Cellular distribution and ontogeny of insulin-like growth factors (IGFs) and IGF binding protein messenger RNAs and peptides in developing rat pancreas

D J Hill^{1,2,3,4}, J Hogg^{1,2}, J Petrik^{1,2,4}, E Arany^{1,3} and V K M Han^{1,4,5,6}

¹Lawson Research Institute, St Joseph's Health Centre, London, Ontario, Canada N6A 4V2

²Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5A5

³Department of Medicine, University of Western Ontario, London, Ontario, Canada N6A 5A5

⁴Department of Paediatrics, University of Western Ontario, London, Ontario, Canada N6A 5A5

⁵Department of Anatomy and Cell Biology, University of Western Ontario, London, Ontario, Canada N6A 5A5

⁶Department of Obstetrics and Gynaecology, University of Western Ontario, London, Ontario, Canada N6A 5A5

(Requests for offprints should be addressed to D J Hill, Lawson Research Institute, St Joseph's Health Centre, 268 Grosvenor Street, London, Ontario, Canada N6A 4V2)

Abstract

To determine the role of insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) in the development of the pancreas, and specifically of the islets of Langerhans, we have examined the cellular distribution and developmental changes in the expression of IGFs and IGFBPs in the pancreas of the fetal and neonatal rat between 19.5 days of gestation and postnatal day 28. This represents a period of substantial growth and restructuring of the β cell component in islets of this species. IGF-I, IGF-II, and IGFBPs-1 to -6 mRNAs were localized by *in situ* hybridization, and peptides by immunohistochemistry, in histological sections. IGF-II mRNA was highly expressed in islet cells and some ductal epithelial cells in late fetal and early neonatal life, but was barely detectable by postnatal day 28. IGF-II peptide showed a similar distribution. IGF-I mRNA was barely detected in the fetus or neonate and was localized predominantly in the ductal and acinar tissues after postnatal day 7. IGF-I immunoreactivity was associated with some islet cells in the fetus and neonate, suggesting an endocrine rather than a paracrine source.

We performed co-localization studies to assess whether the distribution of IGFs within the pancreas might be due to a sequestration by locally produced IGFBPs. The presence of mRNAs for both IGFBPs-1 and -2 was minimal in the pancreas prior to postnatal day 7, although subsequently IGFBP-1 mRNA was seen in islet cells, while IGFBP-2 mRNA was localized in both islets and acinar tissues. In contrast, both IGFBPs-1 and -2 immunoreactivities were identified in islets from late fetal life, suggesting a circulatory source for these IGFBPs during early pancreatic development. IGFBPs-3 to -5 mRNAs and immunoreactivities were identified within islet cells throughout fetal and neonatal life, with IGFBPs-3 and -5 being mainly associated with the α cell-rich islet mantle. The results show a compartmentalization of IGFs within pancreatic tissue, reflecting both paracrine and endocrine sources. The localization and action of IGFs in pancreas likely involves sequestration and distribution by endogenous as well as circulating IGFBPs.

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Introduction

The ability to increase β cell mass in the pancreatic islets of Langerhans by cell proliferation of existing β cells, as well as islet neogenesis (differentiation of new β cells), is essential for the increasing insulin requirements of the growing fetus and neonate (Finegood *et al.* 1995). Substantial evidence supports a role for insulin-like growth factors (IGFs) in islet cell replication and function. We and others have shown that human or rat fetal and neonatal islets release IGF-I and IGF-II (Rabinovitch *et al.* 1982, Romanus *et al.* 1985, Hill *et al.* 1987, Swenne *et al.* 1987,

