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Studies on the Effect of Cyproheptadine on Growth Hormone Secretion (40629)

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There is evidence that several monoamines, including dopamine, norepinephrine, and serotonin, have roles in regulating pituitary growth hormone (GH) secretion (1–3). Evidence on the role of serotonin remains unclear. Nakai *et al.* (4) and Smythe *et al.* (5) reported that 5-hydroxytryptophan administered to humans stimulated GH secretion, but Handwerger *et al.* (6) failed to confirm this observation. Much of the evidence that serotonin directly influences GH release has been derived from indirect experiments in which so-called specific serotonin-receptor antagonists have been used. In this study we investigated the effect of serotonin and cyproheptadine on GH synthesis and release by normal rat pituitary glands and by dispersed pituitary tumor cells.

Materials and methods. Female and male rats (Wistar–Furth, 200–220 g) were housed four to five per cage in a temperature-controlled room (22–23°) under artificial illumination (0600–1800 hr). Tumor homogenate from mixed prolactin (PRL-)- and GH-secreting tumor, MtTW15 was injected into the scapular region 6–8 weeks before the animals were studied, as described previously (7).

For *in vivo* experiments on hemipituitary glands, the animals were decapitated at 1000 hr and the anterior pituitary was quickly removed and bisected. Flasks containing three hemipituitary glands from different rats were incubated in 1 ml of tissue culture medium 199 (Microbiological Associates, Inc.) containing 10 μCi [4,5-^{3}H]leucine (30 Ci/mmole). Each group contained four flasks. The flasks were incubated at 37° in a Dubnoff shaker in an atmosphere of 95% O_2, 5% CO_2 (vol/vol). After 5-hr incubation the medium was removed and the glands were rinsed and homogenized in 1 ml distilled water containing 1% Triton X-100. Aliquots of the incubation medium and of pituitary homogenates were subjected to polyacrylamide gel electrophoresis (8). The separating gel was 7% acrylamide, pH 8.9, and the electrode compartments contained 0.5 *M* Tris–glycine buffer, pH 8.3. Duplicate gels were run at 4 mA/gel for each sample. The protein bands were stained in 7% acetic acid containing 0.5% amido black 10B; excess stain was removed electrophoretically. The location of GH and PRL on the gels was ascertained by subjecting NIH reference hormones to the same electrophoretic separation. The stained protein bands were cut from the gel columns and placed in scintillation vials containing 1 ml of NCS (tissue solubilizing agent; Amersham Searle) and water solution (92:8, vol/vol). A toluene-based scintillation fluid was added to each vial at room temperature and the samples were counted in a liquid scintillation spectrometer. The total amount of newly synthesized GH was computed as the amount released into the medium plus that found in the pituitary gland.

Isolated pituitary tumor cells were prepared by mincing 5 g of the MtTW15 tumor in 10 ml medium 199 containing 1.5 mg/ml trypsin (Worthington) and 100 μg/ml bovine serum albumin. Tissue fragments were incubated for 20 min at 37° under an atmosphere of 95% O_2, 5% CO_2. Following incubation the tissue fragments were brought to room temperature and then drawn up and expelled 25 times through a plastic Pasteur pipet. The debris and large fragments were allowed to settle 5 min and the supernatant containing the isolated cells was aspirated. Trypsin inhibitor (Worthington) was added to this suspension (1.5 mg/ml). The acutely dispersed cells were pipetted into plastic vials (final volume 1 ml) and incubated for either 2.5 or 5 hr in a Dubnoff shaker at 37° in an atmosphere of 95% O_2, 5% CO_2, followed by centrifugation at 3500*g* for 10 min. After centrifugation the medium and cell pellet were separated and stored.

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The tumor cell content of the incubation medium was counted in a hematological counting chamber. The percentage of viable cells, as determined by trypan blue exclusion, was 95% prior to and 90% after the 5-hr incubation.

The incubation medium and rat serum GH content was measured by a double-antibody radioimmunoassay using materials and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. The reference hormone used was GH-RP-1 growth hormone.

The results are expressed as mean ± SEM and statistical analysis was performed by analysis of variance.

Results. The intraperitoneal administration of cyproheptadine (3 mg/kg) decreased serum GH in normal female rats: 15 min after injection serum GH was significantly ($P < 0.01$) suppressed compared with control values (Table I). The pituitary glands from these animals were removed 15, 30, and 45 min after cyproheptadine injection. *In vitro* release of both radioimmunoassayable GH and newly synthesized [^{3}H]GH was significantly inhibited from pituitary glands taken from animals 45 min after injection (Table II).

