Functional Active Receptors for Insulin-Like Growth Factor-I (IGF-I) and IGF-II on Insulin-, Glucagon-, and Somatostatin-Producing Cells

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Insulin-like growth factors I and II (IGF-I and IGF-II) are expressed at high levels in the endocrine pancreas during development and tissue regeneration. However, their effects at the endocrine pancreas are poorly understood. We searched for receptors of IGF-I and IGF-II and possible biological effects on clonal insulin-secreting (HIT), glucagon-secreting (INR1G9), and somatostatin-secreting (RIN 1027 B2) cell lines. Our data showed that HIT cells and RIN 1027 B2 cells express specific type I and type II IGF receptors. INR1G9 cells possess type II IGF receptors and IGF-I binding sites with the same affinity for both IGF-I and IGF-II. In HIT cells, insulin secretion was not influenced by either peptide. Proinsulin gene transcription was stimulated by IGF-II but not by IGF-I. IGF-I potently inhibited proglucagon gene transcription and glucagon secretion in INR1G9 cells, whereas IGF-II only inhibited glucagon release. In RIN 1027 B2 cells, IGF-I but not IGF-II increased somatostatin output, whereas both stimulated somatostatin gene expression. These data demonstrate the presence of classic type I and type II IGF receptors on insulin-, glucagon-, and somatostatin-secreting cells. Both peptides may be important regulators of endocrine pancreatic function in terms of islet hormone release and gene expression. Therefore, both peptides may be involved in the regulation of intraislet cellular homeostasis.

INSULIN-LIKE growth factors I and II (IGF-I and IGF-II) share a high degree of sequence homology with insulin. Especially during embryonic development but also in some tissue in the adult organism, they are produced in a variety of tissues, including the endocrine pancreas, and it is believed that they play important roles during organ development, differentiation, regeneration, and cell proliferation.[1-7]

Several lines of evidence exist indicating that IGF-I has metabolic effects similar to insulin's effects.[8-11] It decreases plasma glucose concentration by stimulating peripheral glucose uptake and reducing hepatic glucose production.[8,9] Furthermore, it decreases plasma C-peptide and glucagon levels.[8,9] Based on these data, direct actions of IGF-I on the different cell types of pancreatic islets were assumed. In addition, a previous study suggested the presence of receptors for IGF-I on endocrine pancreatic A and B cells.[12] However, our knowledge about the direct actions of IGFs at the endocrine pancreas is limited. No consensus exists concerning the effects of IGF-I on pancreatic B cells. These contrasting results are most probably due to the different experimental models applied. Swenne et al[13,14] found no effect of exogenous IGF-I on insulin secretion and biosynthesis in fetal and adult rat islets. This contrasts with another study demonstrating stimulatory effects on the same parameters in the same experimental setting.[4,15] In studies using the isolated perfused rat pancreas and purified rat B cells, IGF-I was described as an inhibitor of glucose- and arginine-stimulated insulin secretion,[16,17] but no data have been published concerning the actions of IGF-I and A on D cells.

Information about the role of IGF-II in the endocrine pancreas is scanty. This is surprising, since in the developing pancreas IGF-II is much more abundant than IGF-I.[2] We know that in cultured fetal and neonatal rat islets, IGF-II stimulates the DNA synthetic rate.[4,18] However, no more data are available concerning the actions of IGF-II on endocrine pancreatic cells. In the present study, we searched for receptors for IGF-I and -II in clonal insulin-, glucagon-, and somatostatin-secreting cell lines. We studied the effects of IGF-I and -II on islet hormone gene transcription on the release of insulin, glucagon, and somatostatin and the growth of HIT, INR1G9, and RIN 1027 B2 cells.

MATERIALS AND METHODS

Peptides, Plasmids, and Chemicals

^{125}I-IGF-I and ^{125}I-IGF-II were obtained from Amersham (Braunschweig, Germany); IGF-I (human recombinant) and IGF-II (human recombinant) were from Boehringer Mannheim (Penzberg, Germany). ^{3}H-thymidine and ^{14}C-chloramphenicol were from Amersham. Plasmid RINS-CAT contains 410 basepairs of the 5′ flanking region, 43 basepairs of exon 1, plus six basepairs of the first intron of the rat insulin I gene; plasmid gluc-CAT contains 350 basepairs of the rat proglucagon promotor; and plasmid SMS-CAT contains 900 basepairs of the rat somatostatin promotor. All three promotor sequences were directly linked to the reporter gene, chloramphenicol acetyltransferase (CAT). They were generously provided by Dr J.F. Habener (Boston, MA). All other chemicals used were purchased from Sigma (Deisenhofen, Germany).

