BIOCELL
2002, 26(1): 49-60

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Prolactin-releasing activity of GHRP-5 (Momany peptide) on lactotrophs *in vivo* and *in vitro*

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Key words: PRL secretion, GHRP-5, lactotroph, immunocytochemistry, mRNA PRL.

ABSTRACT: In the present study the *in vivo* and *in vitro* effects of GHRP-5 on the PRL-releasing activity in correlation with the morphological changes of lactotroph cells and their transcriptional activity were evaluated. The *in vivo* treatment (12 µg/100g BW/day for 3 days) of male rats with GHRP-5 does not induce any significant changes in serum PRL levels. In contrast, the addition of GHRP-5 to pituitary cell cultures increased significantly the release of PRL. This effect is enhanced in cell cultures of enriched lactotrophs, increasing significantly the secretion of PRL, the concentrations of which were 50% higher than that of untreated control cells. The administration of GHRP-5 provokes several changes in the fine structure of lactotrophs, compatible with an increased secretory activity. After the GHRP-5 treatment the different lactotroph subtypes persist but the subtype I displaying secretory granules of larger size (500-900nm) and a significant development of the Golgi apparatus and RER were more frequently observed. These results can be correlated with a significant augmentation in PRL mRNA after the GHRP-5 treatment. In spite of that no variations in serum PRL levels were observed *in vivo*, following GHRP-5 treatment, the lactotroph population experienced evident fine structure modifications, concordant with an upsurge of PRL synthesis. These observations confirmed a direct action of GHRP-5 on receptors expressed by lactotrophs. The differential actions of GHRP-5 on *in vivo* and *in vitro* designs confirm a different effectiveness of this secretagogue to induce PRL secretion.

Introduction

Growth hormone-releasing peptides (GHRPs) are potent GH-secretagogues, characterized by their small size, stability and low toxicity. The clinical applications of GHRPs have been envisioned in three main areas: therapy of GH-deficiencies (GHD), diagnosis of GHD and non-endocrinological aspects (Micic *et al*., 1999). It is generally accepted that GHRPs stimulate the GH release by acting at hypothalamic and pituitary levels (Codd *et al*., 1989; Pong *et al*., 1996) via a specific receptors (GHS-R), different from those of the growth hormone-releasing hormone (GHRH), the endogenous hypothalamic secretagogue. (Blake and Smith, 1991; Howard *et al.,* 1996; Smith *et al*., 1996; Pong *et al*., 1996). The cloning of GHS-R strongly suggested the existence of an endogenous ligand for regulating GH release, probably different from GHRH (Howard *et al*., 1996; McKee *et al*., 1997). More recently an endogenous ligand for the GHS-R named *ghrelin* was identified in the rat stomach with releasing effects on GH *in*

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Received on July 5, 2001. Accepted on November 22, 2001.

vivo and *in vitro* (Kojima *et al*., 1999). The discovery of *ghrelin* in rat and human indicates that the release of GH from the pituitary might be regulated not only by hypothalamic GH releasing hormone but also by *ghrelin* (Kojima *et al.,* 2001).

Concurrent with the development of more potent GH releasing agents and their use in human subjects (Korbonits *et al.*, 1995; Ghigo *et al.,* 1997; Locatelli and Torsello, 1997; Rahim *et al.*, 1999) it soon became clear that the endocrine effects of GHRPs are relatively specific for GH release; however, this specificity is not absolute. Several studies have confirmed that GHRPs are not free from adverse events (Arvat *et al.*, 1997a-b), and most of them also release small but significant amounts of cortisol, ACTH and PRL, in humans (Massoud *et al.,* 1996; Ciccarelli *et al.,* 1996; Arosio *et al*., 1998; Korbonits *et al.*, 1999; Muccioli *et al.,* 2000). Although several reports on various GHRPs provided a wide range of information on GH secretion, the mechanism of action of GHRPs on lactotroph cell activities still remains unknown (Hickey *et al.,* 1996; Jacks *et al*., 1996).

The present study was focused on the effects of (Y-W-A-W-F- NH_2), termed GHRP-5 on lactotrophs of male rat pituitary gland. This GHRP, also known as Momany peptide, was one of the earliest synthesized but scarcely investigated synthetic GH-secretagogue (Momany *et al*., 1981). It was of interest to compare *in vivo* and *in vitro* the PRL-releasing activity of GHRP-5, and to correlate the morphological changes of lactotroph cells with their transcriptional activity.

Material and Methods

Adult male rats of the Wistar strain, aged 2 month-old were used in this investigation. They were housed in air-conditioned quarters with a light-dark cycle (14 h-10 h) and provided with free access to tap water and rodent chow (Nutric, Córdoba, Argentina).

Animal procedures were in compliance with the Guidelines on the Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare, and the local Institutional Animal Care Committee.

Rats were injected intraperitoneally with 12 μg of GHRP-5 in saline per 100 g body weight/day for 3 days. Controls were injected with the solvent. Eight rats were used in each experimental trial and the data presented were representative of at least three independent experiments.

The rats were sacrificed two hours after the last injection. Animals were decapitated within 10 s after removal from their cage, avoiding any stress or external stimuli. Arterial and venous blood drained from head and trunk were collected in a centrifuge tube, allowed to clot at 4ºC and spun down. The serum was removed and save frozen at –20 ºC until PRL measurements. Then, the pituitary gland was rapidly excised and split into two halves by a medial section with a razor blade. One hemipituitary was processed for electron microscopy and the other for immunocytochemistry.

Electron microscopy

Changes in the ultrastructure of PRL cells in GHRP-5 treated rats were studied in three hemipituitaries from each experimental group. The tissues were fixed by immersion in 4% (v/v) glutaraldehyde, 4% (w/v) formaldehyde in a cacodylate buffer, for 2-4 h at room temperature. The tissue was then treated with 1% osmium tetroxide for 2 h at room temperature, dehydrated with increasing concentrations of acetone and embedded in Araldite. Thin sections cut with a diamond knife on a Porter-Blum MT2 and a JEOL, JUM-7 ultramicrotome was examined in a Zeiss 109 electron microscope.

Immunocytochemistry

Three hemipituitaries obtained from rats treated with GHRP-5 for 3 days were fixed in 2% (v/v) glutaraldehyde and 4% (w/v) formaldehyde in 0.1 M cacodylate buffer pH 7.3 at room temperature for 5-6 h. Each fixed hemipituitary was dehydrated in increasing concentrations of ethanol and embedded in acrylic resin (LR White, London Resin Corporation). Electron microscope immunocytochemistry was performed on thin sections of LR-White embedded pituitaries and immunostained for PRL with rabbit anti-rat PRL (diluted 1:4000) used as primary antiserum (all kindly donated by Dr. A. F. Parlow, National Institute of Diabetes and Digestive and Kidney Diseases, NIDDKD, Bethesda, MD. Prolactin immunoreactive sites were labelled with anti-rabbit IgG adsorbed to colloidal gold particles. To validate the specificity of the immunostaining the following controls were performed: (1) Adsorption of antibodies to highly enriched PRL. (2) Replacement of primary antiserum with 1% bovine serum albumin (BSA) in 0.1 M phosphate buffer, pH 7.3 plus 0.15 M sodium chloride (PBS). (3) Replacement of primary antiserum with diluted preimmune serum followed by the protein A/colloidal gold complex.

