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Insulin-Like Growth Factor-I and Its Receptor in Mouse Pituitary Glands

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ABSTRACT—Insulin-like growth factor-I (IGF-I) is produced in the liver and other peripheral tissues in response to growth hormone (GH) stimuli. IGF-I regulates diverse physiological functions in an autocrine and/or paracrine manner. IGF-I and IGF-I receptor (type-I receptor) are expressed in human and rat pituitary glands. However, the cell types of IGF-I-expressing cells and target cells of IGF-I in the pituitary glands are not known. The present study was aimed to identify the cell types of IGF-I-expressing cells and of its type-I receptor-expressing cells in mouse pituitary glands. In the mouse pituitary glands, IGF-I mRNA and IGF-I receptor mRNA were detected by reverse transcription-polymerase chain reaction (RT-PCR). IGF-I-expressing cells and its receptor-expressing cells were detected by non-radioisotopic *in situ* hybridization using mouse IGF-I cDNA and IGF-I receptor cDNA probes, and their cell types were immunocytochemically determined using antibodies raised against pituitary hormones. We found that somatotrophs expressed both IGF-I and IGF-I receptors, and some of corticotrophs expressed IGF-I receptors. Co-localization of IGF-I and GH in the same cultured pituitary cells was observed by dual-labelling immunocytochemistry. The present study demonstrated that pituitary IGF-I produced in somatotrophs regulated functions of somatotrophs and corticotrophs in an autocrine and/or paracrine manner.

INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a 70-amino acid polypeptide that regulates the proliferation and differentiation of various cells (Herington, 1991). IGF-I is mainly produced in the liver and circulating IGF-I acts as a mediator of actions of growth hormone (GH). However, a large number of reports showed that IGF-I was synthesized in multiple tissues and its synthesis was regulated by various factors besides GH (D'Ercole *et al.*, 1984). LeRoith *et al.* (1995) described that IGF-I receptors (IGF type-I receptors) were distributed in various tissues. Therefore, IGF-I is now considered to play diverse physiological roles not only in an endocrine manner, but also in an autocrine and/or paracrine manner.

There were several reports demonstrating that IGF-I was synthesized in human and rat pituitary glands (Ren *et al.*, 1994; Bach and Bondy, 1992) and its receptor was also detected in the pituitary glands (Goodyer *et al.*, 1984a; Rosenfeld *et al.*, 1984; Bach and Bondy, 1992). In the rat pituitary glands, IGF-I mRNA was detected in folliculo-stellate cell-like cells (Bach and Bondy, 1992). However, they did not immunocytochemi-

cally identify the cell types of IGF-I-synthesizing cells. Recently, in the rat pituitary glands IGF-I receptors were immunocytochemically detected in FSH-synthesizing gonadotrophs (Unger and Lange, 1997). This finding indicated that pituitary IGF-I was involved in gonadotropin secretion. As another physiological role of IGF-I, IGF-I inhibits GH expression and secretion (Berelowitz *et al.*, 1981; Goodyer *et al.*, 1984b; Yamashita and Melmed, 1986; Melmed and Yamashita, 1986; Morita *et al.*, 1987; Fletcher *et al.*, 1995). A rat pituitary tumor cell line, GH₃ cells, expresses IGF-I (Fagin *et al.*, 1987) and IGF-I receptors (Rosenfeld *et al.*, 1985; Yamasaki *et al.*, 1991), and IGF-I production is regulated by GH (Fagin *et al.*, 1989). However, IGF-I receptors have not been reported in normal somatotrophs as far as we know. The present study was planned to clarify the cell types of IGF-I-synthesizing cells and IGF-I receptor-expressing cells in the mouse pituitary glands to investigate physiological roles of pituitary IGF-I. IGF-I mRNA and IGF-I receptor mRNA were determined by non-radioisotopic *in situ* hybridization and pituitary hormones were immunocytochemically determined.

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MATERIALS AND METHODS

Animals

Two month-old female mice of ICR strain (CLEA Japan, Inc., Osaka, Japan) were used. They were kept in a temperature-controlled animal room, and given a commercial diet CE-7 (CLEA Japan, Inc.) and tap water *ad libitum*. All animal care and experiments were performed in accordance with the Guidelines of Animal Experimentation, Faculty of Science, Okayama University, Japan.

Cell culture

Anterior pituitary cells Mouse pituitary cells were isolated following the method of Oomizu and Takahashi (1996). Briefly, anterior pituitary glands were cut into small pieces with a sterile razor blade. These small pieces were digested with trypsin (Type III, 10,400 IU/mg, 0.5%, w/v, Sigma, St. Louis, MO, USA) in Hanks' solution containing 20 mM HEPES (HSH)-0.3% bovine serum albumin (BSA, fraction V, Sigma) at 37°C for 15 min and treated with DNase I (Sigma) at 37°C for 1 min. Enzymatic dissociation of pituitary cells was stopped with soybean trypsin inhibitor (0.1%, w/v, Sigma) at 37°C for 15 min. The fragments were washed in Ca^{2+} - Mg^{2+} -free (CMF) HSH-0.3% BSA, and incubated with 2 mM and 1 mM EDTA CMF HSH-0.3% BSA at 37°C for 5 and 15 min, respectively. The pituitary cells were dispersed by pipetting. After gentle pipetting, the dispersed cells were collected by centrifugation. Cell yield was calculated by counting the cells with a haemocytometer, and cell viability was checked using a trypan blue exclusion test (usually more than 95%). The dispersed pituitary cells were suspended in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium without phenol red (DME/F-12 medium, Sigma) containing heat-inactivated fetal bovine serum (10%, v/v). They were seeded on poly-L-lysine (Sigma)-coated glass coverslips (diameter 13 mm, Matsunami Glass Ind., Osaka, Japan) in 24-well tissue culture plates (Becton Dickinson, Lincoln Park, NJ, USA) at a density of 5×10^5 cells/ml/well. These cells were cultured in DME/F-12 medium containing heat-inactivated fetal bovine serum (10%, v/v) at 37°C for 5 days in a humidified atmosphere of 5% CO_2 and 95% air.