Tissue Culture

Hamster glucagonoma INR1G9 cells and rat somatostatin-secreting RIN 1027 B2 cells were cultured in RPMI 1640 medium containing 11 mmol/L glucose supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL (vol/vol) streptomycin. Hamster insulinoma HIT cells were grown in RPMI 1640 medium containing 11 mmol/L glucose supplemented with 10% horse serum (vol/vol), 2.5% fetal calf serum (vol/vol), 100 U/mL penicillin, and 100 μg/mL streptomycin. All reagents were ob-

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Submitted June 14, 1995; accepted December 7, 1995.

Supported in part by grants from the Deutsche Forschungsgemeinschaft.

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0026-0495/96/4506-0015$03.00/0

tained from Gibco (Eggenstein, Germany). Cells were split 1:3 or 1:4 every 3 to 4 days.

Binding Studies: Characterization of Type I and Type II IGF Receptors

Cells were detached from the plastic dish with ice-cold Krebs-Ringer bicarbonate buffer (KRB) containing 10 mmol/L EDTA, centrifuged, and carefully resuspended in incubation buffer (Tris-hydrochloride 2.5 mmol/L, NaCl 120 mmol/L, $MgSO_4$ 1.2 mmol/L, KCl 5 mmol/L, and CH_3COONa 15 mmol/L, pH 7.40) containing 1% human serum albumin (Behring, Marburg, Germany), 0.1% bacitracin, and 1 mmol/L EDTA. Approximately 10^6 cells per tube (total vol, 300 μL) were incubated for 5 minutes in the presence of unlabeled hormone (1 pmol/L to 1 μmol/L) followed by addition of tracer (^{125}I-IGF-I or ^{125}I-IGF-II 25,000 cpm). The experiments were performed at 20°C. After 25 minutes of total incubation time, cells were centrifuged through a mixture of dibutylphthalate/dinonylphthalate (1:1 vol/vol). Radioactivity in the pellet was counted in a gamma-counter. Specific binding was defined as total binding minus nonspecific binding (tracer bound in the presence 1 μmol/L unlabeled hormone). Under these conditions, steady-state binding is reached after 30 minutes. Scatchard analysis was performed using the computer program, Inplot (Graph Pad, San Diego, CA).

Studies on Hormone Release

Cells were washed three times with KRB, detached from the plates with KRB-EDTA, and resuspended in KRB. Approximately 10^6 cells were incubated for 1 hour in KRB containing the test substances as indicated or the respective solvents at 37°C. Finally, cells were centrifuged, and insulin, glucagon, and somatostatin were determined in the supernatant using commercially available radioimmunoassay (RIA) kits. Insulin and glucagon RIA kits were from Biermann (Bad Nauheim, Germany). This insulin assay does not cross-react with IGF-I or IGF-II. The somatostatin RIA kit was obtained from Peninsula (Heidelberg, Germany).

Studies on Insulin, Glucagon, and Somatostatin Gene Transcription

Cells were detached from the culture plates with trypsin/EDTA and transfected in solution in one tube with 20 μg plasmid using the diethyl-aminoethyl-dextran technique. After transfection, cells were plated onto 35-mm dishes and incubated for 36 hours in serum-free media. Treatment was started 12 hours after transfection using 100× stock solutions of the growth factors. Finally, cells were harvested and CAT activity was determined. Acetylated and nonacetylated (^{14}C)chloramphenicol on the chromatography plates was identified and counted with a digital autoradiograph using the WINDAR software (Berthold, Bad Wildbad, Germany). The counting time was 30 minutes.