Scharfmann *et al.* 1989, Hogg *et al.* 1993), while exogenous IGFs-I or -II promote increased islet cell DNA synthesis (Rabinovitch *et al.* 1982, Swenne *et al.* 1987, Hogg *et al.* 1993). Isolated rat islet α and β cells express the high affinity IGF type 1 signalling receptor (Van Schravendijk *et al.* 1987), which is also present in islet-derived cell lines synthesizing insulin, glucagon or somatostatin (Fehmann *et al.* 1996). IGF-I inhibits insulin release by adult islet β cells *in vitro* and *in vivo* (Guler *et al.* 1989, Van Schravendijk *et al.* 1990). However, perfusion of adult islets with IGFs-I or -II causes biphasic effects on glucose-stimulated insulin release (Hill *et al.* 1997). We

previously reported, based on Northern blot hybridization, that the major IGF expressed within whole pancreas in the fetal and neonatal rat was IGF-II, but that expression declined two weeks following birth, to be replaced by IGF-I expression (Hogg *et al.* 1994). This developmentally regulated alteration in IGF expression is seen in many other tissues, and is reflected in the circulating peptides, although the timing of a change from IGF-II to IGF-I expression differs between tissues (Brown *et al.* 1986).

The IGFs are usually found associated with up to six high affinity binding proteins (IGFBPs-1 to -6) in tissues and biological fluids. In addition to extending the biological half-life of IGFs in blood and extravascular fluids, the IGFBPs also modulate the biological actions of IGFs on isolated cells and tissues (McCusker & Clemmons 1992). Using isolated fetal rat islets, we demonstrated a release of IGFBPs-1 to -4, with IGFBPs-1 and -2 release being upregulated by glucose or amino acids (Hogg *et al.* 1993). Exogenous IGFBPs-1 or -2 were able to potentiate the mitogenic actions of IGF-II on fetal islets. Similarly, in the mid-trimester human fetus, IGF peptides and IGFBPs-1, -2 and -3 are localized to islets, including β cells (Hill & Clemmons 1992). When we examined the ontogeny of IGFBP mRNA expression in the whole rat pancreas using Northern blotting, IGFBP-1 mRNA was undetectable and IGFBP-2 mRNA was very low in the fetal and neonatal pancreas, but both IGFBP mRNAs transiently appeared between 2–3 weeks of age and then declined in the adult. IGFBPs-3 and -4 mRNAs are expressed in the pancreas throughout development (Hogg *et al.* 1994).

These analyses, however, do not reveal cellular compartmentalization of IGFs or IGFBPs within the developing pancreas. Pancreatic β cell mass progresses in early life by a combination of islet cell neogenesis and the proliferation of existing islet cells, balanced by developmentally regulated β cell apoptosis (Scaglia *et al.* 1997). It is therefore important to determine whether IGFs and their binding protein mRNAs are expressed, and the proteins localized, to islet cells or adjacent acinar or ductal epithelial cell populations in order to postulate their likely contribution to islet neogenesis, hyperplasia, or both. The present studies report a distinct anatomical and developmental pattern of expression and peptide localization of IGF-I, IGF-II and IGFBPs in the fetal and neonatal rat pancreas.

Materials and Methods

Animals

Pregnant Wistar rats (Charles River, Montreal, PQ, Canada) were killed at days 19.5 or 21.5 of gestation, or were allowed to deliver and the offspring killed between postnatal days 2 and 28. Rat fetuses were decapitated with scissors, while the pregnant females and all rats at postnatal ages were killed by CO₂ asphyxiation. Pregnant and

suckling rats were allowed free access to food and water. All procedures were performed with ethical approval of the Animal Care Committee of the University of Western Ontario. Pancreata were dissected quickly and fixed in ice-cold fixative (4% paraformaldehyde and 0.2% glutaraldehyde buffered with 70 mM phosphate buffer, pH 7.4) for 16 h at 4 °C, followed by four washes at 4 °C in phosphate-buffered saline (PBS) over a 48-h period. Fixed tissues were dehydrated in 70% (v/v) ethanol and embedded in paraffin.

