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.

Materials and methods

Amplification of cDNA for IRR from rat tissues

For cDNA synthesis, 16 µg RNA from rat kidney and pancreas were incubated at 37°C for 60 min in a reaction mixture (20 µl) 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 µg of random hexa-deoxyribonucleotide primer (Takara Shuzo Co. Ltd, Kyoto, Japan). For amplification of cDNA, PCR was carried out for 30 cycles in a reaction mixture (25 µl) containing 2 µl (IRR) or 1 µl (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: TCTCCTGGAGGATAATGCC and 1394 to 1413: GATTTCAGCCTTGGTCC) (4) and rat β-actin...
(nucleotides 175 to 194: CAGAGCAAGAGGGATCCT and 565 to 584: TCGGTCAGGATCTTCATGAG) respectively. The reaction products (2 μl 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 μm 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

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**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: 50X174DNA-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 μm) 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 μm.

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Comparison of IRR mRNA levels between rat pancreas and kidney by immunohistochemistry

In the present study, the content of IRR mRNA in the tissues including the kidney and pancreas. However, in this study, 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.

**Acknowledgements**

This work was supported in part by the Kanae Foundation of Research for New Medicine, the Fujisawa Foundation and Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. I wish to thank Professor Nobuyuki Itoh.

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Received 31 December 1997
Accepted 20 April 1998