Studies on DNA Synthesis

Cells were plated onto 60-mm tissue culture dishes and grown overnight in medium containing only the antibiotics. On the next day, the medium was changed and supplemented with the IGFs as indicated. After 2 hours, 10 μCi ^{3}H-thymidine was added to each dish and incubation was performed for another 5 hours. Finally, DNA was precipitated with TCA and radioactivity was determined in a beta-counter.

Statistics

Data are presented as the mean ± SEM of four to eight experiments. Statistical analysis was performed using the one-tailed Student's *t* test for unpaired data. Statistical significance was set at the 5% level.

RESULTS

Clonal Insulin-, Glucagon-, and Somatostatin-Secreting Cells Express Receptors for IGF-I and IGF-II

The presence of receptors for IGF-I and IGF-II on clonal insulin-, glucagon-, and somatostatin-producing cells was demonstrated by receptor-binding assays. ^{125}I-IGF-I bound specifically to all three cell lines. The tracer was displaced from HIT cells and RIN 1027 B2 cells with higher affinity by IGF-I than by IGF-II (HIT cells: IGF-I, 6×10^{-10} mol/L, and IGF-II, 2×10^{-9} mol/L; RIN 1027 B2 cells: IGF-I, 7×10^{-10} mol/L, and IGF-II, 3×10^{-9} mol/L; Figs 1A, 2A, and 3A and Table 1). In INR1G9 cells, ^{125}I-IGF-I was

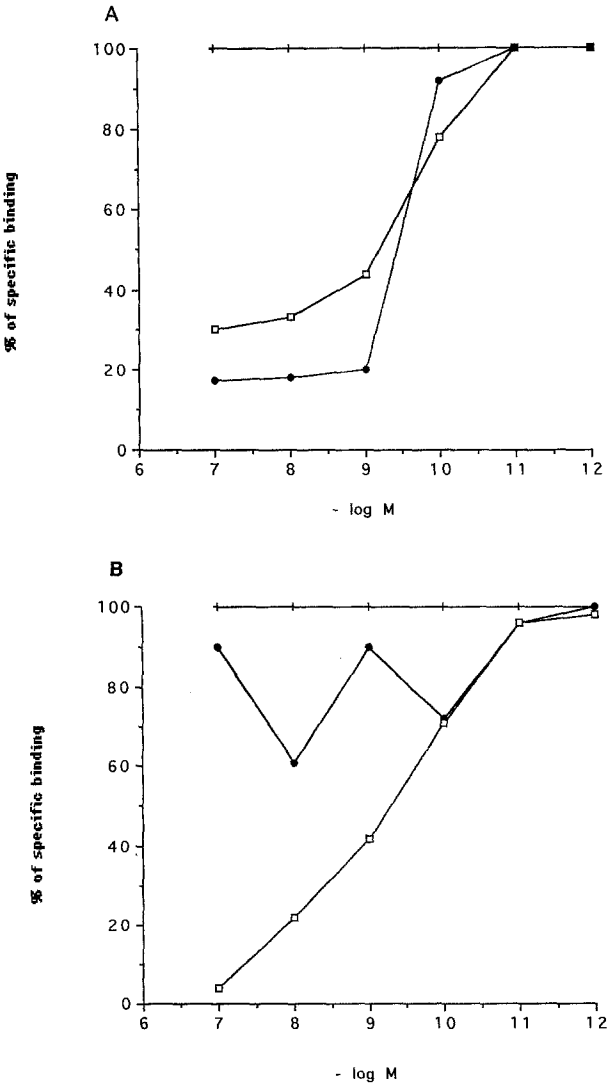

Fig 1. Characterization of type I and type II IGF receptors on insulin-producing HIT cells. (A) Cells were incubated with ^{125}I-IGF-I, and the tracer was displaced by IGF-I (●), IGF-II (□), and insulin (χ). (B) Cells were incubated with ^{125}I-IGF-II, and the tracer was displaced by IGF-I (●), IGF-II (□), and insulin (χ). Standard deviation <10%; n = 6 experiments.

displaced with the same affinity by IGF-I and IGF-II (IGF-I, 6×10^{-10} mol/L; IGF-II, 8×10^{-10} mol/L). This finding is consistent with the presence of classic IGF-I receptors (type I IGF receptors) on HIT cells and RIN 1027 B2 cells. Typically, these receptors bind IGF-I with a slightly higher affinity than IGF-II, and insulin with a much lower affinity.

