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Expression of α, βA and βB subunits of inhibin or activin and follistatin in rat pancreatic islets

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We first detected the mRNA expression of follistatin and three subunits of inhibin/activin in rat pancreatic islets by reverse transcription-polymerase chain reaction (RT-PCR). Immunohistochemistry using anti-follistatin serum (against residues 123–134) revealed that follistatin was localized only in insulin-producing B cells. Although the βA subunit was detectable in the islets, the immunostainable cell types were completely different with two βA antisera, i.e. anti-βA (1–10)-Tyr stained B cells, while anti-βA (87–99) stained glucagon-producing A cells. This inconsistent immunoreactivity was probably related to follistatin binding to β subunits of inhibin/activin. This study indicates that follistatin and inhibin/activin in the islet serve as paracrine or autocrine modulators in the endocrine pancreas.

Inhibin; Activin; Follistatin; Islet of Langerhans; Rat pancreas

1. INTRODUCTION

Inhibin and activin which were originally purified from gonads and believed to modulate FSH secretion from the pituitary gland [1–4], have potent activities in diverse biological systems such as erythroid differentiation [5,6], mesoderm induction [7,8] and neural differentiation [9,10]. Follistatin, which binds to both inhibin and activin through the common β subunit [11,12], neutralizes activin action in various systems and is thought to be a functional regulator of inhibin/activin [11–14]. These molecules are broadly distributed in the nervous and endocrine systems [15,16]. Although activin stimulates insulin secretion from isolated pancreatic islets in vitro [17], there is no evidence that these molecules are localized in the gastro-entero-pancreatic (GEP) endocrine system. In the present study, we first detected the expression of α, βA, and βB subunits of inhibin/activin and follistatin mRNAs in rat pancreatic islets by reverse transcription polymerase chain reaction (RT-PCR). Furthermore, histochemical reaction to antisera raised against synthetic peptides confirmed the localization of inhibin/activin and follistatin in the islet of Langerhans.

2. MATERIALS AND METHODS

2.1. *Animals and treatment*

Female Wistar Imamichi rats, 200–250 g, were obtained from The Imamichi Institute for Animal Reproduction (Ibaraki, Japan). The animals were injected intravenously with a single dose of 65 mg/kg of streptozotocin (STZ; Wako Chemicals, Osaka, Japan) dissolved in 0.5 ml of 0.1 M citrate buffer, pH 4.5. At 24 h after STZ injection, pancreata were excised from intact or STZ-treated rats. The excised pancreata were fixed in Bouin's fixative by microwave treatment for 20 s at 4°C and immersed in the same fixative for 4–6 h at room temperature. Fixed tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin blocks. Sections were cut on a sliding microtome and then subjected to immunohistochemical procedures.

Pancreatic islets were isolated from another group of animals by collagenase treatment [18]. These islets were subjected to acid guanidium thiocyanate-phenol-chloroform (AGPC) RNA extraction [19]. RNA was stored at −20°C until used.

2.2. *Preparation of antisera*

Antisera against follistatin or βA subunit of inhibin or activin were made in rabbits using synthetic peptides as immunogens. Anti-follistatin antiserum was raised against human follistatin peptide (residues 123–134) as previously described [20,21]. Two antisera against the βA subunit of inhibin/activin were raised with two different synthetic peptides [22,23]. Anti-βA (1–10)-Tyr was raised against the N-terminal of βA subunits. The other antiserum, anti-βA (87–99) was against amino acid residues 87–99 of βA. Guinea pig anti-insulin serum and rabbit anti-glucagon serum were obtained from Linco Research Inc. (St. Louis, MO). Rabbit anti-somatostatin-14 serum was purchased from Cambridge Research Biochemicals Ltd. (Wilmington, DE).

2.3. *Immunohistochemical procedure*

The avidin-biotin-peroxidase (ABC) procedure was performed as described previously [22]. To detect the cross reactivities of anti-follistatin, anti-βA (1–10)-Tyr, and anti-βA (87–99) to insulin or glucagon molecules, primary antisera were preincubated with 5 mg/ml of insulin (Collaborative Research Inc., Bedford, MA) or 0.5 mg/ml of glucagon (Collaborative Research Inc., Bedford, MA) for 1 h at room temperature before applying them to the sections. A double-staining procedure was performed to identify the cell types which have follistatin-

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or activin-like immunoreactivities in the islet of Langerhans. After treatment with primary antisera (anti-follistatin, anti-βA (1–10)-Tyr, or anti-βA (87–99)), the sections were incubated with rhodamine-labeled goat anti-rabbit IgG for 30 min. The specimens were observed with an Olympus BHK-RFK photomicroscope equipped with epifluorescence optics. Then the sections were subjected to ABC procedures using anti-insulin, -glucagon, or -somatostatin antisera.