TABLE I. THE EFFECT OF CYPROHEPTADINE ON THE SECRETION OF GROWTH HORMONE *IN VIVO*[a]

Minutes after injection (min)	Serum growth hormone (ng/ml)
0	42.6 ± 14.5
15	11.7 ± 1.7*
30	23.6 ± 5.3
45	22.1 ± 3.5

[a] The 3 mg/kg cyproheptadine was injected intraperitoneally in female rats, six rats per group; mean ± SEM.
* $P < 0.01$ versus control.

Direct effects of cyproheptadine on pituitary GH synthesis and release were investigated. Cyproheptadine (60–600 n*M*) did not affect GH synthesis (Table III, Expts II and III) or release (Table III, Expts II and III). However, the same concentrations of cyproheptadine produced a dose-dependent inhibition of PRL release *in vitro* (Fig. 1). Serotonin (0.1–10 μ*M*) and dopamine (500 n*M*) were without effect (Table III) on GH release.

The effect of cyproheptadine on GH secretion by a transplantable GH-secreting pituitary tumor was also investigated. It was previously shown that the transplantable pituitary tumor MtTW15 secretes large quantities of both GH and PRL (9). During 5-hr incubation of trypsin-dispersed pituitary tumor cells, a linear release of growth hormone and prolactin was observed (Fig. 1). The addition of dibutyryl-cAMP (5 m*M*) significantly increased GH release ($P < 0.05$), while cyproheptadine (6–12 μ*M*) inhibited the release of the hormone ($P < 0.01$). Interestingly, the secretion of GH and PRL by these isolated tumor cells in response to dbcAMP and cyproheptadine showed a parallel pattern (Fig. 2).

The effect of chronic administration of cyproheptadine (3 mg/kg subcutaneously for 5 days) on serum GH and PRL, tumor growth, and body weight was investigated in rats bearing the MtTW15 tumor. Cyproheptadine injections did not affect the spontaneous increase in serum GH and serum PRL as they occurred in tumor-bearing controls (Table IV). Similarly cyproheptadine administration did not have an effect on body weight and tumor size. At the end of the 5-day period the animals were sacrificed in order to compare the effect of the administration of cyprohep-

TABLE II. THE EFFECT OF CYPROHEPTADINE INJECTION ON THE *IN VITRO* SECRETION OF GROWTH HORMONE[a]

	Incorporation of [^{3}H]leucine into growth hormone (cpm/mg pituitary gland)		Growth hormone measured by radioimmunoassay (μg/mg pituitary)
	Pituitary gland	Medium	Medium
Control	11750 ± 590	970 ± 80	6.60 ± 0.42
15 min after cyproheptadine injection	11690 ± 350	850 ± 30	6.68 ± 0.25
30 min after cyproheptadine injection	11210 ± 630	840 ± 70	6.36 ± 0.36
45 min after cyproheptadine injection	11160 ± 530	540 ± 60*	5.36 ± 0.36*

[a] The 3 mg/kg cyproheptadine was injected in female rats and three hemipituitary glands were incubated 15, 30, and 45 min after injection. Four flasks per group; mean ± SEM.
* $P < 0.05$ vs. control.

TABLE III. EFFECTS OF SEROTONIN, CYPROHEPTADINE, AND DOPAMINE ON GROWTH HORMONE SECRETION BY NORMAL PITUITARY GLANDS *IN VITRO*[a]

	Incorporation of [^{3}H]leucine into growth hormone (cpm/mg pituitary gland)		Growth hormone measured by radioimmunoassay (μg/mg pituitary)
	Pituitary gland	Medium	Medium
Expt. I (male rats)			
Control	8055 ± 960	2830 ± 550	15.16 ± 1.13
Serotonin 0.1 μ*M*	8080 ± 1210	2770 ± 540	15.44 ± 1.57
1 μ*M*	9210 ± 710	2790 ± 600	15.58 ± 0.89
10 μ*M*	9250 ± 210	2650 ± 200	13.68 ± 2.00
Expt II (female rats)			
Control	9325 ± 760	365 ± 70	6.13 ± 0.47
Cyproheptadine 60 n*M*	8930 ± 1230	370 ± 60	5.65 ± 0.61
240 n*M*	9920 ± 510	395 ± 70	6.04 ± 0.52
600 n*M*	9590 ± 600	360 ± 25	5.71 ± 0.85
Expt III (male rats)			
Control	8960 ± 645	2430 ± 200	13.60 ± 1.26
Cyproheptadine 100 n*M*	9045 ± 766	2455 ± 105	14.05 ± 1.05
Expt IV (female rats)			
Control	4640 ± 470	370 ± 100	2.62 ± 0.21
Dopamine 500 n*M*	4740 ± 320	395 ± 100	2.64 ± 0.31

[a] Four flasks per group; mean ± SEM.