Preparations of the immunogold complexes and other details of immunocytochemical procedures were described elsewhere (Maldonado and Aoki, 1986).

The one-step acid-guanidinium method for RNA extraction as described by Chomczynski and Sacchi (1987) was performed. In brief, 0.1 g pituitary was homogenized in 1 ml denaturing solution (4M guanidinium isothiocyanate, 25mM sodium citrate pH 7.0, 0.5 N-lauroyl sarcosine and 0.1% ß-mercaptoethanol). After phenol-chloroform-isoamyl alcohol (50:49:1) extraction, RNA was precipitated in isopropanol, recovered by centrifugation and washed in 80% ethanol. After a further extraction, precipitation and washes, the RNA was dissolved in diethyl pyrocarbonate-treated water quantified and checked for purity by spectrophotometry at 260 and 280 nm.

Northern blot

The procedure was similar to that described by Fourney *et al.* (1988) with minor modifications (Pellizas *et al*., 1998). Twenty micrograms total RNA were electrophoresed in 1% agarose gel containing 0.66 M formaldehyde. The gel was stained with ethidium bromide to visualize ribosomal RNA (rRNA). After electrophoresis, RNAs were transferred to a nylon membrane.

The membranes were incubated in pre-hybridization solution containing 30% deionized formamide-5 X Denhart's solution (0.1% Ficoll type 400-0.1% albumin-0.1% polyvinylpirrolidone (PVP)-5 X SSPE (0.75 M ClNa-0.05 M NaH2PO4 -5mMEDTA) 0.1%SDS-200 µg/ml DNA from herring testes, for 5 h at 42°C in hybridization bags. Hybridization with the probe was performed for 48 h at the same temperature. The entire SP65#1- PRL cDNA linearized with Hind III was used as hybridization probe for Northern blots. The entire cDNA was approximately 3.7 Kb and included the full coding sequence. To ensure an even loading, the same blots were hybridized using the entire pBR 322 with 18S rRNA genomic probe. The probes were labelled by the random primer technique with [(32P) deoxy-ATP(3000 Ci/mmol)]. The specific activity of the labelled probes ranged from 2.6 X 109 - 3.9 X 109 d.p.m./ µg DNA. After hybridization, blots were washed in 2 X SSC (0.3 M NaCl - 0.015 M sodium citrate) -1%SDS for 20 min at room temperature, followed by 2 X SSC - 1% SDS for 20 min at 55°C, 1 X SSC -1% SDS for 20 min at 55°C and 0.2 X SSC -1% SDS for 20 min at 55°C. The membranes were exposed to Kodak X-Omat film at -70°C with intensifying screens, for four hours in the case of the PRL probe, and for six hours in the case of the 18S rRNA probe. The bands were quantified densitometrically (Shimadzu Dual-Wavelenght Chromato Scanner CS-930) at 500 nm and the levels of PRL mRNA expressed as absorbance of the PRL signals normalized with that of the 18S rRNA in the same lane.

Dissociation of anterior pituitary cells

The techniques for cell dissociation and culture of pituitary cells was described in detail previously (De Paul *et al.,* 1997). For each experiment, cell suspensions were prepared from anterior pituitaries of 30 male rats. The pituitaries were rapidly excised, posterior and intermediate lobes discarded and anterior pituitaries placed in Eagle's Minimal Essential Medium (S-MEM) consisting of 1 mg/ml BSA, 13 nM HEPES, 30 µM streptomycin sulphate, 90 µM penicillin G and 2 mM L-glutamine. The medium was filtered through a 0.22 µm Nalgene membrane (Nalge Company, New York) before use; the final solution pH was 7.4. Adenohypophyses were rinsed with S-MEM and then incubated with 0.4% trypsin in a shaker bath at 37ºC, for 20 min. Then, tissue blocks were treated with a trypsin inhibitor for 3 min. One hundred micrograms of deoxyribonuclease was added in all incubation steps to avoid cell clumping. After washing in S-MEM at room temperature the cells were mechanically dispersed with siliconized Pasteur pipettes. The cells were spun down and the pellets resuspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 3% fetal calf serum and 8% horse serum (Gibco). The cell yield was $1.5\text{-}2x10^6$ per pituitary and the cell viability, tested with Trypan Blue exclusion, was always better than 90%. The final suspension was adjusted to 1.10^6 cells/ml of medium.

Enrichment of lactotroph cells

Discontinuous Percoll density gradients were used for enrichment of pituitary cells (Velkeniers *et al.,*1988; Shinkai and Ooka, 1995). Percoll gradients were prepared in conical centrifuge tubes layering 2 milliliters of 1.045, 1.065, 1.080, and 1.090 g/ml Percoll solutions, starting with the highest concentration. Freshly dispersed cells ($2x10^6$ cells/2ml S-MEM) were placed on the top of the gradients and centrifuged at 400 x g for 20 min at room temperature. Coloured marker beads of known sizes were used to control the limits of the layers. The cell fraction containing lactotrophs, recovered at densities between 1.045 - 1.065 was sedimented and washed twice in S-MEM medium. The content of

lactotrophs in this fraction was validated by electron microscope immunocytochemistry.

Experimental procedures

Whole cultures of dispersed cells and enriched lactotrophs were placed in 35-mm sterile culture plates (Corning, New York) at a density of $5x10^5$ cells/2 ml DMEM/well and 6 wells for each treatment were studied. The cell cultures were incubated at 37ºC in a humidified atmosphere of 95% air - 5%CO_2. An additional aliquot of 1ml fresh culture medium was added to each well 48 h later. At 72 and 96 h of incubation, the media were withdrawn and replaced with 2 ml fresh DMEM. On the 5th day, 2ml fresh culture medium plus 2.5 μg/ml was added in each well for additional 24 hours. Controls without GHRP-5 were performed for both whole and enriched pituitary cell cultures. Samples of culture media (1 ml) were collected and stored at -20ºC until radioimmunoassay (RIA). At the end of each experiment, the cell viability was tested with Trypan Blue exclusion test.

Unless stated, all the reagents used in this investigation were purchased from Sigma Chemical Company, St. Louis, MO. USA.

Radioimmunoassay

Serum and culture media PRL were quantified by RIA, applying a double antibody technique at two dose levels (Niswender *et al.,* 1969) following the protocol provided by NIDDKD. The results expressed in terms of rat prolactin-RP-3 (biological potency equivalent to 30 IU/mg). All samples were processed simultaneously to avoid interassay variations. The intra-assay coefficient of variation was lower than 10%. All the reagents used were donated by Dr. Parlow of the NIDDKD.

Statistical analysis

The results were processed statistically by the Student "t" test. Results were expressed as means ± SEM of three different experiments. Significance was reported at $P<0.05$ or higher.

Results

Prolactin radioimmunoassay

As it is illustrated in Fig. 1, the treatment of male rats with GHRP-5 does not induce any significant changes in the levels of serum PRL. In contrast, the addition GHRP-5 to pituitary cell cultures increased significantly ($P<0.001$) the release of PRL into the incubation medium when compared to control cell cultures (Fig. 2). The effects of GHRP-5 is enhanced in cultures of enriched lactotrophs, increasing significantly ($P<0.01$) the release of PRL, the concentrations of which were 50% higher than that of untreated control cells. (Fig. 3).