^{125}I-IGF-II also bound specifically to all three cell types, but this tracer was displaced only by IGF-II (EC_{50}: HIT cells, 9×10^{-10} mol/L, INR1G9 cells, 3×10^{-9} mol/L, > RIN 1027 B2 cells, 8×10^{-10} mol/L), not by insulin or IGF-I (Figs 1B, 2B, and 3B and Table 2). These results are consistent with the expression of IGF-II receptors (type II IGF receptors) in these cells. It has been reported that IGF-II is the major ligand for the type II IGF receptor.

RIN 1027 B2 cells express a high number of IGF-I binding sites. In receptor-binding assays, 14.5% of the

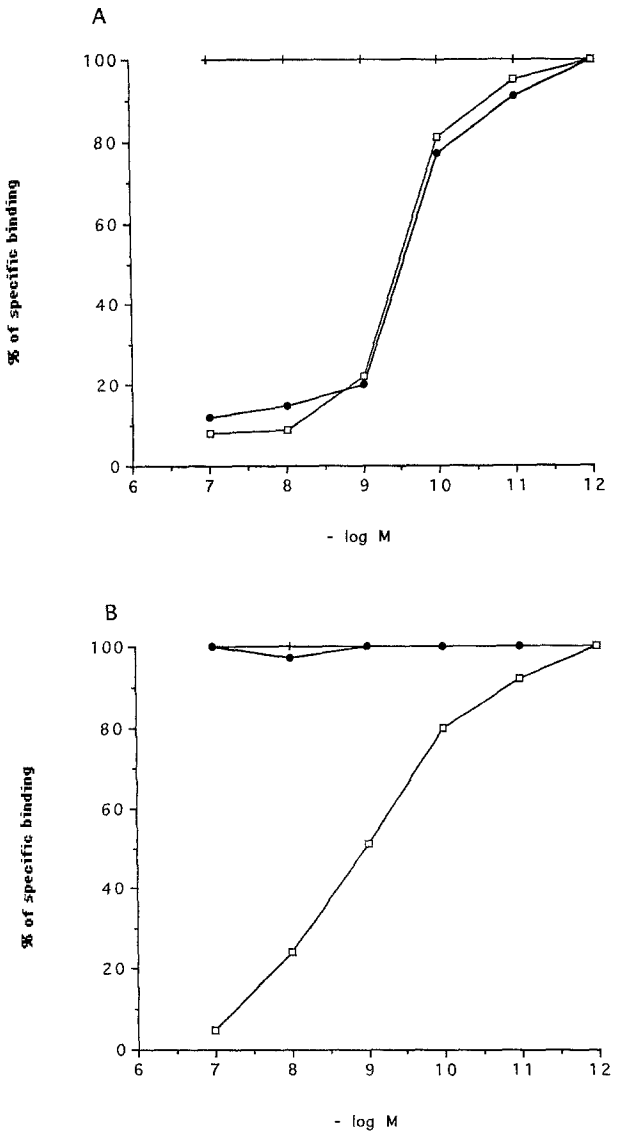

Fig 2. Characterization of type I and type II IGF receptors on glucagon-producing INR1G9 cells. (A) Cells were incubated with ^{125}I-IGF-I, and the tracer was displaced by IGF-I (●), IGF-II (□), and insulin (χ). (B) Cells were incubated with ^{125}I-IGF-II, and the tracer was displaced by IGF-I (●), IGF-II (□), and insulin (χ). Standard deviation <10%; n = 6 experiments.