Immunohistochemistry

Histological sections of pancreas (5 μ m) were prepared and mounted on glass microscope slides (Superfrost Plus, Fischer Scientific, Nepean, ON, Canada). Immunohistochemistry was performed to localize IGFs-I or -II, or IGFBPs using a modified avidin–biotin peroxidase method (Hsu *et al.* 1981) as described by us previously (Petrik *et al.* 1998). Slides were incubated for 48 h with either rabbit anti-human IGF-I or IGF-II (1:2000 dilution) (GroPep Ltd, Adelaide, Australia), rabbit anti-human IGFBPs-1, -2, -3, -4 and -5 (all at 1:1000 dilution), or monoclonal antibody against human IGFBP-6 (1:500 dilution) (Austral Biologicals, San Ramon, CA, USA). Biotinylated goat anti-rabbit immunoglobulin G (IgG) (1:500), or goat anti-mouse IgG (1:100) (Vector Laboratories, Burlingame, CA, USA) were used as second antibodies and diaminobenzidine tetrahydrochloride (DBS tablets, 10 mg, Sigma Chemical Co., St Louis, MO, USA) was used as the chromagen. Tissue sections were counter-stained with Carazzi's haematoxylin. To establish specificity of staining, the primary antisera to IGFs-I or -II were pre-absorbed overnight at 4 °C with 100 nM homologous ligand prior to application to the sections. Antisera against IGFBPs were pre-absorbed with 100 nM homologous or heterologous IGFBP proteins (Austral). All antisera were found to be specific as stated by the supplier, with the exception of antiserum against IGFBP-4, which showed cross-reactivity with IGFBP-2. Further controls included substitution of primary antisera with non-immune serum and omission of the secondary antiserum.

In situ hybridization

In situ hybridization for IGFs-I, -II and IGFBPs-1 to -6 was performed on histological paraffin sections of pancreas as described previously (Petrik *et al.* 1998). Sections were counter-stained with haematoxylin and eosin, and were viewed under dark and bright field microscopy. As controls for non-specific hybridization, hybridization was also carried out (i) using sense probes, and (ii) using antisense probes on sections previously incubated with ribonuclease A (RNase A) to degrade the RNA. Where comparisons were made between pancreata from animals

Figure 1 *In situ* hybridization of IGF-II (A and B) or IGF-I (C and D) mRNAs in tissue sections of rat pancreas from animals of 2 days (A and B) or 28 days (C and D) postnatal age. Hybridizations with antisense cRNAs are shown in panels A and C, and control hybridizations with sense strand cRNAs are shown in panels B and D. Arrows indicate hybridization signal in the islet cells (i) in A, and in the exocrine tissue (e) in C. Magnification bar 10 μ m.

of different ages, all sections were included in the same hybridization reactions to remove interprocedural variability.

Antisense riboprobes were prepared from cDNAs for rat (r) IGF-I (a gift of Dr L Murphy, University of Manitoba, Canada), mouse (m) IGF-II (a gift of Dr G Bell, University of Chicago, USA), rIGFBPs-1 and -2 (gifts of Dr M M Rechler, NIH, MD, USA); rIGFBP-3 (a gift of Dr A Herington, Queensland University of Technology, Brisbane, Qld, Australia) and rIGFBPs-4, -5 and -6 (gifts of Dr S Shimasaki, University of California, San Diego, CA, USA). The restriction enzymes (BRL, Burlington, ON, Canada) and RNA polymerases (Promega) used to linearize the plasmids containing these cDNAs and to generate [35 S] radiolabelled riboprobes are listed as follows: antisense rat IGF-I, HindIII/T7; sense rat IGF-I, PvuII/SP6; antisense mouse IGF-II, HindIII/TSP6; sense mouse IGF-II EcoRI/T7; antisense rIGFBP-1, BamHI/T7; sense rIGFBP-1, AatII/SP6; antisense

rIGFBP-2, HindIII/T7; sense rIGFBP-2, SacI/SP6; antisense rIGFBP-3, HindIII/SP6; sense rIGFBP-3, NdeI/T7; antisense rIGFBP-4, SmaI/T7; sense rIGFBP-4, HindIII/T3; antisense rIGFBP-5, SacII/T7; sense rIGFBP-5, HindIII/T3; antisense rIGFBP-6, SmaI/T7; sense rIGFBP-6, EcoRI/T3. Radiolabelled cRNA probes were synthesised using linearized riboprobe DNA, α -thio [35 S]UTP, and SP6, T3 or T7 RNA polymerase as described previously (Petrik *et al.* 1998). To improve cRNA probe penetration of tissue sections, limited alkaline hydrolysis was used to reduce transcript size to about 150 bases.