Rat pituitary tumor GH₃ cells GH₃ cells, obtained from the Japanese Collection of Research Bioresources (JCRB), National Institute of Health Sciences (NIHS, Tokyo, Japan), were cultured with Ham's F-10 medium (Sigma) containing heat-inactivated fetal bovine serum (2.5%, v/v) and heat-inactivated horse serum (15%, v/v) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was prepared from livers, pituitary glands and GH₃ cells using the method of Chomczynski and Sacchi (1987). Two hundred fifty ng of total RNAs were subjected to RT-PCR using a Gene Amp EZ *rTth* RNA PCR kit (PERKIN ELMER, Forster City, CA, USA) and a thermal cycler (GeneAmp PCR System 9600, PERKIN ELMER). Oligonucleotide primers for IGF-I and IGF-I receptor were based upon the report by Ho *et al.* (1995). The sequences of primers are as follows: IGF-I 5' sense primer; 5'-GGACCAGAGACCCTTTGCGGGG-3', IGF-I 3' antisense primer: 5'-GGCTGCTTTTGTAGGCTTCAGTGG-3', IGF-I receptor 5' sense primer; 5'-ACTGACCTCATGCGCATGTGCTGG-3', IGF-I receptor 3' antisense primer; 5'-CTCGTTCTTGCGGCC-CCCGTTCAT-3'. These primers generated PCR products of 210 and 345 bp for IGF-I and IGF-I receptor, respectively. Upstream and downstream primers of mouse IGF-I are 87.0% and 100% homology compared with rat IGF-I mRNA, respectively. Both upstream and downstream primers of mouse IGF-I receptor are 83.3% homology compared with rat IGF-I receptor mRNA. Messenger RNA of β -actin was detected by RT-PCR as an internal control. Upstream and downstream primers of mouse β -actin were constructed based on a sequence of mouse β -actin cDNA and their sequences are as follows: 5' sense primer; 5'-CCAACCGTGAAAAGATGACCCAGA-3', 3' antisense

primer; 5'-GTACTCCTGCTTGCTGATCCACAT-3'. The set of these primers generated 804-bp PCR products. Upstream and downstream primers of mouse β -actin share 83.3% and 100% identity with rat β -actin mRNA. All primers were synthesized by the Gibco BRL Custom Primers, Life Technologies Asia Pacific (Yokohama, Japan).

RT was performed at 70°C for 15 min and PCR was performed with a following cycle profile: 35 cycles of reactions including denaturation at 95°C for 10 sec and extension at 60°C for 1 min. RT-PCR products were electrophoresed on 2% (w/v) agarose gel, stained with ethidium bromide, and photographed under ultraviolet illumination, and then compared with a known standard (100 bp DNA Ladder, Gibco BRL, Gaithersburg, MD, USA) for size determination.

Southern blot analysis

Southern blot analysis was performed to verify that the bands obtained by PCR were generated from amplification of IGF-I and IGF-I receptor cDNAs. The PCR products were transferred onto Hybond N⁺ (Amersham, Buckinghamshire, UK) by a vacuum blotter. Southern blot analysis was conducted by ECL random prime labelling and detection systems (Amersham) according to the manufacturer's instructions. Mouse IGF-I cDNA (pmigf1-2) obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and IGF-I receptor cDNA subcloned in our laboratory as described below were used as probes.

The cDNA clone for the mouse IGF-I (pmigf1-2) was 720 bp in length. The IGF-I cDNA was 93.5% homology compared with rat IGF-I messenger RNA. DNA fragments encoding a part of the mouse IGF-I receptor were obtained from total RNA prepared from the uteri of ICR mice by RT-PCR using the primers described above. The cDNA fragment was subcloned into pGEM3Zf(+) and subjected to sequencing. Dideoxynucleotide sequencing was performed using fluorescent primers and an automated DNA sequencer. One clone, designated as pcrlIGF-IR, was 354 bp in length and encoded 118 amino acid protein, sharing 93.0% and 87.1% identity in nucleic sequence and sharing 92.6% and 88.1% identity in amino acid sequence with the rat or human IGF-I receptor, respectively. We concluded that pcrlIGF-IR was a cDNA encoding a part of the mouse IGF-I receptor. The nucleotide sequence of pcrlIGF-IR will appear in the DDBJ, EMBL and GenBank nucleotide sequence data bases with the following accession number AB006442. The products generated by RT-PCR using mouse β -actin primers were also verified by Southern blot analysis using mouse β -actin cDNA probe (Ambion, Austin, TX, USA). This probe has 72.2% homology compared with rat β -actin mRNA.