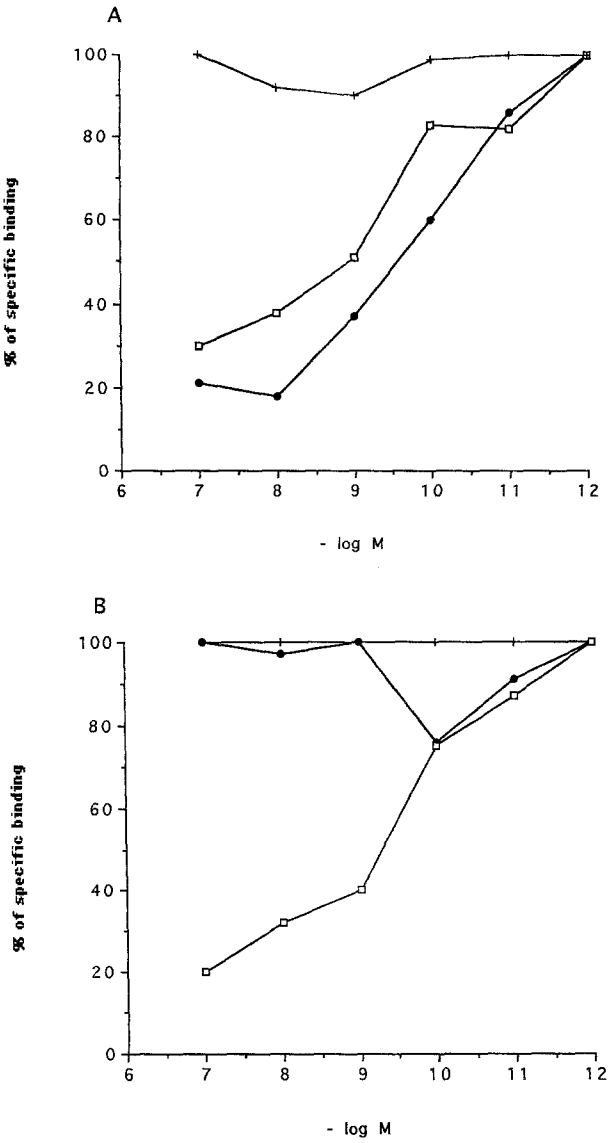

Fig 3. Characterization of type I and type II IGF receptors on somatostatin-producing RIN 1027 B2 cells. (A) Cells were incubated with ^{125}I-IGF-I, and the tracer was displaced by IGF-I (●), IGF-II (□), and insulin (χ). (B) Cells were incubated with ^{125}I-IGF-II, and the tracer was displaced by IGF-I (●), IGF-II (□), and insulin (χ). Standard deviation <10%; n = 5 experiments.

Table 1. EC_{50} Values (mol/L) for Binding Experiments With ^{125}I-IGF-I, IGF-I, and IGF-II Using HIT Cells (B cells), INR1G9 Cells (A cells), and RIN 1027 B2 Cells (D cells)

Cell Type	IGF-I	IGF-II
HIT	6×10^{-10}	2×10^{-9}
INRIG9	6×10^{-10}	8×10^{-10}
RIN 1027 B2	7×10^{-10}	3×10^{-9}

NOTE. The tracer was not displaced by insulin.

Table 2. EC_{50} Values (mol/L) for Binding Experiments With ^{125}I-IGF-II and IGF-II Using HIT Cells (B cells), INR1G9 Cells (A cells), and RIN 1027 B2 Cells (D cells)

Cell Type	IGF-II
HIT	9×10^{-10}
INR1G9	3×10^{-9}
RIN 1027 B2	8×10^{-10}

NOTE. The tracer was not displaced by insulin.

tracer bound to 10^6 cells. By Scatchard plot analysis, the number of IGF-I binding sites was calculated as approximately 400,000 per cell. Significantly less IGF-I binding sites reside on INR1G9 cells (0.8% of the tracer bound to 10^6 INR1G9 cells, ~15,000 sites per cell). All three cell types contain the same number of IGF-II binding sites (~15,000 per cell).

IGF-I and IGF-II Regulate Islet Hormone Gene Expression

In the next set of experiments, we characterized the effects of IGF-I and IGF-II on islet hormone gene expression. Cells were transiently transfected with constructs that each contained the promotor of the insulin, glucagon, or somatostatin gene linked to the reporter gene, CAT. In HIT cells, activity of the rat insulin I promotor was weakly stimulated by IGF-II, with a maximum at 0.1 nmol/L (42% above control levels, $P < .05$; Fig 4), whereas IGF-I did not influence CAT activity (controls, 100%; 1 pmol/L IGF-I, 108%; 10 pmol/L IGF-I, 97%; 100 pmol/L IGF-I, 103%; 1 nmol/L IGF-I, 87%; 10 nmol/L IGF-I, 104%; 100 nmol/L IGF-I, 92%). Gene transcription of the proglucagon gene was potently suppressed by IGF-I (40% inhibition at 100 nmol/L IGF-I, $P < .05$; 37% inhibition at 10 nmol/L IGF-I, $P < .05$; Fig 5), but it remained unaffected by IGF-II (10 pmol/L IGF-II, 95%; 100 pmol/L IGF-II, 92%; 1 nmol/L IGF-II, 81%; 10 nmol/L IGF-II, 91%; 100 nmol/L IGF-II, 90%). Somatostatin promotor activity was increased by both IGF-I and IGF-II. The maximal effect of