Morphometric and statistical analysis

Morphometric analysis was performed using a Zeiss transmitted light microscope at a magnification of $\times 250$. Analyses were performed with Northern Eclipse version 2.0 morphometric analysis software (Empix Imaging Co.,

Figure 2 *In situ* hybridization of IGF-II (A, B and C) or IGF-I (D, E and F) mRNAs in tissue sections of rat pancreas from animals of 19.5 days gestation (A and D), 7 days postnatal (B and E), or 28 days postnatal age (C and F). i, islet; e, exocrine tissue; en, vascular endothelium. Arrows indicate hybridization signal in the islet cells in A and B, and in the acinar tissue in E and F. Magnification bar 10 μ m.

Mississauga, ON, Canada). The percentage of islet cells immunopositive for IGF-I, IGF-II or IGFBPs-1 to -6 was calculated at each age from five sections of each pancreas

representing both head and tail regions. Sections chosen contained at least five islets, and pancreata from at least five animals were examined for each age.

Figure 3 *In situ* hybridization to visualize IGFBP-2 mRNA (A, B and C) in tissue sections of rat pancreas from animals of 7 (A), 14 (B), or 28 (C) days postnatal age. Control hybridization with a sense strand cRNA is shown in panel D. i, islet; e, exocrine tissue. Magnification bar 10 μ m.

Results

IGF-II mRNA was identified in high abundance in the pancreatic islets, and in low abundance in the acinar and ductal tissues of the two-day postnatal rat pancreas (Fig. 1A). Hybridization with a sense IGF-II probe (Fig. 1B), or incubation of tissue sections with RNase prior to hybridization with an antisense IGF-II probe (not

shown), resulted in no hybridization signal. IGF-I mRNA was detected in the acinar and ductal cells of the pancreas at 28 days postnatal life (Fig. 1C). The hybridization signal was not widespread within the islets of Langerhans, but was greater in cells on the outer edge of the islets. No hybridization signal was seen when a sense strand cRNA for IGF-I was used (Fig. 1D) or when the sections were pre-treated with RNase. This demonstrated that

Figure 4 *In situ* hybridization of mRNAs encoding IGFBP-1 (A), IGFBP-3 (B), IGFBP-4 (C) or IGFBP-5 (D) in tissue sections of rat pancreas from animals of 14 days postnatal age. i, islet; e, exocrine tissue. Arrows indicate hybridization signal in the islet cells. Magnification bar 10 μ m.

both IGFs-I and -II mRNAs were identified within specific compartments of the rat pancreas and that the hybridization was specific.

Comparison of sections of pancreata taken at differing ages showed clear ontological patterns of IGF expression in pancreas. In pancreata taken between 19.5 days fetal life and 7 days postnatal life, IGF-II mRNA was abundant in the islets of Langerhans (Fig. 2A,B). IGF-II hybridization

signal was also associated with ductal epithelial cells and some acinar cells. At postnatal days 17, 21 and 28, a much reduced IGF-II expression was seen in islets, with little signal remaining in acinar and ductal tissue (Fig. 2C). At postnatal days 10–14, an intermediate hybridization intensity was seen. Conversely, little or no hybridization for IGF-I mRNA was seen in fetal and neonatal pancreas in either islet or acinar cells (Fig. 2D). In pancreas at postnatal

Table 1 Semi-quantitative assessment of the abundance of IGFBP mRNAs within cellular compartments of the rat pancreas with age

	Age (days)						
	F19.5	F21.5	P2	P7	P14	P21	P28
IGFBP-1							
Islet	—	—	—	—	+	++	++
Acinar	—	—	—	—	—	—	—
IGFBP-2							
Islet	—	—	—	—	+	++	+
Acinar	—	—	—	+	++	+++	++
IGFBP-3, -4							
Islet	+	+	+	+	+	+	+
Acinar	—	—	—	—	—	—	—
IGFBP-5							
Islet	+	+	+	+	+	+	+
Acinar	+	+	+	+	+	+	+

