SHORT COMMUNICATION

Insulin receptor-related receptor in rat islets of Langerhans

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Abstract

The physiological aspects and roles of the insulin receptor-related receptor (IRR), a novel member of the insulin receptor family, are unknown. Published work shows a characteristic localization of IRR mRNA at the cellular level in the kidney, stomach and brain. In our previous study, a specific antibody against the rat IRR β subunit was generated and used to identify the IRR-expressing cells as B-type intercalated cells in the kidney. In this study, IRR cDNA was amplified by PCR to demonstrate IRR mRNA expression in the rat pancreas. Immunohistochemical analysis with the anti-IRR antibody revealed that IRR immunoreactivity is localized in the islets of Langerhans. The IRR-immunoreactive cells were most likely to be the islet B cells which were stained with anti-insulin antibody. This is the first finding on the involvement of IRR in the physiological functions of insulin-secreting cells.

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Introduction

Insulin receptor-related receptor (IRR) has been isolated as a gene encoding the third member of the insulin receptor (IR) family (1). The predicted IRR protein is similar to the IR family protein consisting of $\alpha 2 \beta 2$ heterotetramers. This IRR protein contains a cysteine-rich domain (α subunit) for the ligand binding and a tyrosine kinase domain (β subunit). However, the physiological ligand and role of IRR are currently unknown. Insulin, proinsulin, insulin-like growth factor-I (IGF-I), IGF-II and relaxin (insulin family peptides) do not behave as specific IRR ligands on binding and phosphorylation studies (2, 3).

The expression of IRR mRNA has been demonstrated in the kidney, stomach, brain and sympathetic/sensory neurons; IRR transcripts are present at much higher levels in the kidney than in any other tissues (4–7). Quantitative competitive PCR analyses have also revealed that IRR transcripts in human kidney are 3- to 10-fold greater than those in thymus, brain, heart and stomach (8). At the cellular level, IRR-expressing cells have been detected by *in situ* hybridization as a subpopulation of epithelial cells within the distal tubular segments in the kidney (5), enterochromaffin-like cells in the stomach (8, 9) and cholinergic neurons co-expressing TrkA, a high affinity receptor for nerve growth factor (NGF) in the forebrain (6, 7, 10). In a previous study, we have examined the cellular localization of IRR mRNA and its protein in the rat kidney by using both an *in situ* hybridization technique and immunohistochemistry with anti-IRR antibody, and the IRR-expressing cells were identified as B-type intercalated cells (11). Bates *et al.* (12) concurrently reported

that the murine IRR is a specific marker for non-A intercalated cells in the kidney (12).

This study focused on IRR in the pancreas. Using Northern blot analysis, the presence of IRR mRNA in human pancreas has previously been demonstrated by Zhang & Roth (3). IRR transcripts are similarly found in the kidney, heart, skeletal muscle, liver and pancreas. However, the precise localization of IRR in the pancreas remains to be elucidated. In this paper, the presence of IRR mRNA and localization of IRR immunoreactivity in rat pancreas were demonstrated.

Materials and methods

Amplification of cDNA for IRR from rat tissues

For cDNA synthesis, 16 μ g RNA from rat kidney and pancreas were incubated at 37 °C for 60 min in a reaction mixture (20 μ l) with 300 units of Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Life Technologies, Tokyo, Japan), 10 units of human placenta RNase inhibitor (Wako Pure Chemicals, Osaka, Japan), and 2 μ g of random hexadeoxyribonucleotide primer (Takara Shuzo Co. Ltd, Kyoto, Japan). For amplification of cDNA, PCR was carried out for 30 cycles in a reaction mixture (25 μ l) containing 2 μ l (IRR) or 1 μ l (β -actin) of the above cDNA solution, 0.625 units Takara Ex Taq DNA polymerase (Takara Shuzo Co. Ltd) and 10 pmol each of the forward and reverse primers corresponding to the nucleotide sequences of the cDNA for the rat IRR (nucleotides –39 to –20: TTCCTGGAGGATACCTAGAA and 1394 to 1413: GATTTCAGCCTTGCTTTGCC) (4) and rat β -actin

(nucleotides 175 to 194: CAGAGCAAGAGAGGCATCCT and 565 to 584: TCGGTCAGGATCTTCATGAG) (13) respectively. The reaction products (2 μ l of each reaction mixture) were fractionated on a 1% agarose gel. The

identity of PCR products for IRR from rat tissues was established by nucleotide sequencing (ABI PRISM 310 Genetic Analyzer, Perkin-Elmer Japan, Chiba, Japan).

Immunohistochemistry

Rabbit polyclonal anti-IRR antibody was generated against the carboxy-terminal 90 amino acids of rat IRR β subunit as described previously (11). Male Wistar rats (8 weeks old) were anesthetized before perfusing through the ascending aorta with PBS, followed by 0.1 mol/l sodium phosphate buffer (pH 7.0) containing 2% paraformaldehyde. After perfusion, the pancreas was removed and placed in the above buffer containing 20% sucrose for at least 1 day at 4 °C. The tissue was cut into 16 μ m sections on a cryostat prior to mounting on gelatin-coated glass slides for immunostaining. The anti-IRR antibody was used as a primary antibody. Slide-mounted tissue sections were pretreated with PBS containing 2% BSA for 2 h before incubating overnight with the diluted primary antibody (1:400) at 4 °C. The sections were then incubated overnight with horse radish peroxidase (HRP) conjugated to goat anti-rabbit IgG antibody (Cappel, Organon Teknica, Durham, NC, USA) (1:500) at 4 °C. After washing with PBS, they were immersed in 50 mmol/l Tris-HCl (pH 7.5) containing 0.05% diaminobenzidine tetrahydrochloride (DAB) for 20 min at 4 °C. Immunolabeled peroxidase was visualized by placing slides in an incubation medium containing 50 mmol/l Tris-HCl (pH 7.5), 0.05% DAB and 0.01% hydrogen peroxide for 10 min at room temperature prior to studies with a light microscope. For staining pancreatic B cells, adjacent sections were incubated with a diluted polyclonal guinea pig anti-insulin antibody (Zymed Lab. Inc., San Francisco, CA, USA) and stained with HRP-conjugated goat anti-guinea pig IgG secondary antibody (Cappel) (1:500) as described above.

