

The Effect of GHRH, GHRP-2 and Somatostatin on GH Secretion by fetal pituitary*

LIU Qin (刘 勤), BAI Xiangjun(白祥军)*, LIU Kui (刘 睽)*,
LIN Wen (林 雯), LEI Ting (雷 雯)*

Department of Pediatrics, Xiehe Hospital, Tongji Medical University, Wuhan 430022

* Department of Neurosurgery, Tongji Hospital, Tongji Medical University, Wuhan 430030

Summary: Growth hormone releasing peptide (GHRP-2) is a synthetic hexapeptide which specifically stimulates secretion of growth hormone (GH) by fetal pituitary somatotrophs through a new membrane receptor, which is different from growth hormone releasing hormone (GHRH) and somatostatin (SMS) receptors. We used cell cultures of human fetal pituitary somatotroph cells to investigate the effect of GHRH, GHRP-2 and somatostatin on GH secretion. The results showed that the mechanism of GHRH/SMS and GHRP-2 was different. This indicated that a different intracellular signal transduction system might also play a crucial role in the regulation of GH secretion.

Key words: fetal pituitary; GH; GHRP-2; GHRH; somatostatin

Growth hormone release hormone (GHRH) and somatostatin (SMS) secreted by hypothalamus play a primary role in control of GH secretion. Growth hormone releasing-peptide (GHRP-2) is a synthetic hexapeptide, which specifically stimulates GH-secretion by human pituitary somatotrophs. Many evidences showed that GHRP-2 mediated its effects mainly via hydrolysis of membrane phosphatidylinositol (PI) and the subsequent activation of protein kinase C (PKC). In this study we investigated the effects of GHRH, GHRP-2 and SMS on regulation of GH secretion by fetal pituitary cells *in vitro*.

1 MATERIALS AND METHODS

1.1 Pituitary Cell Culture

Experiments were performed on 4 embryos from induction of labor by water bag. For cell culture, freshly resected tissues were washed immediately in phosphate-buffered saline (Oxoid, Bashingstoke, United Kingdom) containing 200 mg/L strep-

tomycin, 200 U/ml penicillin and 2.5 mg/ml Fungizone (Gibco-BRL, Freiburg, Germany) and cut into small pieces with scalpels. The tissue fragments were incubated at 37 °C with 1 mg/ml collagenase (Boehringer Mannheim, Germany) in an orbital incubator shaker for 30 min. The dispersed cells were washed and resuspended in Minimum Essential Medium containing 10 % fetal calf serum (FCS), nonessential amino acids, 20 mmol/L HEPES, 0.75 % NaHCO₃, 100 mg/L streptomycin, and 100 U/ml penicillin (all ingredients from Gibco-BRL), hereafter referred to as culture medium (CM). For each embryo studied, equal aliquots of pituitary cells were distributed into 6—15 glass culture tubes and allowed to equilibrate during the next 18—24 h. After this period, the cells were washed with CM and further equilibrated at 37 °C for 1 h in fresh CM (2 ml). Experiment was then commenced on the cell cultures.

1.2 Modulation of GH Secretion

Equilibrated pituitary cell cultures were washed with modified CM (CM-SFCS), in which the 10 % FCS was replaced with 5 % charcoal-stripped FCS^[2]. Cells were then incubated in fresh basal CM-SFCS without additives (controls), CM-SFCS containing

LIU Qin, Female, born in 1962, Doctor in Charge

* This project was supported by the grants from Health Ministry (No. 96-2-14) and Educational Committee of China (No. 97-436).

1–100 nmol/L GHRP-2, and (or) 100 nmol/L GHRH, 100 nmol/L SMS (Sandoz) respectively. At least 3 cultures were used for each variable. After 4 h incubation at 37 °C, media were removed and stored at –20 °C until assayed for GH content by RIA technique. GH secretion by treated fetal pituitary cell cultures were expressed as a percentage of that achieved by the control cultures during the same period, and the later was standardized to 100 %. The data was processed statistically by *t* test.

2 RESULTS

As showed on fig. 1 and 2, GHRH and SMS exerted effects of stimulation and inhibition on GH secretion by human fetal pituitary respectively. GHRP-2 at dose of 100 nmol/L also significantly stimulated the GH secretion by 4 samples of fetal pituitary. In terms of interactions between GHRP-2 and GHRH, SMS, GHRP-2 can enhanced the effect of GHRH, while SMS not only inhibited the effect of GHRH, but also abolished the effect of GHRP-2 on GH secretion.

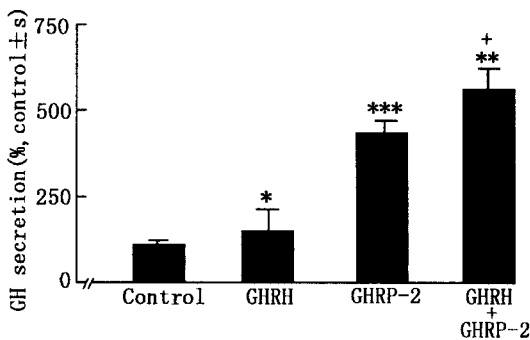


Fig. 1 The effect of GHRH (100 nmol/L) and GHRP-2 (100 nmol/L) on GH secretion by fetal pituitary cells
 * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ as compared with control group,
 + $P < 0.05$ as compared with GHRH