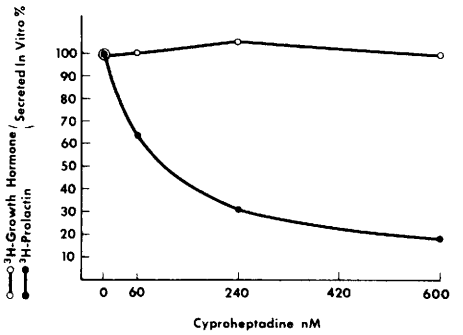

FIG. 1. Effect of cyproheptadine *in vitro* on the secretion of radioactive prolactin and growth hormone. Hemipituitary glands from three different rats were incubated in medium 199 containing [^{3}H]leucine for 5 hr as described. Aliquots of the incubation medium were subjected to polyacrylamide gel electrophoresis and the radioactivity in the stained bands of prolactin and growth hormone measured. Four flasks per group.

tadine on organ weights. Chronic cyproheptadine administration did not affect the organomegaly (liver, spleen, adrenal) which occurs as a reaction to the hypersecretion of hormones by the mixed GH-PRL producing tumor MtTW15.

Discussion. The evidence for a positive role of serotonin on growth hormone secretion is partly derived from indirect experiments in which so-called specific serotonin antagonists such as cyproheptadine are used. In man, cyproheptadine suppresses sleep-related GH release (10) and inhibits in normal individuals the release of GH induced by insulin hypoglycemia (11, 12), arginine infusion (4), physical exercise (13), and glucagon (14). In normal children the mean GH response to an oral glucose administration was significantly reduced by cyproheptadine (14). Systemic administration of cyproheptadine suppressed GH secretion in neonatal rats (15). Our findings of suppressed GH secretion within 15 min after the administration of cyproheptadine agree with these observations. Following systemic cyproheptadine administration pituitary GH release was significantly inhibited.

This action of cyproheptadine was shown not to be exerted directly at the level of the pituitary gland. In addition, serotonin by itself had no effect on pituitary GH synthesis and release *in vitro*. The neuropharmacological specificity of cyproheptadine is uncertain. In addition to antiserotoninergic actions of the drug, histamine-, choline-, and dopamine-blocking actions have been described. The ability of cyproheptadine to block L-dopa-induced GH release might indicate an antidopaminergic action of the drug on GH secretion (3, 16).

The evidence for a positive role of dopamine as a hypothalamic regulator of GH

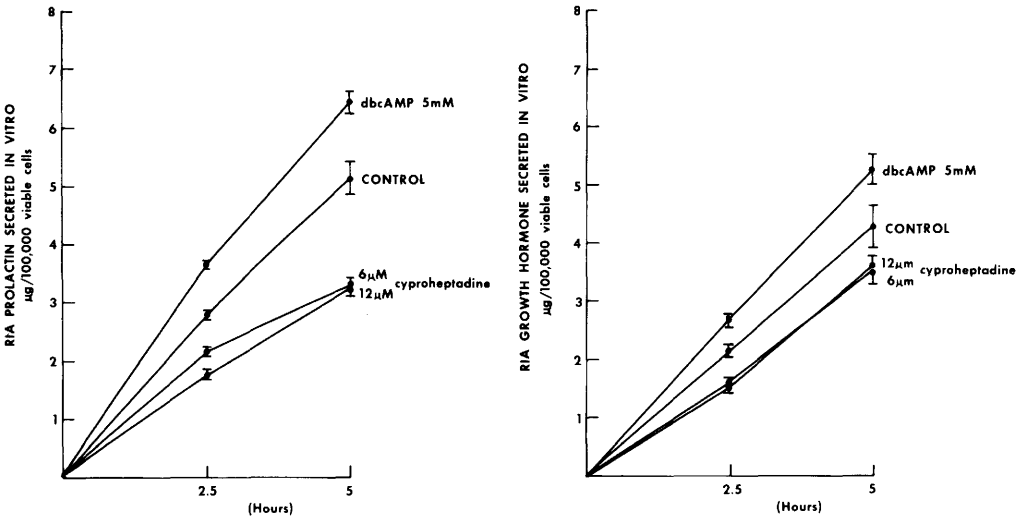

FIG. 2. Effect of DBcAmp and cyproheptadine on prolactin and growth hormone secretion by trypsin-isolated pituitary tumor cells *in vitro*. Approximately 0.5 × 10^6 cells were incubated for the indicated times in the presence of DBcAMP or cypropeptadine.