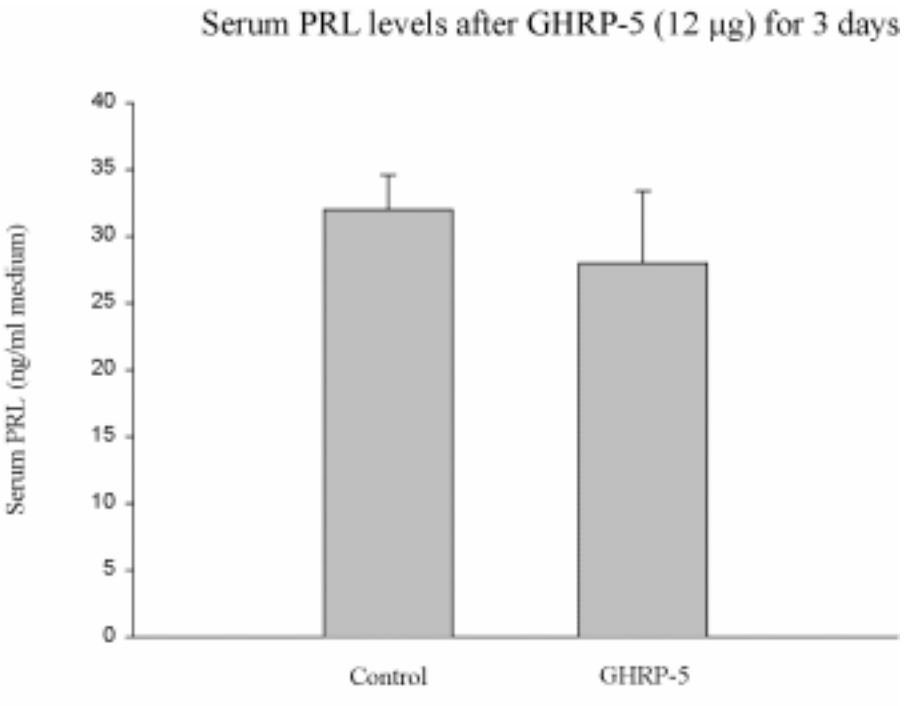


FIGURE 1. Serum PRL levels of rats treated with GHRP-5 (12 μg/100g BW/day) for 3 days. The treatment did not change the serum PRL concentration. Data are expressed as mean ± SEM for eight rats in each group of three different experiment. Student "t" test.

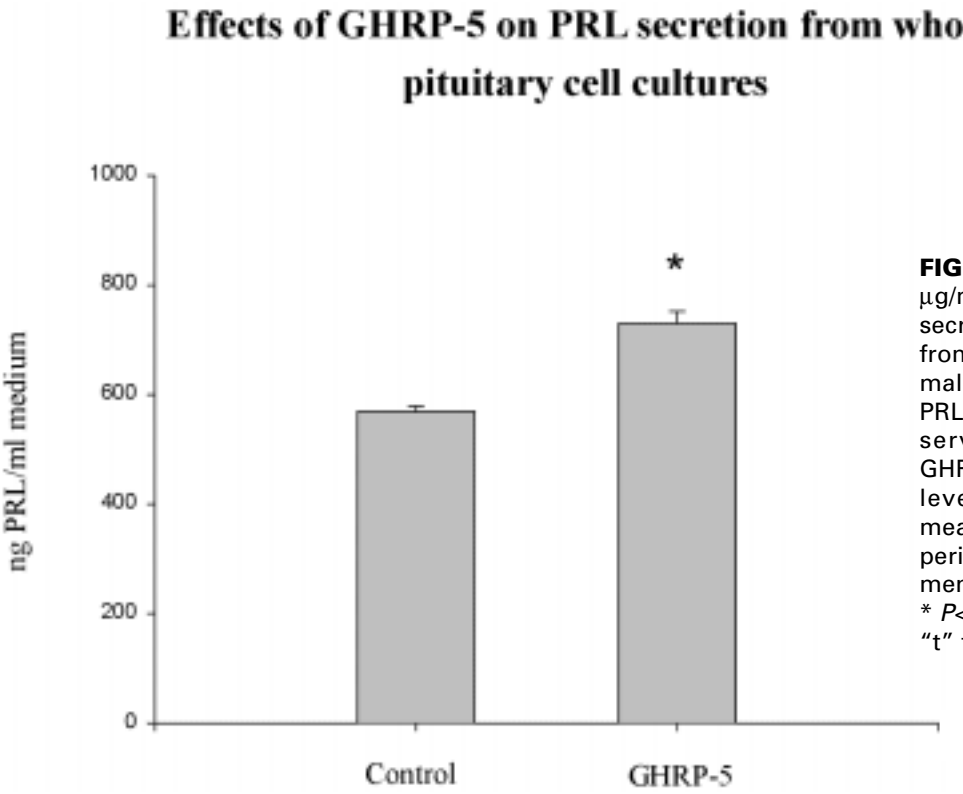


FIGURE 2. Effects of GHRP-5 (2.5 μg/ml medium) for 24 hours on PRL secretion of pituitary cell cultures from whole pituitary gland of adult male rats. Striking augmentation of PRL levels in culture media was observed in cells incubated with GHRP-5 compared with the control level. Results are expressed as means ± SEM of three different experiment (6 wells/treatment/experiment). Significance was reported at * $P<0.001$ vs control wells. Student "t" test.

