acromegaly by an unknown mechanism that is different from serotonergic and dopaminergic systems. In accordance with this view, Lamberts & MacLeod (1978) reported that cyproheptadine-mediated inhibition of in vitro synthesis and release of Prl by rat pituitary cells was occasioned by a non-serotonin-linked mechanism. Desmethylcyproheptadine, a metabolite of cyproheptadine which is neither a serotonin nor a histamine antagonist (Rickert & Fischer 1975), was shown to suppress the in vitro release of bioassayable ACTH from the neurointermediate lobe, but not from the anterior lobe of the rat pituitary gland (Lamberts et al. 1983). In human corticotroph adenoma cells, the effect of cyproheptadine on ACTH release was inhibited by the addition of serotonin (Ishibashi & Yamaji 1981). Whether the apparent discrepancy may be attributed to the difference between somatotroph and corticotroph adenoma cells or between normal and neoplastic corticotroph cells remains unknown at present.

Cyproheptadine was also shown to have a direct action on rat pancreatic β cells to inhibit insulin release in vitro (Richardson et al. 1975). Of interest in this regard is the finding that calcium uptake of Langerhans islet cells induced by D-glucose and

Fig. 4.
Effects of cyproheptadine and dopamine alone or in combination on GH and Prl secretion by cultured adenoma cells from 2 patients with acromegaly. Results are the mean ± SEM. *P < 0.05, **P < 0.01. See text in detail.
high K+ was completely blocked by cyproheptadine suggesting that the drug inhibited insulin secretion by blockade of depolarization-dependent calcium entry into islet cells (Joost et al. 1976; Donatsch et al. 1980). Such a mechanism may operate in the cyproheptadine-mediated suppression of GH and Prl release by pituitary adenoma cells of acromegaly demonstrated in the present study.

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References

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