In situ hybridization

Pituitary glands obtained from 7 female mice were immediately fixed with 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.6) at 4°C overnight. They were processed for paraffin embedding. The tissues were sectioned at 5- μ m thickness. The sections were digested with 10 mg/ml proteinase K (Merck, Darmstadt, Germany) at 37°C for 30 min and reaction of proteinase K was stopped with 0.2% (w/v) glycine in 0.01 M PBS. They were postfixated with 4% paraformaldehyde in 0.01 M PBS, followed by an acetylation treatment.

Preparation and detection of cDNA probes were performed using a digoxigenin (DIG) DNA labelling and detection kit (Boehringer Mannheim, Germany). Mouse IGF-I cDNA (pmigf1-2) and human IGF-I receptor cDNA (pIGF-I-R.8), obtained from ATCC, were labelled with DIG-11-UTP by random-primer method at 37°C for 20 hr. pBR328 plasmids were labelled and used as a negative control for *in situ* hybridization. After the random-labelling reaction, DIG-labelled DNA probes were purified as follows. The 20 μ l labelling mixture was added to 2 μ l of 5 M LiCl, 3 μ l of 10 mg/ml herring sperm DNA and 75 μ l of prechilled ethanol, and placed at -80°C for at least 30 min, followed by a centrifugation ($11,000 \times g$, 4°C, 20 min) to precipitate only the DIG-labelled DNA probes.

The pituitary sections were placed in a moist chamber and hy-

bridized at 42°C overnight in a solution containing 5 × SSPE, 1 × Denhardt's solution, 10% (w/v) sodium dextran sulfate, 50% (v/v) deionized formamide, 120 µg/ml denatured herring sperm DNA and 0.3 ng/µl DIG-labelled probe. The slides were washed in 1 × SSC at room temperature for 10 min, then in 1 × SSC at 45°C for 15 min. The final wash was carried out in 0.5 × SSC at 45°C for 15 min. Non-specific binding was blocked with 0.5% (w/v) BSA in TN buffer (0.1 M Tris-HCl, 0.3 M NaCl, pH 7.5) for 30 min. Anti-DIG-alkaline phosphatase conjugate (1:2000) was applied to the sections at room temperature for 1 hr. The slides were washed with TN buffer containing 0.2% (v/v) Tween 20 for 10 min 3 times. Hybridization signal was visualized using Nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) as chromogens. Colour development was performed for 20 hr.

Immunocytochemistry

After the detection of *in situ* hybridization, the sections were immunocytochemically stained with antibodies raised against anterior pituitary hormones: antibodies to rat GH (1:2000; Takahashi, 1992), mouse PRL (1:4000; mP-001P, Shikibo, Kusatsu, Japan), pig ACTH (1:4000; ADVANCE, Tokyo, Japan), rat TSHβ (1:2000; NIDDK-anti-rBetaTSH-IC-1, NIDDK, Bethesda, MD, USA), rat LHβ (1:1000; NIDDK-anti-rBetaLH-IC-2, NIDDK) and rat FSHβ (1:1000; NIDDK-anti-rBetaFSH-IC-1, NIDDK). Immunostaining was abolished by omission of each primary antibody or by the use of primary antibodies (working dilution, 100 µl) preabsorbed with 10 µg of each highly purified pituitary hormone (rat GH, rat PRL, rat ACTH, rat TSH, rat LH, rat FSH, respectively) at 4°C for 24 hr. The specificity of antibodies to GH and PRL were also checked with the immunoblotting assay (Takahashi, 1992). The pituitary cells were incubated with one of antibodies to GH, PRL, ACTH and TSHβ at room temperature for 2 hr or one of antibodies to LHβ and FSHβ at 4°C for 48 hr. The primary antibodies were localized with fluorescein-labelled antibodies.

For double-labelling immunocytochemistry of IGF-I and GH, anterior pituitary cells cultured on glass coverslips were fixed in Bouin's solution at room temperature for 2 hr. Somatotrophs were stained using monkey antibodies raised against mouse GH (#35, NIDDK). The glass coverslips were incubated with antibodies to mouse GH (1:2000) at room temperature for 1 hr. Detection was carried out using Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA, USA) and 3,3'-diaminobenzidine tetrahydrochloride (DAB) as a chromogen. Immunocytochemical detection of IGF-I was performed using rabbit antibodies raised against human IGF-I (UB286, NIDDK) at a dilution of 1:250. The glass coverslips that had already been immunostained with antibodies to mouse GH were incubated with the antibodies to human IGF-I at room temperature for 2 hr. After washing with PBS, they were incubated with secondary FITC-labelled antibodies raised against rabbit immunoglobulin G. To ascertain the validity of the dual-labelling immunocytochemistry, control study was performed by omitting either the antibody to mouse GH or the antibody to human IGF-I. In either case, immunostaining of IGF-I or GH was not altered.