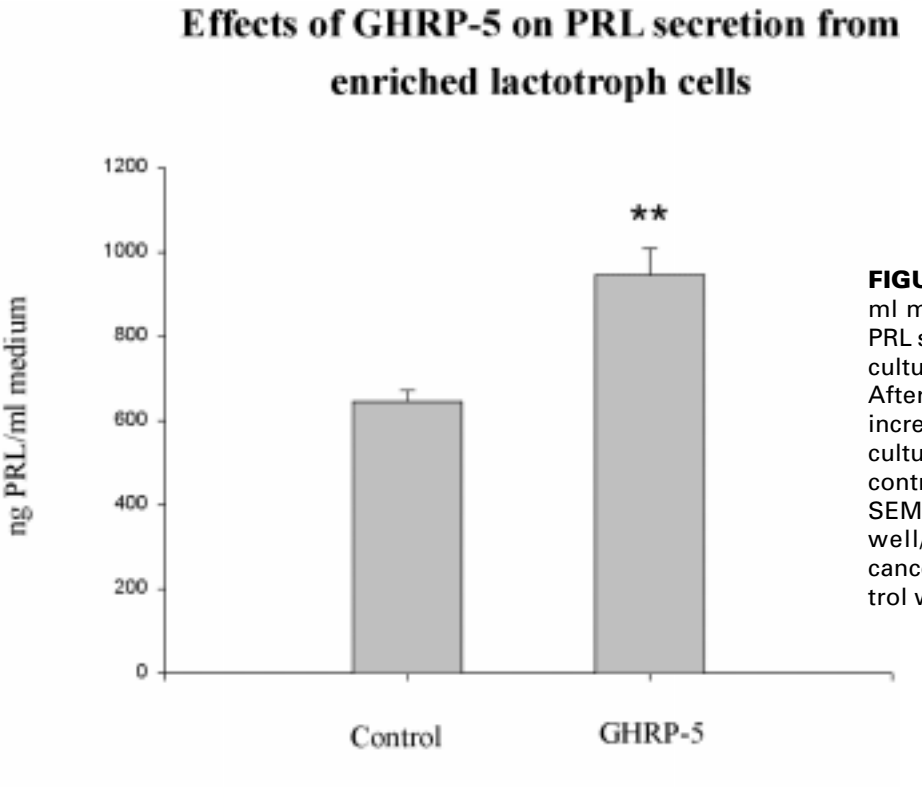


FIGURE 3. Effects of GHRP-5 (2.5 μg/ml medium) treatment for 24 hours on PRL secretion of enriched lactotroph cell cultures obtained from adult male rats. After GHRP-5 treatment a remarkable increase of PRL levels was observed in culture media when compared with the control. Data are expressed as means ± SEM of three different experiment (6 well/treatment/experiment). Significance is reported at ** $P<0.01$ vs control wells. Student "t" test.

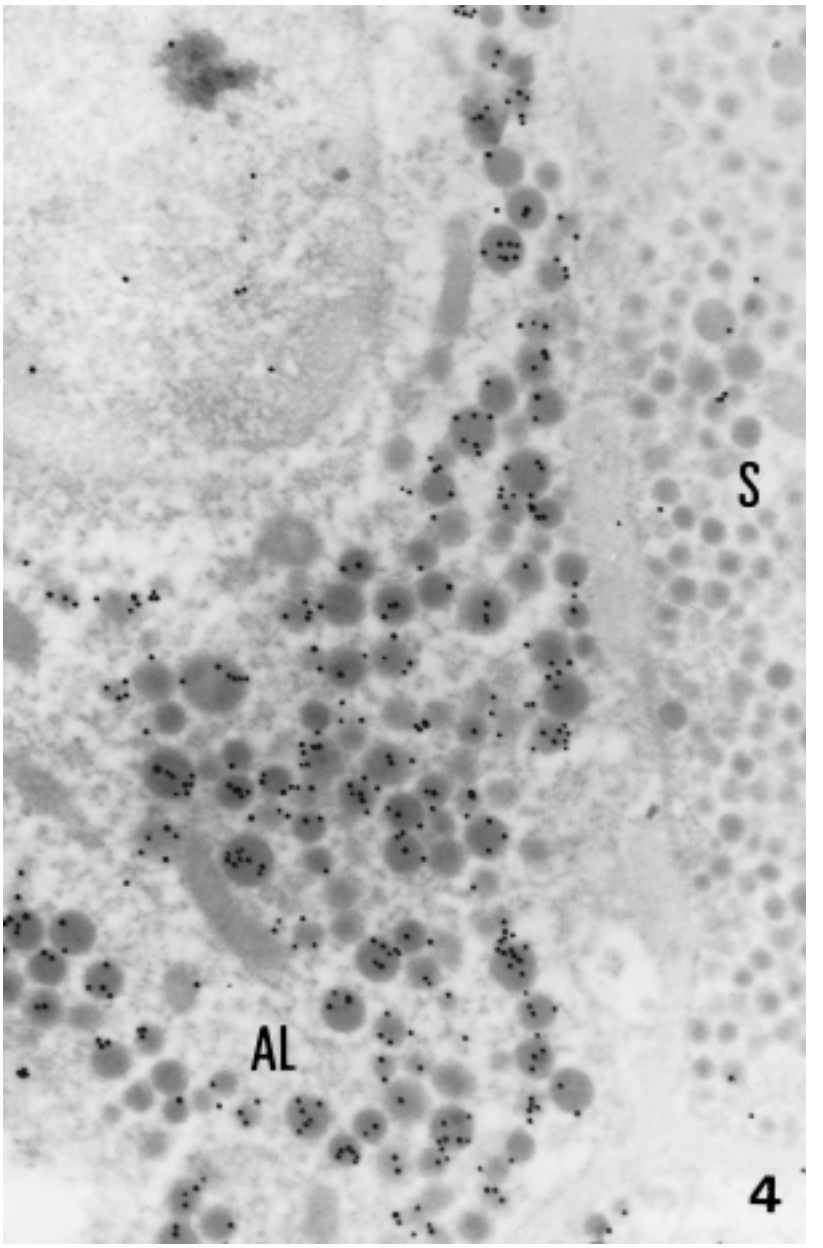
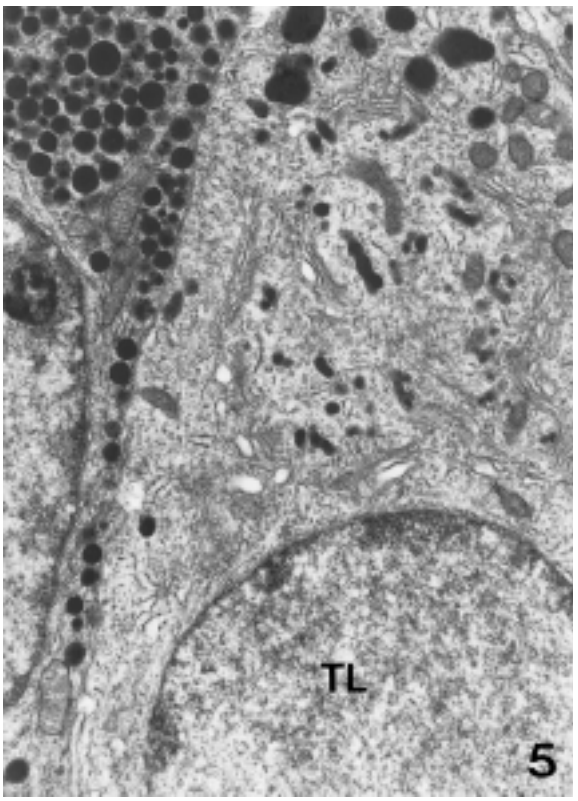


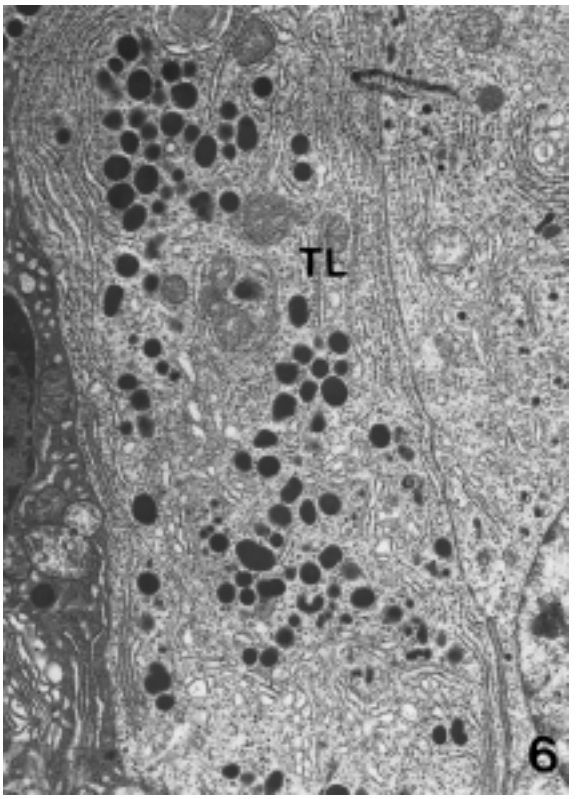
FIGURE 4. Electron microscope immunocytochemistry of a pituitary gland section from control male rat. Atypical lactotroph cell (AL) is characterized by the accumulation in the cytoplasm of immunolabelled spherical secretory granules the size of which was between 100-250 nm. An unlabelled somatotroph cell (S) with uniform size and round profile similar to those found in lactotroph is also seen at the bottom of the figure and serves as a control for immunostainig background. X 28,000.