F, fetal age; P, postnatal age; —, No mRNA detectable; +, ++, +++, increasing abundance of mRNA hybridization signal.

day 7, IGF-I mRNA hybridization was now seen in ductal and acinar cells, with a low level of hybridization in islet cells (Fig. 2E), and this was maintained up to postnatal day 28 (Fig. 2F). Therefore, we have observed a relatively high expression of IGF-II mRNA within islets in late fetal and neonatal life which decreased between one and two weeks following birth, and was almost absent by weaning. IGF-I mRNA expression was low in all cell compartments of the pancreas in early life, but appeared in acinar tissue and in some islet cells by weaning.

IGFBP-2 mRNA was of low abundance in islets and absent from acinar tissue at fetal ages and up to postnatal day 7 (Fig. 3A). At postnatal days 14 and 21, the mRNA was more abundant in acinar tissue and in the outer cell mantle of islets (Fig. 3B), and became less prominent at postnatal day 28 (Fig. 3C). IGFBP-1 mRNA was also of low abundance in the pancreas prior to postnatal day 7. At postnatal days 14 to 28, IGFBP-1 mRNA was located evenly within cells of the islets (Fig. 4A). IGFBPs-3 and -4 mRNAs were located throughout the islets and did not obviously change in abundance with age (Fig. 4B,C). IGFBP-5 mRNA was seen at all ages and in all regions of the islet, although the signal intensity was greatest in the outer mantle of predominantly α cells (Fig. 4D). IGFBP-5 was also expressed in the acinar cells. No difference in hybridization signal for IGFBP-6 was seen at any age with antisense probe compared with sense control, suggesting a low level of abundance for this mRNA. A semi-quantitative assessment of the changes in IGFBP mRNA expression with age in pancreas is shown in Table 1.

Immunohistochemistry for IGF-II showed strong staining of the fetal and neonatal islets and occasional

ductal epithelial cells up to postnatal day 2 (Fig. 5A). Within the islets, the staining was most apparent in the central, β cell-rich core, whereas the periphery of the islets was immunonegative. The staining was abolished by pre-incubation of the primary antiserum with excess IGF-II (Fig. 5B), demonstrating that this was specific and unlikely to represent cross-reactivity with insulin. The distribution of IGF-II peptide differed from that of the IGF-II mRNA, which was present in all regions of the islets. By postnatal day 10, staining for IGF-II immunoreactivity in the islets was less uniform (Fig. 5C) and by day 21 it was barely detectable (Fig. 5D). The change in the area of islets immunopositive for IGF-II with age is shown in Fig. 6. Immunoreactivity for IGF-I in pancreas was low in the fetus, but steadily increased with postnatal age (Fig. 6). At older ages, a diffuse staining was seen in many cells of the islets, and in the acinar cell lobules (Fig. 7A). Again, a discrepancy existed between the presence of some IGF-I immunoreactivity in fetal and neonatal islets and a lack of mRNA for IGF-I. Immunoreactive IGFBP-1 was diffusely present in islet cells in the fetus, and the area of immunopositive cells declined with age (Fig. 6). IGFBP-1 was also present in certain acinar and mesenchymal cells at all ages, but staining was most intense at postnatal day 21 (Fig. 7B). IGFBP-2 immunoreactivity was predominantly associated with the outer mantle of islet cells, comprised mostly of α cells, and vascular endothelium. The extent of islet cell IGFBP-2 immunoreactivity did not change greatly with age (Figs 6 and 7C). IGFBP-3 immunoreactivity was seen at all ages within the pancreas, in both islets and mesenchymal tissues, but not in most acinar cells (Fig. 7D). Many islet

Figure 5 Immunohistochemistry of IGF-II in sections of rat pancreas from animals of 2 (A), 10 (C), or 21 (D) days postnatal age. The absence of staining observed when the primary antiserum was pre-absorbed with excess IGF-II is shown in panel B. i, islets; e, exocrine tissue. Arrows indicate immunoreactivity associated with islet cells. Magnification bar 10 μ m.

cells showed a pronounced nuclear localization of IGFBP-3, and the proportion of islet cells immunopositive for IGFBP-3 increased with postnatal age (Fig. 6). IGFBP-3 immunoreactivity was greater in the outer cells of the islets. IGFBP-4 was similarly distributed, but showed no nuclear localization. Immunoreactivity for IGFBP-5 was also seen at all ages and was identified in the α cells of the islets and with ductal epithelial cells (Fig. 7E).