RESULTS

RT-PCR analysis of IGF-I and its receptor

RT-PCR was performed to determine whether IGF-I mRNA and IGF-I receptor mRNA were expressed in mouse livers, pituitary glands and GH₃ cells. We observed single bands in ethidium bromide-stained gels for those amplification (Fig. 1A). The 210-bp and 345-bp products corresponded to amplified cDNAs of IGF-I and IGF-I receptors, judging the size of the products, respectively. RT-PCR using the total RNA from GH₃ cells showed that IGF-I and IGF-I receptor cDNAs were amplified with a set of primers prepared for mouse IGF-

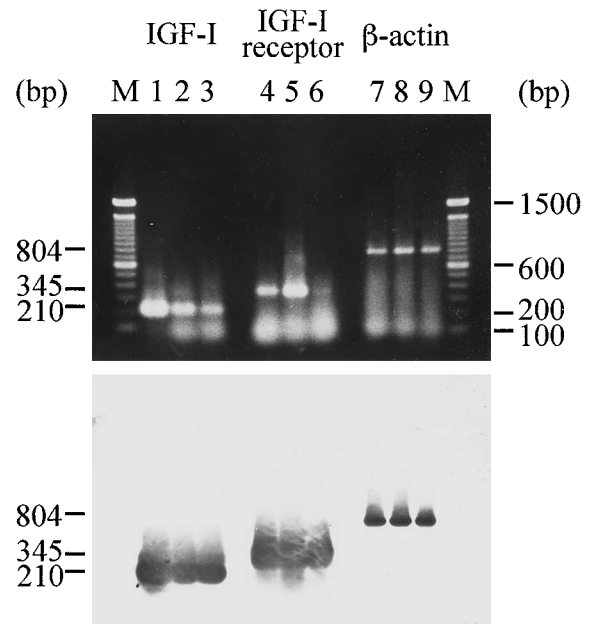


Fig. 1. Identification of IGF-I and IGF-I receptor mRNAs in mouse livers, pituitary glands and GH₃ cells. **(A)** RT-PCR was performed using 250 ng total RNAs derived from mouse livers (lane 1, 4 and 7), pituitary glands (lane 2, 5 and 8) and GH₃ cells (lane 3, 6 and 9). RT-PCR products of IGF-I mRNA (lane 1-3), IGF-I receptor (lane 4-6) and β-actin (lane 7-9) were electrophoresed and stained with ethidium bromide. **(B)** Southern blot analysis of RT-PCR products was carried out using probes of mouse IGF-I cDNA (lane 1-3), IGF-I receptor cDNA (lane 4-6) and β-actin cDNA (lane 7-9). All products of RT-PCR were hybridized with each probe.

I and its type-I receptor. In PCR reaction without RT reaction, no PCR products were found (data not shown). Southern blot analysis using mouse IGF-I cDNA and IGF-I receptor cDNA probes confirmed that bands of RT-PCR products corresponded to IGF-I mRNA and IGF-I receptor mRNA (Fig. 1B). These results indicate that IGF-I mRNA and its receptor mRNA were expressed in mouse livers, pituitary glands and GH₃ cells. β-actin as internal control was also detected by RT-PCR and Southern blot analysis.

In situ hybridization

IGF-I mRNA and IGF-I receptor mRNA in mouse pituitary glands were studied by non-radioisotopic *in situ* hybridization. IGF-I and IGF-I receptor mRNA were expressed in the anterior lobe of pituitary glands (Fig. 2A, B). As control experiments of *in situ* hybridization, no signal was detected using DIG-labelled pBR328 as probes (Fig. 2C). The cells expressing IGF-I and IGF-I receptor mRNA were round or oval in shape, and occupied 33.5% of the total anterior pituitary cells in the adult female mice. These results show that IGF-I and IGF-I receptor mRNAs were expressed in mouse anterior pituitary glands.

Cell types of IGF-I and IGF-I receptor-expressing cells

Pituitary hormones produced in IGF-I mRNA- or IGF-I receptor mRNA-expressing cells were immunocytochemically