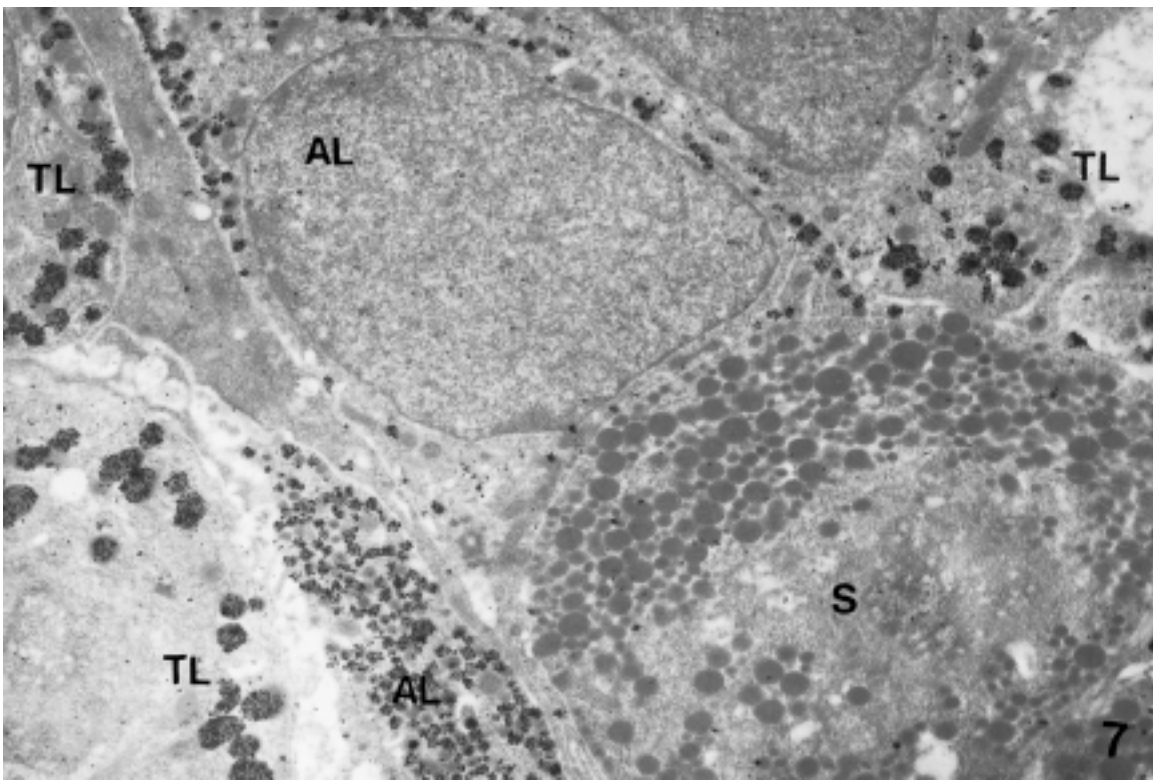
FIGURE 5. Electron micrograph of typical lactotroph cell (TL) of a pituitary gland from male rat treated with GHRP-5 (12 μg/100g BW/day) for three days. The cytoplasm contains a highly developed Golgi complex and RER. Numerous immature secretory granules are assembled. X 12,800.

FIGURE 6. Electron micrograph of typical type I lactotroph cell (TL) after three days of treatment with GHRP-5. The remarkable proliferation of RER and Golgi complex cisternae is accompanied by a noticeable accumulation in the cytoplasm of polymorphic immature and large mature secretory granules. X 22,000.

FIGURE 7. Electron microscope immunocytochemistry of a pituitary gland from a male rat treated with GHRP-5. A marked heterogeneity among the lactotroph population is clearly seen. The cytoplasms of typical and atypical lactotrophs show a conspicuous accumulation of secretory granules of different sizes. On the left, an unlabelled somatotroph cell (S) serves as negative control. X 13,800.


TL
5


TL
6


TL
AL
TL
S
TL
AL
7

Electron microscopy

Lactotrophs of adult male rat pituitary gland exhibit several distinctive features which have been described in detail in an early paper (Maldonado and Aoki, 1994). To avoid repetition only pertinent data will be described here. In contrast to the female, the main characteristics of adult male lactotrophs are the stability of their ultrastructural organization and the predominance of a subpopulation containing small secretory granules (100-250 nm in diameter). The quiescent appearance acquired by this lactotroph subtype was validated by the accumulation of PRL secretory granules with uniform size and round profile similar to those observed in somatotroph cells (Fig. 4).

The administration of 12 μg/100g BW/day for 3 days of GHRP-5 provokes several changes in the fine structure of lactotrophs particularly in the cytoplasmic