Discussion

These findings demonstrate that the developing rat pancreas has a dynamic and differential expression of IGFs and their binding proteins. As in most fetal and neonatal rat tissues (Brown *et al.* 1986, Lund *et al.* 1986), the expression of IGF-II mRNA in pancreas, as detected by *in situ* hybridization, is much higher than that of IGF-I, which was virtually undetectable in any cell type prior to

postnatal day 7. This agrees with our previous report that IGF-I mRNA was very low when assessed by Northern blot hybridization of total RNA from whole pancreata (Hogg *et al.* 1994). In the fetus and neonate, IGF-II mRNA is largely associated with pancreatic endocrine cells, but was not restricted to any single islet cell type, since the hybridization signal was seen in both the inner zone of the islets, which is rich in β cells, and the outer mantle, which has an abundance of α cells. IGF-II immunoreactivity showed a similar distribution, but was more intense in the inner core than the outer mantle of the islets. It is possible that this distribution is dictated by islet-associated IGFBPs, but at least three of these, IGFBPs-2, -3 and -5 are more clearly associated with the α cells than the central β cells. Alternatively, IGF-II peptide distribution could be determined, in part, by binding to the type II IGF/mannose 6-phosphate receptor which is abundant on β cells (Fehmann *et al.* 1996, Ishiwa *et al.* 1997).

Figure 6 Percentage area of islets immunopositive for IGF-I and IGF-II (A), IGFBPs-1, -2 and -3 (B), or IGFBPs-4 and -5 (C) in rats from 19.5 days gestation (F) to 21 days postnatal (P) age. Panels show the mean values ($\pm$ S.D.) for at least five sections from each of five pancreata at each age.

IGF-II synthesis is a common feature of isolated rodent and human islets derived in fetal or neonatal life (Rabinovitch *et al.* 1982, Bryson *et al.* 1989, Hogg *et al.* 1993), and from β cell lines which maintain a fetal-like, proliferative phenotype (Asfari *et al.* 1995). The role of

IGF-II in the pancreas of the fetus and neonate is likely to be that of a paracrine or autocrine mitogen. Recently, we reported that an IGF-II transgenic mouse model exhibited fetal overgrowth with increased pancreatic weight and a five-fold increase in islet cell mass (Hill *et al.* 1998). Islets were bigger with an increased complement of β cells, but islet number was unaltered, suggesting that IGF-II did not act as an islet neogenic factor, but served as a growth factor for existing islets.