2.4. *RT-PCR and sequencing*

cDNA was synthesized from total RNA of isolated islets by Super-Script Preamplification System (BRL, Gaithersburg, MD). To identify follistatin and three subunits of inhibin/activin proteins, several oligonucleotide primers corresponding to the amino acid sequence of those protein molecules were subjected to RT-PCR using mRNA obtained from rat pancreatic islets. The oligonucleotide primers were prepared according to the amino acid sequences of rat follistatin [16] or α, βA and βB subunits of rat inhibin/activin [2] as follows, 5′-GGGCAGGATCCATTGGATTAGCCTAT-3′ and 5′-ACACTGC-TGGACAGTTTACCACTCT-3′ for follistatin, 5′-TTCATTTTCC-ACTACTGCCATGGTAGCT-3′ and 5′-GATACAAGCACAGTG-TTGTGTAATGAG-3′ for inhibin α subunit, 5′-TCAACAGTCAT-TAACCACTACCGCATGA-3′ and 5′-AGCCACACTCCTCCA-CAATCATGTT-3′ for inhibin/activin βA subunit, and 5′-AGGCAA-CAGTTCTTCATCGACTTTCGGCT-3′ and 5′-AGCCACACTCC-TCCACAATCATGTT-3′ for inhibin/activin βB subunit. The reaction was cycled 30–45 times with a cycle profile of 1 min at 94°C, 1 min at 55°C and 1.5 min at 72°C. Bands of corresponding size were cloned into pUC18 plasmid and sequenced by the *Taq* method

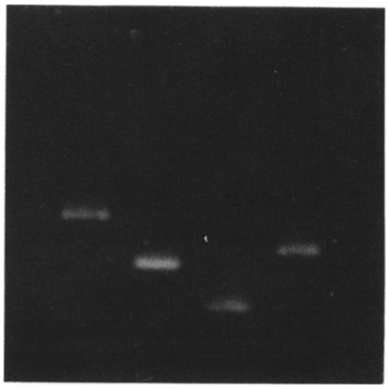

Fig. 1. Detection of follistatin and α, βA and βB subunits of inhibin/activin mRNA in islet of Langerhans by RT-PCR. The follistatin products (expected size 370 bp) were generated from isolated islets (lane 1). The corresponding length of α (240 bp), βA (165 bp) and βB (300 bp) cDNA products were cloned from isolated islets by RT-PCR (lanes 2–4). The cDNA sequence of each subunit and follistatin was identical to the corresponding region of rat inhibin, activin [2] and follistatin [14], respectively.

3. RESULTS

3.1. *Identification of α, βA and βB subunits of inhibin/activin and follistatin in islet of Langerhans*

Expression of follistatin and three subunits of inhibin/activin mRNAs were demonstrated in isolated pancreatic islets by RT-PCR. RT-PCR of islet RNA yielded a single band of cDNA corresponding to each protein fragment (Fig. 1). The cDNA sequence of each molecule was identical to the corresponding region of rat inhibin, activin and follistatin, respectively.

3.2. *Histochemical localization of immunoreactive βA subunit of inhibin/activin and follistatin in islet of Langerhans*

The immunohistochemical reaction of islet cells with anti-follistatin antiserum is shown in Fig. 2. Cells located widely in the islet of Langerhans exhibited intense immunoreaction with anti-follistatin (Fig. 2A). These cells were identified as insulin-producing pancreatic B cells by double-staining the same section with anti-insulin antiserum (Fig. 2A,B). The intense reaction of B cells with anti-follistatin could not be abolished by pread-

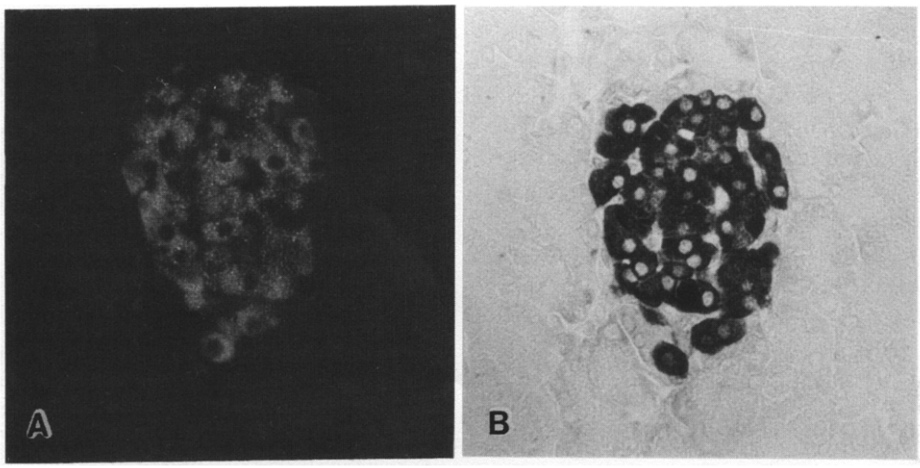

Fig. 2. Histochemical micrographs showing localization of immunoreactive follistatin in rat pancreatic islets. (A) Fluorescence micrograph indicating the cells, round in shape, which are located centrally in the islet exhibit intense immunoreactions with anti-follistatin. (B) The same section which is subjected to avidin-biotin-peroxidase stainig with anti-insulin antiserum showing the cells stained with anti-follistatin antiserum are identified with insulin-producing cells, pancreatic B cells.