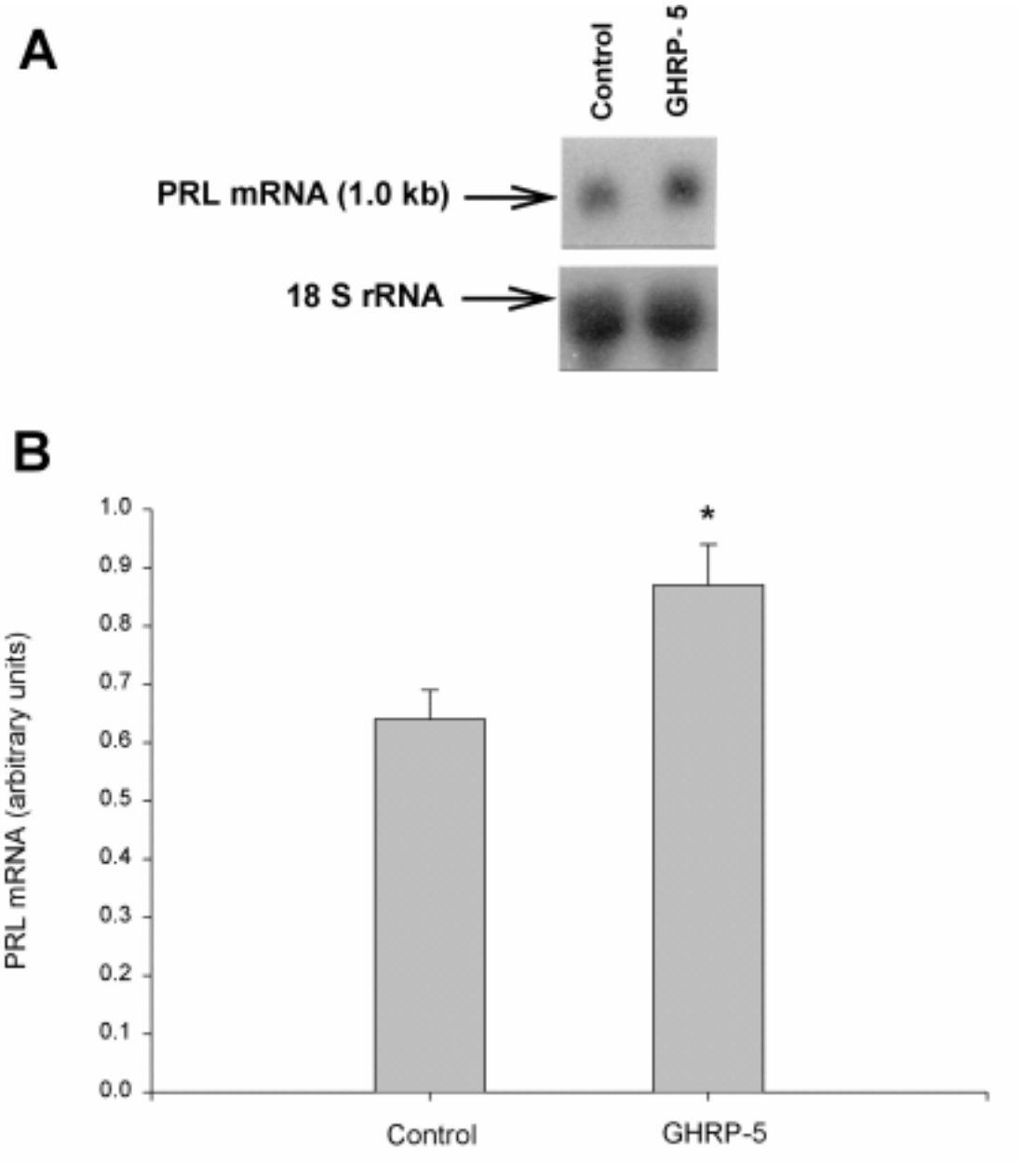


FIGURE 8. (A) Northern blot for PRL mRNA after administration of GHRP-5 (12 μg/100g BW/day) for 3 days. Twenty micrograms total RNA were applied to each lane. Blots were hybridized with PRL (upper panel) and 18 S rRNA probes (lower panel). (B) Densitometric analysis of Northern blots. Data are given as the ratio between the absorbance of PRL specific signal and the absorbance of the 18 S rRNA signal in the same lane. Results are expressed as mean ± SEM of eight rats per group. * $P < 0.05$ compared with the control group by Student "t" test.

organelles engaged in synthesis and processing of proteins. These modifications are compatible with an increased secretory activity as judged by the proliferation or the RER and Golgi complex membranes and the appearance of abundant immature granules (Fig. 5). There are numerous PRL cells storing large secretory granules and numerous immature granules associated with Golgi area. After the GHRP-5 treatment the presence of lactotrophs types persist but the subtype I displaying secretory granules of larger size (500-900 nm) and polymorphic profiles were more frequently observed (Fig. 6).

The specific identification of PRL cells by electron microscope immunocytochemistry was crucial to identify and characterize the lactotroph subtypes depicting a dissimilar metabolic activity (Fig 7).

PRL mRNA

The changes in the levels of pituitary PRL mRNA in male rats treated with daily injections of 12 μg GHRP-5 per 100g BW for 3 days are exposed in Figure 8. A significant augmentation in PRL mRNA (37% over control level) was observed after the GHRP-5 treatment. A single PRL mRNA transcript of about 1kb was observed.

Discussion

The present investigation was proposed to study the effects of GHRP-5 on lactotroph cell secretory activity. In spite that no variations in serum PRL levels were observed *in vivo* following GHRP-5 treatment, the lactotroph population displayed noticeable fine structure alterations, which were concurrent with an upsurge of PRL synthesis.

It has been reported that in pituitary glands of male rats a 63% of PRL cell population corresponds to a lactotroph atypical subtypes (subtypes II and III) individualized by its secretory granules, the size of which 100-250 nm in diameter can easily be mistaken with granules of other cell pituitary cells (Maldonado and Aoki, 1994). After GHRP-5 administration to adult male rats, the fine structure of lactotrophs reveals signs of activation of the hormonal synthesis, as judged by the remarkable development of organelles involved in protein synthesis and the appearance of numerous immature secretory granules. The GHRP-5 treatment increased the predominance of typical lactotrophs, the most active secreting cell among lactotroph subtypes. The latter is the prevalent lactotroph in the pituitary gland of both adult female rats and male rats stimulated with estrogens. The main features of lactotroph type I are the presence of large and polymorphic mature secretory granules, about 500-900 nm in diameter, many of them in exocytosis and the striking development of RER and Golgi apparatus cisternae, characteristics of an enhanced biosynthetic activity (Maldonado and Aoki, 1994). Supporting these morphological observations, the GHRP-5 also induced an increase in PRL biosynthesis as confirmed by the significantly higher levels of mRNA.

Striking differences were found in the activity of GHRPs and nonpeptidyl GHRPs; moreover, they appear to be not fully specific for GH release. A TSH-inhibiting effect has been reported for GHRPs (Jaffe *et al*., 1993; Laron *et al*., 1993) in addition to a slight but significant and dose-dependent PRL-, ACTH- and cortisol-releasing activity (Bowers, 1993; Aloi *et al*., 1994; Ghigo *et al*., 1994; Imbimbo *et al.,* 1994; Copinschi *et al*., 1996; Carmignac *et al*., 1998).

A specific high-affinity binding site that mediates the activity of GHRPs has been identified in hypothalamic membranes and anterior pituitary (Codd *et al.,* 1989). A direct action of GHRP-5 lactotrophs was verified by the significant release of PRL into the incubation medium of dispersed pituitary in cell culture. This effect was remarkable enhanced when enriched lactotrophs were used as target cells. These observations confirmed a direct action of GHRP-5 on receptors expressed in lactotrophs as described by others (Korbonits *et al*., 1998; Barlier *et al*., 1999).

The mechanisms underlying these effects are still unresolved. Some evidences suggested that the stimulatory effects on PRL secretion in human pituitary tumors may involve direct effect on somatomammotrope cells (Adams *et al*., 1995), but in the rat, the existence of somatomammotrophs is still under dispute (Pasolli *et al.,* 1994). On the other hand, opioids, serotonin and histamine are known for their important role in the control of PRL and ACTH secretion (Muller and Nistico, 1989). Nevertheless both the PRL- and the ACTH/cortisol-releasing activity of hexarelin was not modified by serotonin and histamine antagonists such as naloxone, cyproheptadine, or diphenhydramine (Korbonits *et al.,* 1995; Arvat *et al*., 1997c). Thus, the PRL- and ACTH-releasing effects of GHRPs do not appear to be mediated by these transmitters.

