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The Effects of ACTH 1–17 on GH Secretion In Vitro

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Summary

Recently we demonstrated that ACTH 1–17 infusion in normal subjects is able to stimulate growth hormone (GH) secretion. In order to study the mechanism by which ACTH 1–17 induces this hormonal secretory pattern, we examined the effects of ACTH 1–17 addition to primary cultures of rat anterior pituitary cells and of two human pituitary adenomas (a mixed GH- and PRL-secreting adenoma and a prolactinoma) on GH and PRL secretion. Normal rat pituitary cells responded to rGRF with a dose-dependent increase of rGH: ACTH 1–17 induced a slight not significant increase of rGH secretion even at micromolar concentrations. Furthermore no additive effect of ACTH 1–17 on rGRF-stimulated GH release was observed. No significant stimulatory effect was also documented in the human tumors studied. These results suggest that the GH releasing activity of ACTH 1–17 observed in vivo is mediated via a direct action on CNS.

Key-Words: *ACTH 1–17 – GRF – Primary Pituitary Cultures – PRL – GH Secretion*

Introduction

Since the original observations of *Zahnd* et al. in 1969, several investigators have reported a stimulation of GH release by ACTH 1–24 (*Zahnd, Nadeau* and *von Muhlendahl* 1969; *Neri, Bartorelli, Ambrosi, Beck-Peccoz* and *Faglia* 1970; *Pandos, Strauch* and *Bricaire* 1970; *Byyny, Orth, Nicholson* and *Liddle* 1972; *Stjernholm, Alsever* and *Beck* 1975; *Kobberling, Juppner* and *Hesch* 1976) and by Gly-ACTH octadecapeptide amide (*Takahara, Asaoka* and *Ofuji* 1973). Recently we have investigated the ability of an analogue of corticotropin, the heptadecapeptide ACTH 1–17 (Synchrodin) to alter pituitary hormone secretion (*Valenti, Banchini, Denti, Polotti* and *Ceda* 1986). A clear increase in GH levels after the infusion of this peptide was documented in almost all the patients studied, while prolactin levels decreased during the study period; no significant change in testosterone, estradiol or DHEA-S levels was observed, while serum cortisol concentrations increased.

In order to study the mechanism by which ACTH 1–17 induces this hormonal secretory pattern in vivo, the effects of ACTH 1–17 on GH and PRL secretion were studied in primary cultures of rat pituitary cells and of two human pituitary adenomas (a mixed GH- and PRL-secreting adenoma, and a prolactinoma).

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Materials and Methods

Primary cultures of dispersed anterior pituitary cells derived from adult, male Sprague-Dawley rats and from two patients, one with acromegaly and one presenting with the amenorrhea-galactorrhea syndrome, were prepared by our previously described methodology (*Ceda, Hoffman, Silverberg, Wilson* and *Rosenfeld* 1985). All cells were grown in 2cm² 24-multiwell plates in monolayer cultures in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 ug/ml) at 37°C in an atmosphere of 95% air and 5% CO_2. The plating density ranged between 1–5 x 100,000/ml; the cells were allowed to attach to culture dishes for at least three days before the medium was changed for the experimental incubations.

GH and PRL Release Studies

After 3–5 days in culture, the medium was removed from the cells and was replaced with fresh DMEM supplemented with 1% BSA, with or without various hormones.

A minimum of 4 replicates was used for each experimental condition. rGH was assayed using reagents provided by the National Hormone and Pituitary Program (NIH); hGH and hPRL were assayed with a double antibody RIA method using commercial kits purchased from Biodata-Serono Immunochemicals (Milan, Italy).

Rat GRF and human GRF 1–44 were purchased from Peninsula Laboratories (Belmont, CA); ACTH 1–17 (Synchrodyn) was obtained from Hoechst (Scoppito, L'Aquila, Italy).

Statistical analysis of the results was performed with analysis of variance and Student's t-test, when appropriate.

Results

1) Normal rat pituitary cultures

Normal rat pituitary cells responded to rGRF with a dose-dependent increase in rGH secretion; an approximate 6-fold increase in GH release was observed with the highest dose of GRF. ACTH 1–17 induced a slight and not significant increase in GH secretion even when micromolar concentrations of the peptide were employed (Fig. 1). Furthermore, no additive effect of ACTH 1–17 on rGRF-stimulated release was seen (data not shown).

2) Human pituitary adenomas

In the mixed GH- and PRL-secreting adenoma, 100 nM GRF 1–44 induced a slight increase in GH secretion both after 4 and 24 hours of incubation; ACTH 1–17 (1 μM) was without any significant effect on either GH or PRL secretion (Fig. 2).