We have shown previously that IGF-II mRNA levels decrease in the pancreas between 14 and 21 days after birth (Hogg *et al.* 1994), to become undetectable. The present study shows that this represents specifically a loss of expression from pancreatic islet cells. The timing of the loss of expression of IGF-II in pancreatic islets corresponds to a wave of developmental β cell apoptosis (Scaglia *et al.* 1997), and we have demonstrated that IGF-II functions as a survival factor for isolated islets from the neonatal rat which will prevent the induction of β cell apoptosis by cytokines (Petrik *et al.* 1998). A fall in IGF-II expression within neonatal islets may, therefore, be functionally linked to developmental β cell apoptosis, and the replacement of these cells through islet cell neogenesis from pancreatic ductal epithelial cells. The mechanism by which the IGF-II expression in islets is lost during neonatal life is not known, but could be linked to altered nutrition, or a maturation of the immune system and the colonization of the pancreas with macrophages and dendritic cells at this time (Grove *et al.* 1991). The fall in IGF-II expression in pancreas may also be linked to changes in the ontogeny of glucocorticoids and the glucocorticoid receptor at this time. Corticosterone levels rise appreciably in the rat circulation between two and three weeks of postnatal life at a time when glucocorticoid receptors appear in the pancreas (Lu *et al.* 1987, Matthes *et al.* 1994), to be found mainly on β cells in the adult (Fischer *et al.* 1990). In the fetal sheep, a sharp decrease in the expression of IGF-II prior to birth, at least in the liver, has been shown to be functionally linked to a rise in circulating cortisol (Li *et al.* 1998). In the hyperinsulinaemic Goto-Kakizaki (GK) rat, which develops a non-insulin-dependent diabetes, inappropriately processed IGF-II of high molecular weight continues to be synthesized by large, irregular shaped islets at 2–3 months of age (Hoog *et al.* 1996). Immunoreactive IGF-II was localized, together with insulin, to secretory granules in a sub-set of islet β cells in the GK rat (Hoog *et al.* 1997). In situations of β cell hyperplasia, therefore, a prolonged expression of IGF-II may occur in the pancreas.

IGF-I mRNA was absent from the fetal and neonatal pancreas, but appeared by postnatal day 7, predominantly in exocrine tissue and with a low abundance in islets. Acinar tissue contains type-1 and type-2 IGF receptors, and insulin receptors (Mossner *et al.* 1984), the type 1 receptors mediating weak mitogenic actions of IGFs and an activation of phospholipase D (Rydzewska & Morisset

Figure 7 Immunohistochemistry of IGF-I (A), IGFBP-1 (B), IGFBP-2 (C), IGFBP-3 (D) or IGFBP-5 (E) in sections of rat pancreas from animals of 21 days postnatal age. i, islets; e, exocrine tissue. Arrows indicate immunoreactivity associated with islet cells. Magnification bar 10 μ m.

1995). The ontogeny of IGF-I gene expression in acinar tissue may relate to the maturation of the growth hormone receptor, and the onset of growth hormone-dependent IGF-I expression (Mathews *et al.* 1986). Isolated islets from

the 20–22 day gestation rat fetus release insulin and demonstrate increased DNA synthesis in response to GH, the latter mediated partly by a release of IGF-I (Svenne *et al.* 1987). However, such cultures are maintained *in vitro*

for 7–10 days prior to experimentation, suggesting that the timing of onset of growth hormone-dependent IGF-I expression is maintained *ex vivo*. A contribution of pancreatic IGF-I expression to the proliferation of both exocrine and endocrine tissue is supported by an increased IGF-I synthesis during the compensatory islet hyperplasia and acinar regrowth which follows sub-total pancreatectomy in the young rat or dog (Hayakawa *et al.* 1996, Calvo *et al.* 1997). In the regenerating rat pancreas, IGF-I mRNA was localized to epithelial cells of proliferating ductules, the source of new islets by a process of neogenesis, and to endothelial cells of the supporting vasculature (Smith *et al.* 1991). In the present study, immunoreactive IGF-I was seen at low abundance in islet cells in fetal and early neonatal life, but it increased in intensity with age. IGF-I was also located in a sub-population of acinar and ductal cells. This is in agreement with a localization of IGF-I to α , β and somatostatin cells in adult rat islets of Langerhans, although a localization to somatostatin cells was reported to be strongest (Hansson *et al.* 1988). The localization of IGF-I to islet cells despite very low levels of mRNA suggests a sequestration of endocrine IGF-I at the cell surface by IGFBPs as a likely explanation.