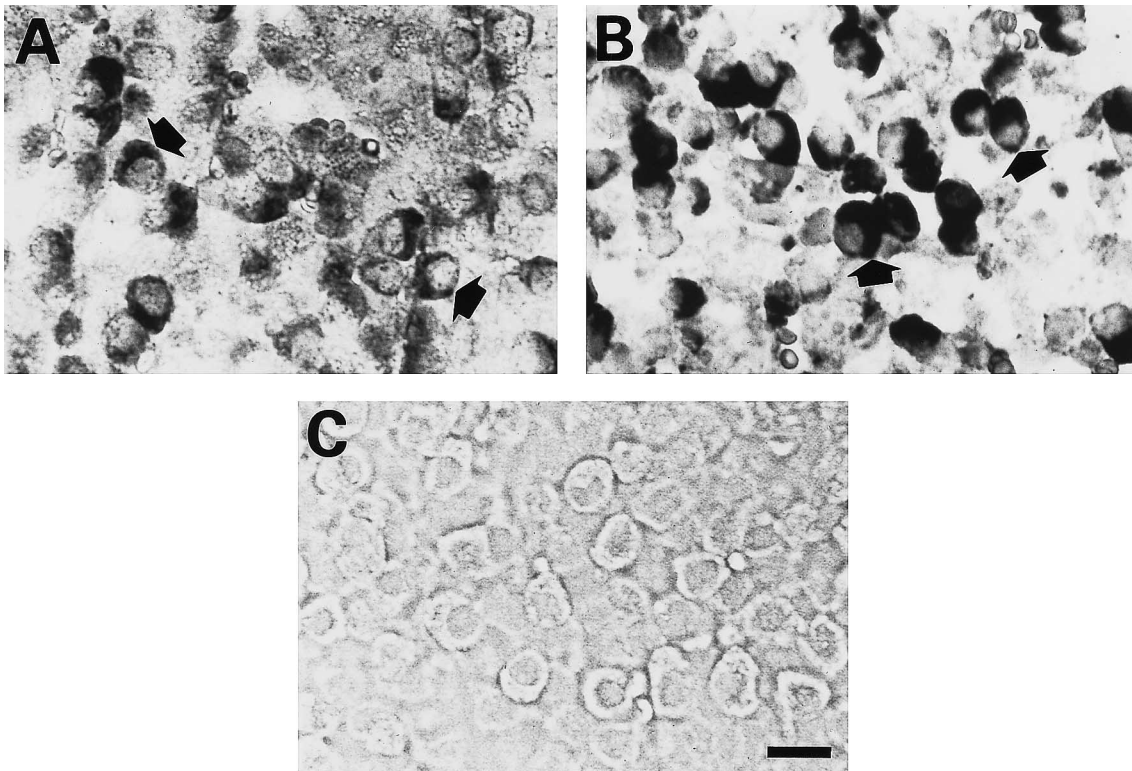


Fig. 2. Expression of IGF-I and IGF-I receptor mRNAs in mouse pituitary glands. *In situ* hybridization of IGF-I (A) or IGF-I receptor (B) in mouse pituitary glands. IGF-I mRNA was detected in anterior lobes of pituitary glands (arrow). IGF-I receptor mRNA was detected in anterior lobes of pituitary glands (arrow). *In situ* hybridization using pBR328 as probes (C) detected no signal. Bar = 20 μ m.

determined. IGF-I mRNA-expressing cells contained immunoreactive GH (Fig. 3A, B). IGF-I mRNA was not detected in corticotrophs, mammotrophs, thyrotrophs and gonadotrophs (data not shown).

IGF-I receptor mRNA-expressing cells contained immunoreactive GH (Fig. 3C, D). Some of corticotrophs (Fig. 3E, F, arrowhead) expressed IGF-I receptor mRNA, but most of corticotrophs did not show any IGF-I receptor mRNA signal (Fig. 3E, F). IGF-I receptor mRNA was not detected in mammotrophs (Fig. 3G, H), thyrotrophs and gonadotrophs (data not shown).

Immunoreactive IGF-I was detected in the cytoplasm of cultured somatotrophs which were immunocytochemically identified (Fig. 4A, B). When IGF-I mRNA was identified by *in situ* hybridization, the same cells contained immunoreactive GH (Fig. 3A, B). Therefore, these results clearly indicate that somatotrophs produced IGF-I.

DISCUSSION

We demonstrated using RT-PCR and *in situ* hybridization in the present study that the mouse anterior pituitary glands expressed IGF-I mRNA and that they also expressed IGF-I receptor mRNA. These findings are in an agreement with reports on the human and rat pituitary IGF-I (Bach and Bondy, 1992; Ren *et al.*, 1994; Ray and Melmed, 1997). The present results together with those previous reports indicate that pituitary

IGF-I is involved in regulation of pituitary functions. However, we could not eliminate the possibility that circulating IGF-I that is synthesized in the liver may regulate pituitary functions.

Simultaneous detection of IGF-I mRNA and pituitary hormones clearly showed that IGF-I mRNA was expressed in pituitary cells that contained immunoreactive GH. And immunoreactive IGF-I was clearly detected in somatotrophs of the cultured pituitary cells. These observations indicate that somatotrophs synthesize IGF-I. IGF-I receptor mRNA was also detected in somatotrophs and some of corticotrophs. Thus, IGF-I-expressing cells express IGF-I receptors, suggesting that IGF-I acts in an autocrine and/or paracrine manner to regulate pituitary functions. These are the first demonstration concerning colocalization of IGF-I and GH in the normal mouse pituitary secretory cells.

In human pituitary glands, IGF-I-expressing cells did not contain pituitary hormones (Ren *et al.*, 1994). Bach and Bondy (1992) found by *in situ* hybridization that rat anterior pituitary glands expressed IGF-I mRNA, but they did not clearly determine cell types of IGF-I mRNA-expressing cells. They claimed that IGF-I system was not selectively associated with somatotrophs, and that IGF-I might be expressed in folliculostellate cell-like cells, although direct evidence for these assumptions were not presented.

Unger and Lange (1997) recently demonstrated immunocytochemically that IGF-I receptor-expressing cells were