In our experiments the effects of GHRP-5 on PRL release appear to be conditioned to the experimental design. In male rats, the administration of 12 μg of GHRP-5 for three days did not induce PRL release, but

it is capable of stimulating the PRL gene transcription and boost the PRL mRNA accumulation. On the other hand, GHRP-5 *in vitro* provokes a significant secretion of PRL to the culture medium. The differential effects of GHRP-5 on PRL secretion could be explained by the action of neuromodulators which regulate the lactotroph secretory activity *in vivo* but they are absent in *in vitro* systems.

The existence of a functional receptor for GHRPs in somatotrophs, mammosomatotrophs, lactotrophs and corticotroph adenomas (Korbonits *et al.,* 1998; Barlier *et al*., 1999) poses new queries on the role played by GHRP-R in pituitary adenomas, particularly those involving no GH secretion. The discovery of the endogenous ligand of GHRP-R *(ghrelin)* opens a new area for clinical and basic GH research, where the relevance of GHRPs, as diagnostic tools or therapeutic applications in different GH deficient states must still be elucidate.

Acknowledgements

The authors are grateful to Dr. A.F. Parlow, NIDDKD, for the gift of rat GH antiserum for immunocytochemistry and rat GH antigen, rat GH antiserum and rat GH reference preparation for RIA. We also wish to thank Dr. J. Martial (Laboratoire central de la Génie génétique, Université de Liège, Belgium) for providing rat PRL cDNA and to Mercedes Guevara, Irma Alegre and Lucía Artino for their excellent technical assistance. This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR), the Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECyT). ALD and CGP are Post-Doctoral Fellows and AIT and AA are established investigators of CONICET.

References

ADAMS EF, PETERSEN B, TING L, BUCHFELDER M, FAHLBUSCH R (1995). The growth hormone secretagogue, L-692,429, induce phosphatidylinositol hydrolysis and hormone secretion by human pituitary tumors. Biochem Biophys Res Commun 208: 555-61.

ALOI JA, GERTZ BJ, HARTMAN ML, HUHN WC, PEZZOLI SS, WITTREICH M, KRUPA DA, THORNER MO (1994). Neuroendocrine responses to a novel growth hormone secretagogue, L-692,429, in healthy older subjects. J Clin Endocrinol Metab 79: 943-49.

AROSIO M, CASATI G, BIELLA O, PORRETTI S, IMBIMBO BP, FAGLIA G (1998). Lack of effect of hexarelin on TRH-induced TSH response in normal adult man. J Endocrinol Invest 21(4): 239-44.

ARVAT E, DI VITO L, MACCAGNO B, BROGLIO F, BOGHEN MF, DEGHNGHI R, CAMANNI F, GHIGO E (1997a). Effects of GHRP-2 and hexarelin, two synthetic GH-releasing peptides, on GH, prolactin, ACTH and cortisol levels in man. Comparison with the effects of GHRH, TRH and hCRH. Peptides 18 (6): 885-91.

ARVAT E, RAMUNNI J, BELLONE J, DI VITO L, BAFFONI C, BROGLIO F, DEGHENGHI R, BARTOLOTTA E, GHIGO E (1997b). The GH, prolactin, ACTH and cortisol response to hexarelin, a synthetic hexapeptide, undergo different age-related variations. Eur J Endocrinol 137 (6): 635-42.

ARVAT E, MACCAGNO B, RAMUNNI J, GIANOTTI L, DI VITO L, DEGHENGHI R, CAMANNI F, GHIGO E (1997c). Effects of histaminergic antagonists on the GH-releasing activity of GHRH or hexarelin, a synthetic hexapeptide, in man. J Endocrinol Invest 20: 122-127.

BARLIER A, ZAMORA AJ, GRINO M, GUNZ G, PELLEGRINI-BOUILLER I, MORANGE-RAMOS I, FIGARELLA-BRANGER D, DUFOUR H, JAQUET P, ENJALBERT A (1999). Expression of functional growth hormone secretagogue receptors in human pituitary adenomas: polimerase chain reaction, triple in-situ hybridization and cell culture studies. J Neuroendocrinol 11(7): 491-502.

BLAKE AD, SMITH RG (1991). Desensitization studies used perifused rat pituitary cells show that Growth hormone-releasing hormone and His-D-Trp-D-Phe-Lys-NH2 stimulate GH release through different receptor sites. J Endocrinol 129: 11-19.

BOWERS CY (1993). GH releasing peptides Structure and kinetics. J Pediatr Endocrinol 6: 21-31.

CARMIGNAC DF, BENNET PA, ROBINSON ICAF (1998). Effects of growth hormone secretagogues on prolactin release in anesthetized dwarf (dw/dw) rats. Endocrinology 139: 3590-96.

CICCARELLI E, GROTTOLI S, RAZZORE P, GIANOTTI L, ARVAT E, DEGHENGHI R, CAMANNI G, GHIGO E (1996). Hexarelin, a synthetic growth hormone releasing peptide, stimulate prolactin secretion in acromegalic but not in hyperprolactinaemic patients. Clin Endocrinol (Oxf) 44 (1): 67-71.

CODD EE, SHU AYL, WALKER RF (1989). Binding of growth hormone releasing hexapeptide to specific hypothalamic and pituitary binding sites. Neuropharmacol 28: 1139-44.

COPINSCHI G, VAN ONDERBERGEN A, L'HERMITE-BALERIAUX M, MANDEL CM, CAUFRIEZ A, LEPROULT R, BOLOGNESE JA, DE SMET M, THORNER MO, VAN CAUTER E (1996). Effects of 7-day treatment with a novel, orally active, growth hormone

(GH) secretagogue, MK-677, on 24-hour GH profiles, insulin-like growth factor I and adrenocortical function in normal young men. J Clin Endocrinol Metab 81: 2776-82.

CHOMCZYNSKI P, SACHI N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochem 162: 156-159.

DE PAUL AL, PONS P, AOKI A, TORRES AI (1997). Different behavior of lactotroph cells in response to Angiotensin II and Thyrotrophyn-releasing hormone. Cell Mol Neurobiol. 17 (2): 245-258.

FOURNEY RM, MIYAKOSHI J, DAY III RS, PATTERSON MC (1988). Northern Blotting: efficient RNA staining and transfer. Focus 10: 5-7.

GHIGO E, ARVAT E, GIANOTTI L, IMBIMBO BP, LENEARTS V, DEGHENGHI R, CAMANNI F (1994). Growth hormone-releasing activity of Hexarelin, a new synthetic hexapeptide, after intravenous, subcutaneous, intranasal and oral administration in man. J Clin Endocrinol Metab 78: 693-98.

GHIGO E, ARVAT E, RAMUNNI J, COLAO A, GIANOTTI L, DEGHENGHI R, LOMBARDI G, CAMANNI F (1997). Adrenocorticotropin- and cortisol-releasing effect of hexarelin, a synthetic growth hormone-releasing peptide, in normal subjets and patients with Cushing's syndrome. J Clin Endocrinol Metab 82: 2439-2444.

HICKEY GJ, DRISKO J, FAIDLEY T, CHANG C, ANDERSON LL, NICOLICH S, MCGUIRE L, RICKES E, KRUPA D, FEENEY W, FRISCINO B, CUNNINGHAM P, FRAZIER E, CHEN H, LAROQUE P, SMITH RG (1996). Mediation by the central nervous system is critical to the in vivo activity of the GH secretagogue L-692,585. J Endocrinology 148: 371-380.