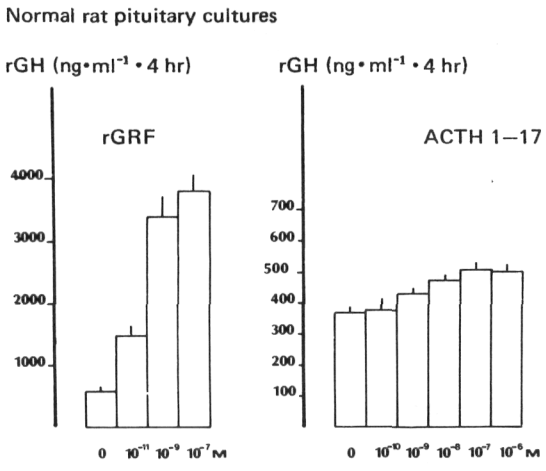

Fig. 1 Rat GRF (rGRF) and ACTH 1–17 stimulation of rGH secretion from cultured rat pituitary cells. The cells were incubated in the presence of varying concentrations of rGRF and ACTH 1–17. A significant increase of GH secretion was documented with each concentration of rGRF only ($P < 0.001$).
Results are the mean±SEM of 4 dishes for each bar.

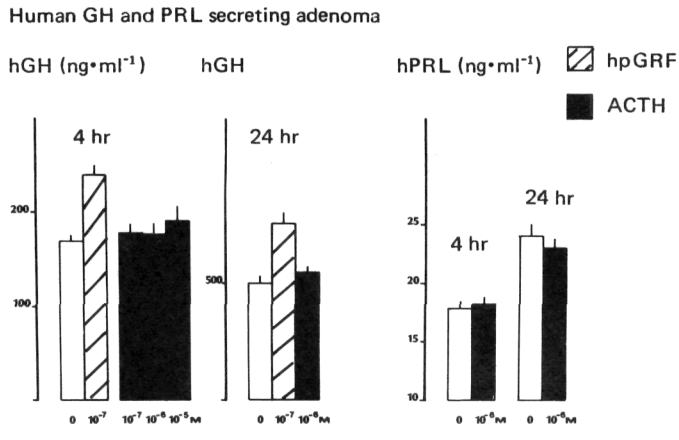

Fig. 2 Effects of GRF 1–44 (hpGRF) and ACTH 1–17 on GH secretion from cells derived from one mixed GH- and PRL-secreting human pituitary adenoma. GRF 1–44 caused a significant increase in GH release ($P < 0.001$) in each case. No significant change was seen in ACTH 1–17-treated cells. The numbers of hours of incubation are noted; *results are the mean±SEM of 4 replicates for each* condition.

Furthermore no significant differences in prolactin secretion in comparison with control cells were observed after ACTH 1–17 was added to the cells derived from the human prolactinoma (data not shown).

Discussion

It is known that ACTH and its fragments are able to exert behavioural effects which are probably mediated via the central nervous system (CNS), possibly through a modulation of the metabolism and actions of classical neurotransmitters (*De Wied* 1980; *Versteeg* 1973). Since no stimulatory action on GH release was documented in vitro in either normal rat pituitary cells or in human pituitary tumor cells, a similar indirect CNS mechanism could be responsible for the GH releasing ability of ACTH 1–17 which we have previously documented in vivo. The prompt and sustained increase of cortisol levels seen after ACTH 1–17 administration in vivo is the only alteration of peripheral steroid pattern which could theoretically explain the augmented GH release. Glucocorticoids increase the synthesis and pituitary cell content of GH in vitro (*Spindler, Mellon* and *Baxter* 1982) and also sensitize the pituitary somatotrophs to GRF stimulation (*Vale, Vaughan, Yamamoto, Spiess* and *Rivier* 1983). Clinically, however, glucocorticoids, suppress the pituitary GH response to various stimuli (*Frantz* and *Rabkin* 1964) and long term treatment with these steroids inhibits somatic growth in both men and animals (*Daughaday, Herrington* and *Phillips* 1975); furthermore, acute exposure of rat pituitary cells in primary culture to corticosterone (*Webb, Szabo* and *Frohman* 1983) and to dexamethasone (*Ceda, Davis* and *Hoffman*, unpublished) inhibits GRF stimulated GH release.

In cultured hypothalamic cells, ACTH 1–24 has been shown to cause a decrease in both intracellular somatostatin levels and somatostatin secretion into the medium (*Robbins, Leidy* and *Landon* 1985). Thus, ACTH might augment GH release in vivo by inhibiting the release of hypothalamic somatostatin. In light of the lack of any direct stimulatory effect of ACTH on pituitary somatotrophs and the presence of an inhibitory effect of an acute glucocorticoid challenge on GH secretion, we believe that the ACTH-directed GH release observed in vivo is mediated via a direct action of ACTH on the CNS.

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