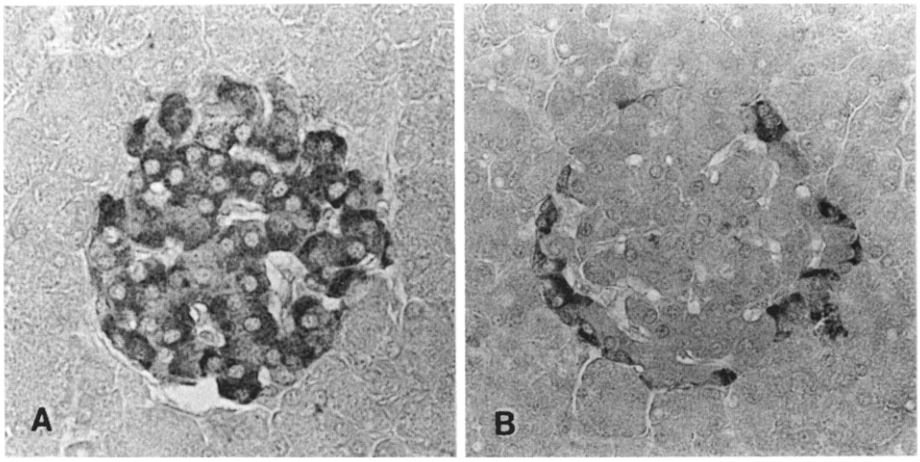

Fig. 3. Histochemical localization of βA subunit in rat islet cells using two different antibodies. (A) Pancreatic B cells were stained with anti-βA (1–10)-Tyr as well as the reactions with anti-follistatin, however, immunoreactions with anti-βA (87–99) (B) were found in pancreatic A cells in contrast to anti-βA (1–10)-Tyr reactions.

sorption of antiserum with 5 mg/ml of insulin, whereas anti-insulin reactions were completely eliminated. At 24 h after STZ treatment, the majority of islet cells were glucagon-producing A cells and somatostatin-producing D cells. Although immunoreactions with anti-insulin persisted in the few remaining B cells, follistatin-immunoreactivity completely disappeared in the same islets.

Localization of immunoreactive βA subunits of inhibin/activin in the islet cells was demonstrated with two different antisera. Although the islet cells were stained with both antisera, the staining characteristics were remarkably different. Immunoreactions to both anti-βA (1–10)-Tyr and anti-follistatin antisera were found in double-stained B cells (Fig. 3A). The reactions also were affected by STZ treatment. However, reactions to anti-βA (87–99) were found only in glucagon producing pancreatic A cells (Fig. 3B), while only B cells reacted with anti-βA (1–10)-Tyr. The reaction in A cells was not affected by STZ treatment. Preadsorption with antiserum containing 5 mg/ml of insulin or 0.5 mg/ml of glucagon did not interfere with these reactions. Somatostatin-producing D cells were not stained with any antisera used in this study.

4. DISCUSSION

The present study demonstrates for the first time that pancreatic islet cells are the site of inhibin, activin, and follistatin production. RT-PCR and immunohistochemistry results suggest that insulin producing B cells are the site of follistatin production. Detecting three subunits of inhibin/activin in pancreatic islets strongly suggests that islet cells secrete both inhibin and activin. Although immunoreactive βA subunits are localized in the islet of Langerhans, it is uncertain why the immunostainable cells react differently with two types of anti-βA antisera (Fig. 3A,B). One possible explanation of this inconsistency is that inhibin/activin in the B cells are bound to follistatin, and the altered βA subunit conformation conceals the 87–99 amino acid region while revealing the N-terminal epitope. In contrast, in A cells where inhibin/activin are free from follistatin, the 87–99 amino acid residues are revealed while concealing the N-termini of their βA subunits. Additional studies are needed to clarify the molecular state of inhibin and activin in the individual type of islet cells.

A major function of the islet of Langerhans is secretion of insulin and glucagon. The role of inhibin/activin and follistatin in the pancreatic islet is not clear. Since these molecules were initially isolated from gonads, they were thought to be a long-loop modulator of FSH secretion in the pituitary-gonadal axis. However, reports of localization and function in various physiological systems [1–10,15,16] suggests that they act as a local regulator in an autocrine or paracrine manner rather than as a modulator in a long-loop fashion. The present study suggests that inhibin/activin and follistatin may play a role in regulation of insulin and glucagon secretion in islet of Langerhans in an autocrine or paracrine manner.

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