HOWARD AD, FEIGHNER SD, CULLY DF, ARENA JP, LIBERATOR PA, ROSENBLUM CI, HAMELIN M, HRENIUK DL, PALYHA OC, ANDERSON J, PARESS PS, DIAZ C, CHOU M, LIU KK, MCKEE KK, PONG SS, CHAUNG LY, ELBRECHT A, DASHKEVICZ M, HEAVENS R, RIGBY M, SIRINATHSINGHJI DJ, DEAN DC, MELILLO DG, VAN DER PLOEG LH (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273: 974-77.

IMBIMBO BP, MANT T, EDWARD M, AMIN D, FROUD A, LENAERTS U, BOUTIGNON F, DEGHENGHI R (1994). Growth hormone releasing activity of hexarelin in human: A dose-response study. Eur J Clin Pharmacol 46: 421-25.

JACKS T, SMITH R, JUDITH F, SCHLEIM K, FRAZIER E, CHEN H, KRUPA D, HORA DJ, NARGUND R, PATCHETT A, HICKEY G (1996). MK-0677, a potent, novel, orally active growth hormone (GH) secretagogue: GH, insulin-like growth factor I, and other hormonal responses in beagles. Endocrinology 137: 5284-89.

JAFFE CA, HO PJ, DEMOTT-FRIBERG R, BOWERS CY, BARKAN AL (1993). Effects of a prolonged growth hormone (GH)-releasing peptide infusion on pulsatile GH secretion in normal men. J Clin Endocrinol Metab 77: 1641-47.

KOJIMA M, HOSODA H, DATE Y, NAKAZATO M, MATSUO H, KANGAWA K (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656-660.

KOJIMA M, HOSODA H, MATSUO H, KANGAWA K (2001). Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol & Metab 12 (3): 118-122.

KORBONITS M, JACOBS RA, AYLWIN SJ, BURRIN JM, DAHIA PL, MONSON JP, HONEGGER J, FAHLBUSH R, TRAINER PJ, CHEW SL, BESSER GM, GROSSMAN AB (1998). Expression of the growth hormone secretagogue receptor in pituitary adenomas and other neuroendocreine tumors. J Clin Endocrinol Metab 83(10): 3624-30.

KORBONITS M, KALTSAS G, PERRY LA, PUTIGNANO P, GROSSMAN AB, BASSER GM, TRAINER PJ (1999). The growth hormone secretoagogue hexarelin stimulates the hypothalamo-pituitary-adrenal axis via arginine vasopressin. J Clin Endocrinol Metab 84 (7): 2489-95.

KORBONITS M, TAINER PJ, BESSER GM (1995). The effect of an opiate antagonist on the hormonal changes induced by hexarelin. Clin Endocrinol (Oxf) 43 (3): 365-71.

LARON Z, BOWERS CY, HIRSCH D, ALMONTE AS, PELZ N, KERET R, GIL-AI (1993). Growth hormone-releasing activity of growth hormone-releasing peptide-1 (a synthetic heptapeptide) in children and adolescents. Acta Endocrinol 129: 424-29.

LOCATELLI V, TORSELLO A (1997). Growth Hormone secretagogues: focus on the Growth Hormone-releasing peptides. Pharmacol Res 36: 414-423.

MALDONADO CA, AOKI A (1986). Influence of embedding media in prolactin labeling with immunogold techniques. Histochem J 18: 429-433.

MALDONADO CA, AOKI A (1994). Occurrence of Atypical Lactotrophs associated with levels or prolactin secretpry activity. Biocell 18(3): 83-95.

MASSOUD AF, HINDMARSH PC, BROOK CG (1996). Hexarelin-induced growth hormone, cortisol and prolactin release: a dose-response study. J Clin Endocrinol Metab 81 (12): 4338-41.

MICIC D, CASABIELL X, GUALILLO O, POMBO M, DIEGUEZ C, CASANUEVA FF (1999). Growth hormone secretagogues: the clinical future. Horm Res 51 (suppl 3): 29-33.

MC KEE KK, PALYHA OC, FEIGHNER SD, HRENIUK DL, TAN CP, PHILLIPS MS, SMITH RG, VAN DER PLOEG LH, HOWARD AD (1997). Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. Mol Endocrinol 11: 415-423.

MOMANY FA, BOWERS CY, REYNOLDS GA, CHANG D, HONG A, NEWLANDER K (1981). Design, synthesis and biological activity of peptides which release growth hormone in vitro. Endocrinology 108: 31-39.

MUCCIOLI G, BROGLIO F, VALETTO MR, GHE C, CATAPANO F, GRAZIANI A, PAPOTTI M, BISI G, DEGHENGHI R, GHIGO E (2000). Growth hormone-releasing peptides and the cardiovascular system. Ann Endocrinol (Paris) 61 (1): 27-31.

MULLER EE, NISTICO G (1989). Brain Messenger and the Pituitary. Muller EE and Nistico' G Eds. Academic Press, San Diego.

NISWENDER GD, CHEN CL, MIDGLEY AR, MEITES J, ELLIS S (1969). Radioimmunoassay for rat prolactin. Proc Soc Exptl. Biol Med 130: 793-797.

PASOLLI HA, TORRES AI, AOKI A (1994). The mammosomatotroph: a transitional cell between growth hormone and prolactin producing cells? An immunocytochemical study. Histochemistry 102(4): 287-96.

PELLIZAS CG, COLEONI AH, COSTAMAGNA ME, DI FULVIO M, MASINI-REPISO AM (1998). Insulin-like growth factor I reduces thyroid hormone receptors in rat liver. Evidence for a feed-back loop regulating the peripheral thyroid hormone action. J Endocrinol 158: 87-95.

PONG SS, CHAUNG LYP, DEAN DC, NARGUNT RP, PATCHETT AA, SMITH RG (1996). Identification of a new G-protein-linked receptor for growth hormone secretagogues. Mol Endocrinol 10: 57-61.

RAHIM A, O'NEILL PA, SHALET SM (1999). The effect of chronic hexarelin administration on the pituitary-adrenal axis and prolactin. Clin Endocrinol (Oxf) 50: 77-84.

SHINKAI T, OOKA H (1995). Effect of angiotensin II on the proliferation of mammotrophs from the adult rat anterior pituitary in culture. Peptides 16 (1): 25-29.

SMITH RG, PONG SS, HICKEY G, JACKS T, CHENG K, LEONARD R, COHEN CJ, ARENA JP, CHANG CH, DRISKO J, WYVRATT M, FISHER M, NARGUND R, PATCHETT A (1996). Modulation of pulsatile GH release trough a novel receptor in hypothalamus and pituitary gland. Recent Prog Horm Res 51: 261-85.

VELKENIERS B, HOOGE-PETERS EL, HOOGHE R, BELAYEW A, SMETS G, CLAEYS A, ROBBERECHT P, VANHAELST L (1988). Prolactin subpopulations separated on discontinuous Percoll gradient: an immunocytochemical, biochemical and physiological characterization. Endocrinol 123: 1619-1630.