TABLE IV. THE EFFECTS OF CYPROHEPTADINE ON SERUM GROWTH HORMONE AND PROLACTIN IN RATS BEARING A MIXED GROWTH HORMONE/PROLACTIN-SECRETING TUMOR (MtTW15)[a]

	Serum growth hormone (μg/ml)		Serum prolactin (μg/ml)	
	Day 1	Day 5	Day 1	Day 5
Control tumor-bearing rats	2.28 ± 0.30[b]	3.13 ± 0.74	11.71 ± 1.71	13.90 ± 2.69
Cyproheptadine-injected tumor rats	2.93 ± 0.14	3.87 ± 0.64	13.80 ± 1.39	18.00 ± 2.64

[a] Six MtTW15-bearing rats per group; cyproheptadine (3 mg/kg; s.c.) was administered for 5 days; 90 min after the last injection the animals were killed by decapitation and blood was collected.
[b] Mean ± SEM.

secretion is strong. Low concentrations of the dopamine receptor agonists apomorphine and piribedil have been reported to stimulate GH secretion both in man (17, 18) and in rats (19). The ability of the specific dopamine receptor antagonist haloperidol to reduce plasma GH levels and to block apomorphine-induced GH release in rats (19) strengthens this hypothesis. Recently comparable human data have been reported in which pimozide was able to block GH release induced by exercise and arginine infusion (13).

Dopamine probably exerts its effect by inhibiting somatostatin release in the external layer of the median eminence (20). Further evidence for the role of dopamine is found in the observations of a positive correlation between the diurnal variations in plasma GH and hypothalamic dopamine content (21). Our experiments also support the view that dopamine acts as a neurotransmitter at the hypothalamic level to regulate GH secretion. We have shown previously that neither norepinephrine, epinephrine, ergocryptine, nor perphenazine has a direct effect on GH synthesis and release by the normal pituitary gland *in vitro* (22, 8). The 500 n*M* dopamine, a concentration which inhibits PRL secretion by the pituitary gland by 85% (23), was shown to have no effect on GH release *in vitro*. This contrasts with observations by Quijada *et al.* (24) who showed a significant inhibitory effect of dopamine (up to 10^{-5} *M*) on *in vitro* pituitary GH secretion.

The direct inhibitory effect of cyproheptadine on GH secretion by dispersed rat pituitary tumor cells has not been described before. Cyproheptadine administration (0.6 mg daily for 2 days) decreased plasma GH concentrations during oral glucose tolerance tests

in four of six patients with active acromegaly (25), but Chiodini *et al.* (26) did not observe a change in plasma GH in response to the administration of a single dose of 8 mg cyproheptadine, nor during chronic treatment of four acromegalic patients for 2 weeks with 12 mg cyproheptadine per day. We have shown before that cyproheptadine has a direct inhibiting effect on prolactin secretion by the normal pituitary gland *in vitro* (27): This effect was shown not to be mediated via a serotonin- or dopamine-receptor mechanism. The observation that cyproheptadine had no direct effect on GH secretion by the pituitary gland *in vitro*, while it does inhibit secretion of GH and PRL by dispersed pituitary tumor cells *in vitro*, suggests that the GH secretion in this mixed GH-PRL-secreting pituitary tumor has acquired the properties of PRL-secreting tumor cells. However, chronic administration of cyproheptadine did not affect tumor growth or serum GH in rats bearing the transplantable pituitary tumor MtTW15.

Summary. The administration of cyproheptadine to female rats transiently decreased serum GH levels. Forty-five minutes after cyproheptadine injection, *in vitro* GH synthesis and release was significantly decreased. Although cyproheptadine decreased PRL release *in vitro*, the drug had no direct *in vitro* inhibitory effect on GH release. Cyproheptadine did not affect the secretion of GH and PRL from transplantable tumor MtTW15, although it inhibited the *in vitro* release of GH from dispersed tumor cells. Cyproheptadine had no effect on either tumor size or the biological actions produced by the tumor hormones.

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