Results

Amplification of the cDNA for rat IRR in the rat pancreas

Amplification of cDNA encoding the extracellular region with the cysteine-rich domain of rat IRR by PCR revealed that IRR mRNA was detectable in the rat pancreas. PCR products of an appropriate size for the used primers

Figure 1 (I) Expression of rat IRR mRNA in kidney and pancreas. Amplification of the cDNAs for IRR (A and B) and β -actin (C and D) from rat kidney (A and C) and pancreas (B and D). M: ϕ X174DNA-HaeIII digest DNA marker (1353, 1078, 872 nucleotides). (II) Localization of IRR and insulin immunoreactivities in the islets of Langerhans in the rat pancreas. Sections (16 μ m) of adult rat pancreas were analyzed by immunohistochemistry with the anti-IRR antibody (A) and anti-insulin antibody on adjacent sections (B). Scale bar = 125 μ m.

were generated from a pool of cDNA obtained from reverse-transcribed rat pancreatic RNA, which was similar to PCR products from the rat kidney (Fig. 1 (I) A and B). As the kidney is the major tissue of IRR expression, the amount of PCR products from the kidney was much larger than that from the pancreas. The nucleotide sequences of these cloned PCR products were identical to that of rat IRR. The expression level of IRR mRNA in the rat pancreas was much lower than that in the kidney, while PCR products for the rat β -actin from both sources were quantitatively similar (Fig. 1 (I) C and D).

Immunohistochemistry

In order to investigate the IRR immunoreactivity in rat pancreas, we performed immunohistochemistry with anti-IRR antibody that had previously demonstrated IRR-immunoreactive cells in the kidney as B-type intercalated cells; the specificity of this antibody has been evaluated in a previous study (11). In the present study, the IRR immunoreactivity was localized only in the islets of Langerhans of rat pancreas; no IRR immunoreactivity was observed in the exocrine pancreas or in the pancreatic ducts (Fig. 1 (II)). The specificity of IRR-labeling was confirmed as the staining was abolished when anti-IRR antibody was absorbed with the antigen used to raise this antibody (data not shown). Most of the islet cells (70–80%) were stained with the antibody. Four major cell types in the islets were identified immunocytochemically in the endocrine pancreas: glucagon-immunoreactive A cells, insulin-immunoreactive B cells, somatostatin-immunoreactive D cells and pancreatic polypeptide-immunoreactive PP(F) cells (14). Both the population and distribution of IRR-positive cells were similar to those of the insulin-immunoreactive cells analyzed on adjacent sections (Fig. 1 (II)). Staining with anti-IRR antibody was less effective compared with the use of anti-insulin antibody. This fact may reflect the expression level for each molecule in the pancreatic islets.

Discussion

The IRR gene has a more limited pattern of expression compared with the insulin receptor and IGF-I receptor, and the highest level of IRR transcript can be found in the kidney (4, 5). In this study, amplification of IRR cDNA by PCR rendered detection of IRR mRNA in the rat pancreas possible (Fig. 1 (I)). Previous Northern blot analyses by Zhang & Roth (3) have revealed that the mRNA for IRR is expressed similarly in various human tissues including the kidney and pancreas. However, in the present study, the content of IRR mRNA in the pancreas was much lower than that in the kidney when the PCR products from both sources were compared. Furthermore, by immunohistochemistry

with anti-IRR antibody against the rat pancreas, the distribution of IRR immunoreactivity was localized in the islets of Langerhans; no IRR immunoreactivity was seen in the exocrine pancreas. The immunostaining with anti-insulin antibody on adjacent sections revealed that IRR immunoreactivity co-existed with insulin immunoreactivity (Fig. 1 (II)). Thus, IRR-expressing cells in the pancreas were most likely to be B cells. This is the first report indicating the involvement of IRR with insulin-producing cells, although this receptor is structurally similar to the insulin receptor, however, does not bind with insulin (2, 3).

On the other hand, the high affinity NGF receptor, TrkA, is also present in pancreatic B cells of adult rats (15). Thus, it is my belief that IRR might be co-localized with TrkA in the islet B cells, similar to NGF-sensitive neurons co-expressing IRR and TrkA (6, 7, 10). A functional linkage of IRR and TrkA receptors may exist in the pancreatic islets. As for the expression of insulin-like peptides in the pancreas, immunohistochemical analyses by Maake & Reinecke (16) have revealed that IGF-I and -II immunoreactivities in the pancreas exist in A (and/or D) and B cells respectively; it was presumed that IGF-I, derived from A cells and/or D cells, acts on the B cells in a paracrine manner. IRR ligand might be a peptide hormone with an autocrine or a paracrine function.

The isolation of a physiological ligand of IRR, however, is still unestablished. Recently, the possible application of IRR-null mice has been reported (12). In these mutants, defects may exist not only in the kidney but also in the pancreas. Furthermore, investigations with normal and IRR-null mice might be useful for understanding the physiological functions of IRR and screening the ligand.

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