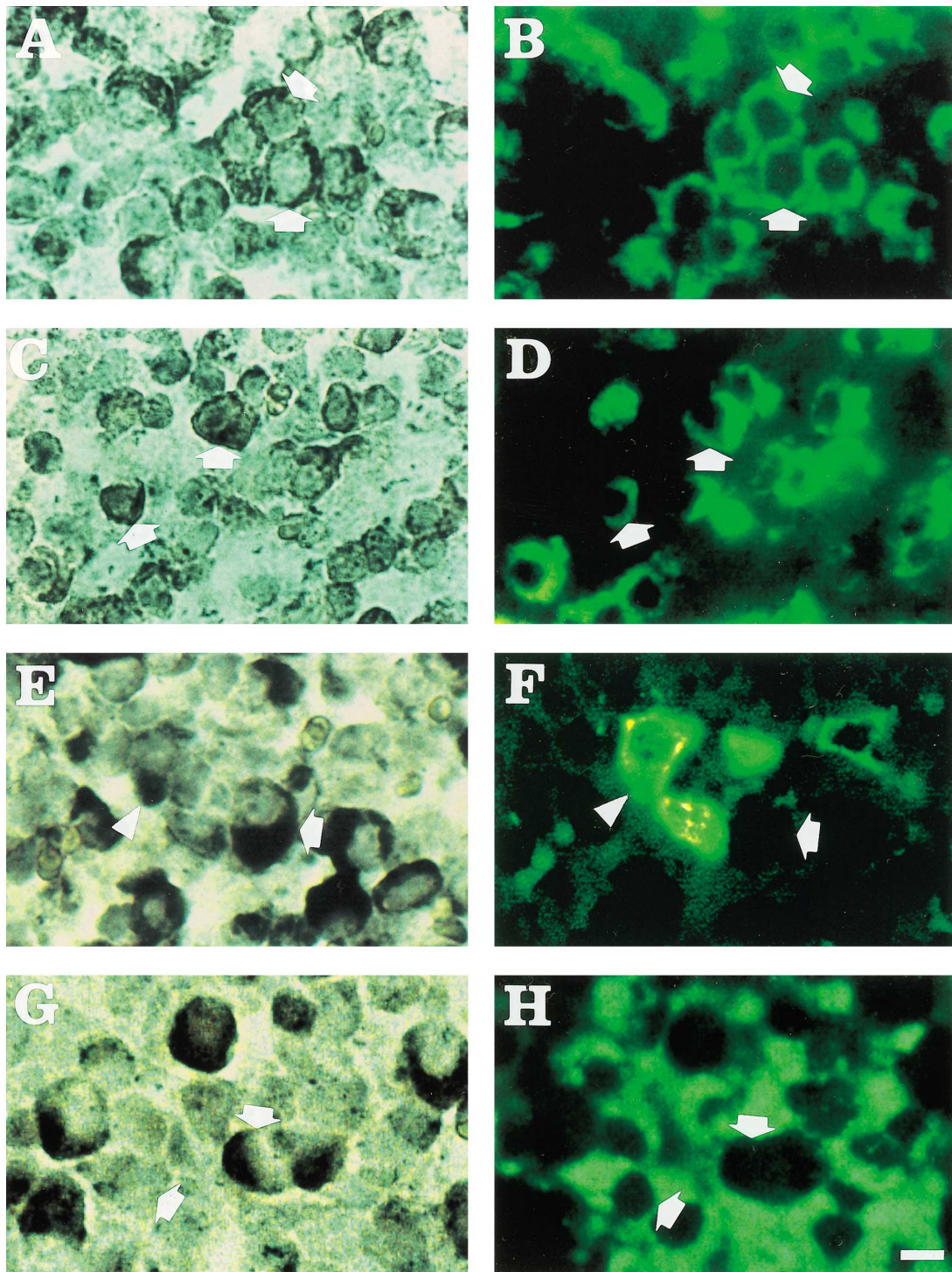


Fig. 3. Analysis of cell types of IGF-I and IGF-I-receptor expressing cells. *In situ* hybridization of IGF-I mRNA (A) and IGF-I receptor mRNA (C, E, G) and immunocytochemistry of GH (B, D), ACTH (F) and PRL (H) were simultaneously performed. A and B, C and D, E and F, G and H are the same sections, respectively. GH-immunoreactive cells (B) expressed IGF-I mRNA (A). GH-immunoreactive cells (D) expressed IGF-I receptor mRNA (C). Some ACTH-immunoreactive cells (F, arrowhead) expressed IGF-I receptor mRNA (E, arrowhead). However other ACTH-immunoreactive cells (F) did not express IGF-I receptor mRNA (E). PRL-immunoreactive cells (H) did not express IGF-I receptor mRNA (G). Bar = 10 μ m.

almost exclusively present in a postero-lateral portion of the male rat anterior pituitary glands. They reported that IGF-I receptors were almost exclusively present on FSH-secreting

gonadotrophs. On the other hand, we studied mRNA signals for IGF-I receptors with *in situ* hybridization, and could not find IGF-I receptor mRNA in LH- or FSH-secreting