3 DISCUSSION

GHRH and SMS are able to control the GH secretion by pituitary cell by combining, separately, with the special membrane G-protein-coupled receptor and subsequent

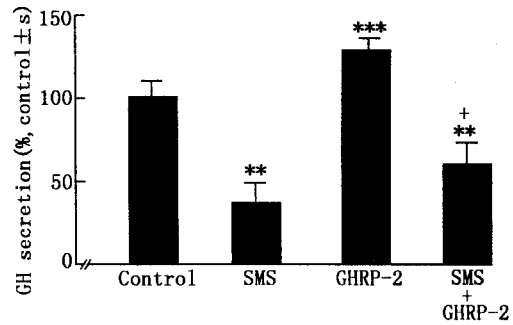


Fig. 2 The effect of somatostatin (100 nmol/L) and GHRP-2 (100 nmol/L) on GH secretion by human fetal pituitary cells
 ** $P < 0.01$ *** $P < 0.001$ as compared with control group, + $P < 0.05$ as compared with GHRP-2

activation of adenylyl cyclase-cAMP-PKA system. GHRP-2 is a synthetic peptide which specifically stimulates the GH secretion by pituitary cells mediated via the special receptor *in vitro* and *in vivo*^[1, 2]. It is one of the growth hormone secretagogues including GH-releasing peptide and nonpeptide. The previous studies showed that GHRP-2 was dependent on PKC to stimulate the GH secretion mediated via hydrolysis of membrane phosphatidylinositol (PI) into inositol - 1, 4, 5 - trisphosphate and diacylglycerol^[1-3, 5]. As second messengers, the former activates PKC directly and the latter mobilizes intracellular Ca^{2+} reserves. So it is referred to as GHSs-PI-PKC regulation system of intracellular signal transduction. Our study showed that GHRH and SMS were able to stimulate and inhibit GH secretion, respectively by fetal pituitary cells. GHRP-2 was also able to stimulate significantly GH secretion and exerted additive effect on GHRH. This result was consistent with that found in pituitary somatotrophinomas. It is inexplicable that the stimulatory effect of GHRP-2 on GH secretion could be abolished by SMS. Our results indicated that in fetal period the mechanism of regulation on GH secretion was different from that after birth, and the GH secretion by fetal pituitary is controlled mainly by GHRH/SMS - cAMP -PKA system. Further study about the possible cross-talk phenomena between above two systems is

needed^[1-7].

REFERENCES

- Howard A D, Feighner S D, Cully D F *et al.* A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science*, 1996,273:974
- Bertherat J. Cloning of the growth hormone secretagogues receptor cDNA: new evidence for a third endocrine pathway controlling growth hormone releasing. *Eur J Endocrinol*, 1997,136:37
- Lei T, Buchfelder M, Fahlbusch R *et al.* Growth hormone releasing peptide (GHRP-6) stimulates phosphatidylinositol (PI) turnover in human somatotroph cell. *J Mol Endocrinol*, 1995, 14: 135
- Lei T, Adams E F, Buchfelder M *et al.* The relationship between PKC and adenylyl cyclase activity in the regulation of growth hormone secretion by human pituitary somatotrophinomas. *Neurosurgery*, 1996,39:569
- Liu Q, Lei T, Adams E F *et al.* Relationship between GHRP-6 and TPA in the regulation of growth hormone secretion by human pituitary somatotrophinomas. *J Tongji Med Univ*, 1997,17:132
- Nishizuki Y. Intracellular signaling by hydrolysis of phospholipid and activation of protein kinase C. *Science*, 1992,258:607
- Adams E F, Lei T, Buchfelder M *et al.* PKC-dependent growth hormone releasing peptides stimulate cAMP production by human pituitary somatotrophinomas expressing gsp oncogenes: evidence for cross-talk between transduction pathways. *Mol Endocrinol*, 1996,10:432

(Received Oct. 19, 1998)

(Continued from page 252)

combination with CH50, the efficacy could be improved, and the dosage of angiogenesis inhibitor could be reduced.

After surgical or chemotherapeutic treatment, it is possible that the residual tumor will be suppressed by CH50 in combination with other drug(s), which will establish a basis and win enough time for the successful inducement of specific immune response against tumor cells. It is now generally accepted by clinicians and research workers that tumor must be treated by combined use of several elements. CH50 may serve as an important element in the combined treatment of tumor.

REFERENCES

- 张桂梅, 冯作化, 李 东等. 双功能结构域重组 FN 多肽表达质粒的构建及其表达产物的功能. *生物工程学报*, 1996,12(suppl):121
- 张桂梅, 冯作化, 张 慧等. 双功能域重组 FN 多肽抑制癌细胞浸润能力的研究. *同济医科大学学报*, 1998, 27:6
- 冯作化, 张桂梅, 张 慧等. 重组 FN 多肽 CH50 对巨噬细胞抗肿瘤功能的促进作用. *中国免疫学杂志*, 1998, 14:268
- 曹雪涛, 章卫平, 周正芳等. 白细胞介素 2 基因转移的肿瘤细胞克隆的建立及其体内外生长特性的研究. *中国免疫学杂志*, 1994, 10:284
- O'Relly M S, Holmgren L, Shing Y *et al.* Angiostatin: a novel angiogenesis inhibitor that medates the suppression of metastases by a Lewis lung carcinoma. *Cell*, 1994, 79: 315
- O'Relly M S, Boehm T, Shing Y *et al.* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell*, 1997; 88:277

(Received June 21, 1999)