At least six IGFBP molecules are capable of binding IGFs-I or -II, of which IGFBPs-1 to -4 have previously been shown to be released from isolated fetal rat islets after one week of culture (Hogg *et al.* 1993). We have previously shown separate ontological patterns of expression of IGFBPs-1 to -4 mRNAs in fetal and neonatal rat pancreas by Northern blot hybridization (Hogg *et al.* 1994), IGFBPs-1 and -2 mRNAs appearing after postnatal day 7 and being maximal by day 21, while IGFBPs-3 and -4 mRNAs were present throughout late fetal and neonatal life with greatest abundance 7–14 days after birth. The present study provides further evidence that IGFBPs-1 to -5 mRNAs are all present within fetal and/or neonatal islets with discrete expression patterns. IGFBP-2 mRNA appeared after postnatal day 7 and was predominantly localized to the outer mantle of α cells, as was the immunoreactive peptide. IGFBP-5 mRNA and peptide and IGFBP-3 also predominated in the outer zones of the islets, but were present from late fetal life. IGFBP-1 mRNA did not appear until around postnatal day 14, although peptide immunoreactivity associated with islets was much greater at earlier ages. This suggests that, in late fetal and early neonatal life, the source of islet-associated IGFBP-1 is from the circulation.

The cellular distributions of IGFBPs suggest functional contributions to IGF action in islet cell growth and function. We previously showed that exogenous IGFBPs-1 and -2 could potentiate the mitogenic actions of IGF-II on isolated, β cell-enriched fetal rat islets (Hogg *et al.* 1993), while IGFBP-3 inhibited IGF-II action (D J Hill, unpublished observation). The expression of human

IGFBP-1 in transgenic mice caused a hyperplasia of pancreatic islet cells, leading to increased β cell mass (Rajkumar *et al.* 1996). Islets isolated from these animals showed an increased release of insulin in response to glucose (Dheen *et al.* 1996), demonstrating a functional role for IGFBP-1 in islet cell growth and function. Since IGFBPs-2 and -3 peptides are predominantly associated with the outer, α cell-rich area of the islets, a contribution to β cell growth is likely to represent a paracrine rather than an autocrine interaction. In contrast, since IGFBP-1 is not expressed locally within the pancreas to any extent prior to postnatal day 14, any influence on islet cell development must be from the circulating IGFBP-1 derived almost exclusively from the liver in early life (Lund *et al.* 1986). A fall in endogenous expression of islet IGF-II after postnatal day 7 is associated with the onset of β cell apoptosis (Petrik *et al.* 1998). A substantial increase in the localization of IGFBP-3 to islet cells at this time may limit IGF-II availability, and potentiate the onset of apoptosis. The IGF axis within islets could also contribute to the endocrine function of non- β cell types, since IGF-I potentially inhibited proglucagon gene expression and glucagon release from a glucagon-secreting islet cell line, while potentiating somatostatin release from RIN 1027B2 cells (Fehmann *et al.* 1996). The association of IGFBPs-2, -3 and -5 with the α cell-rich area of the outer islet would implicate them in the modulation of IGF action in glucagon and somatostatin release, although no direct functional evidence exists as yet. Of the IGFBPs studied, only IGFBP-3 demonstrated a nuclear localization within islet cells. This has been observed for other cell types (Jaques *et al.* 1997, Li *et al.* 1997), and in kidney cells it has been associated with a transport of IGF-I to the nucleus (Li *et al.* 1997). In the pancreas we did not detect any nuclear association of immunoreactive IGFs-I or -II in islet cells, suggesting that any biological functions of nuclear IGFBP-3 are independent of an IGF transport role.

Islet neogenesis from pancreatic ductal epithelial cells is ongoing in the fetus and neonate, and a wave of neogenesis two to three weeks after birth in the rat replaces β cells lost at this time by apoptosis (Finegood *et al.* 1995). We found a substantial localization of IGFBPs-1, -2 and -5 to sub-population of ductal and acinar cells, with IGFBP-2 especially increasing in its presence after postnatal day 7. Since both IGF-II in the fetus, and IGF-I in the neonate, are expressed and localized to similar cells, and IGF-I expression is increased during neonatal pancreas regeneration (Smith *et al.* 1991), it seems likely that interactions of IGFs with IGFBPs also modulate the expansion of new endocrine cell populations.

In summary, these experiments show a dynamic and regional distribution of IGF and IGFBP mRNA expression and peptide immunoreactivity within the developing rat pancreas, which would support an important role for the IGF axis in endocrine pancreatic

maturation, and the determination of β cell mass and insulin availability.

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