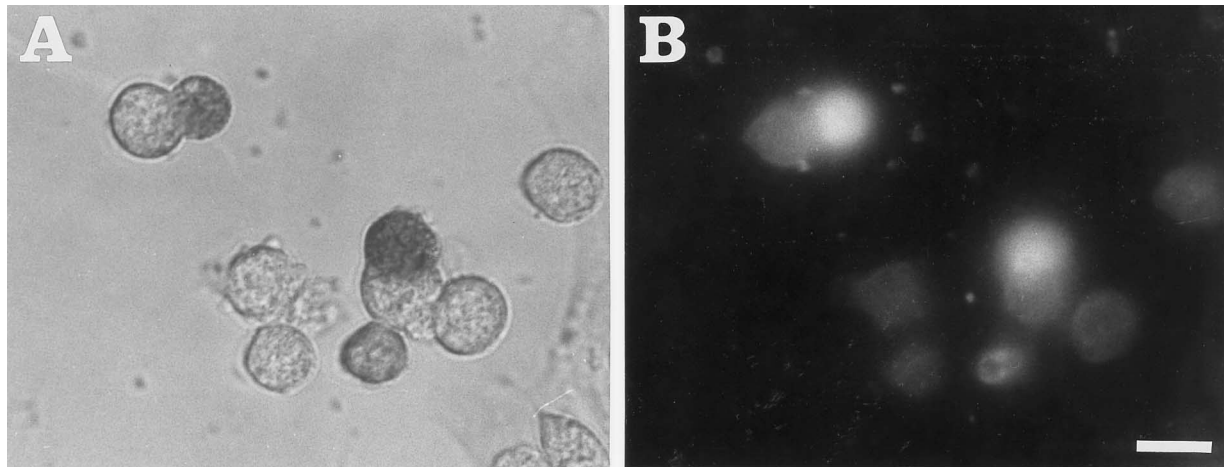


Fig. 4. Dual-labelling of GH (**A**) and IGF-I (**B**) in cultured cells of anterior pituitary glands. **A** and **B** are the same cells. GH-immunoreactive cells (**A**) stained with antibody of IGF-I (**B**). Bar = 10 μ m.

gonadotrophs. We used the female mice for the detection of IGF-I mRNA or IGF-I receptor mRNA. This discrepancy between the present study and their report may be due to the species difference or the sex difference in IGF-I receptor expression.

In hypothalamo-pituitary axis, IGF-I inhibits GH synthesis and secretion *in vivo* and *in vitro*. Intraventricular administration of somatomedin C (IGF-I) suppressed pulsatile GH release (Abe *et al.*, 1983; Tannenbaum *et al.*, 1983). IGF-I stimulated somatostatin secretion (Berelowitz *et al.*, 1981; Sato and Frohman, 1993; Becker *et al.*, 1995) and inhibited GHRH secretion (Sato and Frohman, 1993; Becker *et al.*, 1995) in the hypothalamus. In the pituitary level, IGF-I inhibits GH synthesis and secretion (Berelowitz *et al.*, 1981; Goodyer *et al.*, 1984b; Yamashita and Melmed, 1986; Melmed and Yamashita, 1986; Morita *et al.*, 1987; Fletcher *et al.*, 1995). The present study clearly indicated that IGF-I regulated GH secretion through IGF-I receptors expressed on the somatotrophs.

IGF-I expression was regulated by GH and triiodothyronine in GH₃ cells (Fagin *et al.*, 1989) and transplantation of GH₃ cells in rats induced the expression of pituitary IGF-I (Fagin *et al.*, 1988). Somatotrophs were reported to express GH receptors (Mertani *et al.*, 1994, 1996). These data showed that GH might stimulate the expression of pituitary IGF-I. IGF-I expression was also regulated by estrogen in the rat pituitary glands (Michels *et al.*, 1993). From these recent findings, pituitary IGF-I is considered to act as a mediator of GH, triiodothyronine and estrogen on pituitary cells in an autocrine and/or paracrine manner.

This study shows that parts of corticotrophs expressed IGF-I receptors. Thus, corticotrophs appear to consist of two types: IGF-I receptor-expressing cells, and the cells not expressing the receptor. IGF-I may regulate corticotrophic function and IGF-I secreted from somatotrophs may act as mediators for the interaction between corticotrophs and somatotrophs.

We recently demonstrated that IGF-I stimulated proliferation of corticotrophs and mammotrophs (Oomizu *et al.*, 1998). Therefore, the corticotrophic growth is directly regulated by IGF-I through IGF-I receptors of corticotrophs. IGF-I actions on mammotrophs may be mediated by other growth factors, since mammotrophs did not express IGF-I receptors. IGF-I may stimulate the synthesis of factors in somatotrophs and/or corticotrophs, for example, vasoactive intestinal peptide (VIP), and this factor may regulate proliferation of mammotrophs. IGF-I stimulated prolactin secretion (Lara *et al.*, 1994) and IGF-I-induced PRL secretion may be mediated by VIP. In another possibility, IGF-I may directly regulate mammotroph proliferation through IGF-I receptors that have been already synthesized, but the amount of IGF-I receptor mRNA is decreased to the level below the sensitivity of the present *in situ* hybridization.

In the conclusion, we have shown that IGF-I mRNA and its receptor mRNA were expressed in the mouse anterior pituitary glands, and have demonstrated that IGF-I was synthesized in somatotrophs, and its receptors were present in somatotrophs and parts of corticotrophs. Our results indicate that IGF-I which is synthesized in the pituitary glands regulates functions of somatotrophs and corticotrophs in an autocrine and/or paracrine manner, but do not exclude the possibility that circulating IGF-I may be involved in the control of pituitary functions.

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REFERENCES

- Abe H, Molitch ME, Van Wyk JJ, Underwood LE (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology* 113: 1319–1324
- Bach MA, Bondy CA (1992) Anatomy of the pituitary insulin-like growth factor system. *Endocrinology* 131: 2588–2594
- Becker K, Stegenga S, Conway S (1995) Role of insulin-like growth factor I in regulating growth hormone release and feedback in the male rat. *Neuroendocrinology* 61: 573–583
- Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both hypothalamus and pituitary. *Science* 212: 1279–1281
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159
- D'Ercole AJ, Stiles AD, Underwood LE (1984) Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci USA* 81: 935–939
- Fagin JA, Pixley S, Slanina S, Ong J, Melmed S (1987) Insulin-like growth factor I gene expression in GH₃ rat pituitary cells: messenger ribonucleic acid content, immunocytochemistry, and secretion. *Endocrinology* 120: 2037–2043
- Fagin JA, Brown A, Melmed S (1988) Regulation of pituitary insulin-like growth factor-I messenger ribonucleic acid levels in rats harboring somatomammotropic tumors: implications for growth hormone autoregulation. *Endocrinology* 122: 2204–2210
- Fagin JA, Fernandez-Mejia C, Melmed S (1989) Pituitary insulin-like growth factor-I gene expression: regulation by triiodothyronine and growth hormone. *Endocrinology* 125: 2385–2391
- Fletcher TP, Thomas GB, Dunshea FR, Moore LG, Clarke IJ (1995) IGF feedback effects on growth hormone secretion in ewes: evidence for action the pituitary but not the hypothalamic level. *J Endocrinol* 144: 323–331
- Goodyer CG, De Stéphanio L, Lai WH, Guyda HJ, Posner BI (1984a) Characterization of insulin-like growth factor receptors in rat anterior pituitary, hypothalamus and brain. *Endocrinology* 114: 1187–1195
- Goodyer CG, De Stéphanio L, Guyda HJ, Poner BI (1984b) Effects of insulin-like growth factors on adult male rat pituitary function in tissue culture. *Endocrinology* 115: 1568–1576
- Herington AC (1991) Insulin-like growth factors: biochemistry and physiology. *Baillière's Clin Endocrinol Metab* 5: 531–551
- Ho Y, Wigglesworth K, Eppig JJ, Schultz RM (1995) Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. *Mol Reprod Dev* 41: 232–238
- Lara JI, Lorenzo MJ, Cacicedo L, Tolón RM, Balsa JA, López-Fernández J, Sánchez-Franco F (1994) Induction of vasoactive intestinal peptide gene expression and prolactin secretion by insulin-like growth factor I in rat pituitary cells: evidence for an autocrine regulatory system. *Endocrinology* 135: 2526–2532
- LeRoith D, Werner H, Beitner-Johnson D, Roberts Jr CT (1995) Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr Rev* 16: 143–163
- Melmed S, Yamashita S (1986) Insulin-like growth factor-I action on hypothyroid rat pituitary cells: suppression of triiodothyronine-induced growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* 118: 1483–1490
- Mertani HC, Waters MJ, Jambou R, Gossard F, Morel G (1994) Growth hormone receptor binding protein in rat anterior pituitary. *Neuroendocrinology* 59: 483–494
- Mertani HC, Waters MJ, Morel G (1996) Cellular trafficking of exogenous growth hormone in dwarf rat pituitary. *Neuroendocrinology* 63: 257–268
- Michels KM, Lee W, Seltzer A, Saavedra JM, Bondy CA (1993) Up-regulation of pituitary [¹²⁵I] insulin-like growth factor-I (IGF-I) binding and IGF binding protein-2 and IGF-I gene expression by estrogen. *Endocrinology* 132: 23–29
- Morita S, Yamashita S, Melmed S (1987) Insulin-like growth factor I action on rat anterior pituitary cells: effects of intracellular messengers on growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* 121: 2000–2006
- Oomizu S, Takahashi S (1996) Insulin stimulates the proliferation of mouse anterior pituitary cells in vitro. *Biomed Res* 17: 365–371
- Oomizu S, Takeuchi S, Takahashi S (1998) Stimulatory effect of insulin-like growth factor I on proliferation of mouse pituitary cells in serum-free culture. *J Endocrinol* 157: 53–62
- Ray D, Melmed S (1997) Pituitary cytokine and growth factor expression and action. *Endocr Rev* 18: 206–228
- Ren P, Schelthauer BW, Halper J (1994) Immunohistological localization of TGF α , EGF, IGF-I, and TGF β in the normal human pituitary gland. *Endocr Pathol* 5: 40–48
- Rosenfeld RG, Ceda G, Wilson DM, Dollar LA, Hoffman AR (1984) Characterization of high affinity receptors for insulin-like growth factors I and II on rat anterior pituitary cells. *Endocrinology* 114: 1571–1575
- Rosenfeld RG, Ceda G, Cutler CW, Dollar LA, Hoffman AR (1985) Insulin and insulin-like growth factor (somatomedin) receptors on cloned rat pituitary tumor cells. *Endocrinology* 117: 2008–2016
- Sato M, Frohman A (1993) Differential effects of central and peripheral administration of growth hormone (GH) and insulin-like growth factor on hypothalamic GH-releasing hormone and somatostatin gene expression in GH-deficient dwarf rats. *Endocrinology* 133: 793–799
- Takahashi S (1992) Growth hormone secretion in old female rats analyzed by the reverse hemolytic plaque assay. *Acta Endocrinol* 127: 531–535
- Tannenbaum GS, Guyda HJ, Posner BI (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science* 220: 77–79
- Unger JW, Lange W (1997) Insulin receptors in the pituitary gland: morphological evidence for influence on opioid peptide-synthesizing cells. *Cell Tissue Res* 288: 471–483
- Yamasaki H, Prager D, Gebremedhin S, Moise L, Melmed S (1991) Binding and action of insulin-like growth factor I in pituitary tumor cells. *Endocrinology* 128: 857–862
- Yamashita S, Melmed S (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* 118: 176–182

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