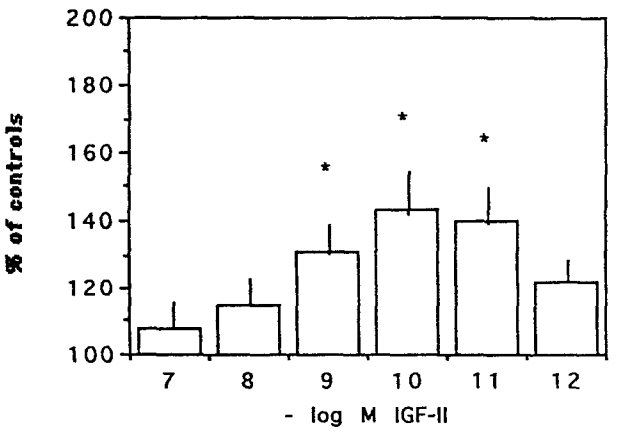

Fig 4. Stimulation of proinsulin gene transcription by IGF-II in HIT cells. Cells were transiently transfected with a plasmid containing 410 bp of the rat insulin I promotor linked to the reporter gene CAT. Treatment with IGF-II was performed for 24 hours. *$P < .05$. n = 6 experiments.

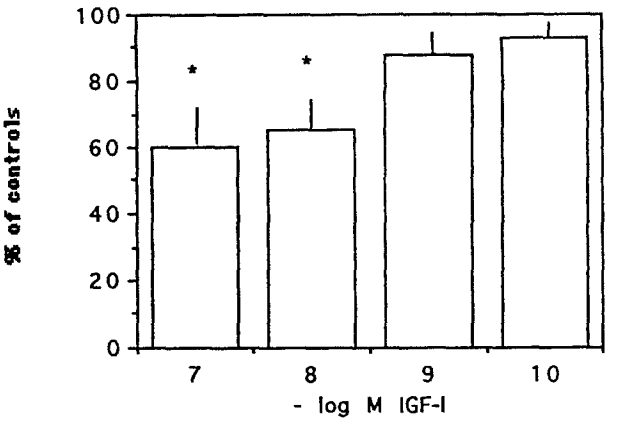

Fig 5. Inhibition of proglucagon gene transcription by IGF-I in INR1G9 cells. Cells were transiently transfected with a plasmid containing 350 bp of the rat proglucagon promotor linked to the reporter gene CAT. Treatment with IGF-II was performed for 24 hours. *$P < .05$. n = 8 experiments.

IGF-I occurred at 10 nmol/L IGF-I (155%, $P < .05$; Fig 6A), and IGF-II action was maximal at 10 nmol/L IGF-II (146%, $P < .05$; Fig 6B).

IGF-I and IGF-II and Islet Hormone Release

Neither IGF-I nor IGF-II influenced insulin release from HIT cells (data not shown). Glucagon release from INR1G9 cells was potently inhibited in a concentration-dependent manner by IGF-I (controls, 100%; 1 nmol/L IGF-I, 50%, $P < .05$; 10 nmol/L IGF-I, 56%, $P < .05$; 100 nmol/L IGF-I, 35%, $P < .05$; Table 3). Insulin is a potent inhibitor of glucagon secretion. At 100 nmol/L, insulin decreased glucagon release less potently than IGF-I (controls, 100%; insulin, 61%; IGF-I, 35%). IGF-II decreased glucagon secretion only at 10 nmol/L (26% inhibition, $P < .05$; Table 3). Somatostatin release from RIN 1027 B2 cells was not influenced by IGF-I (controls, 100%; 100 nmol/L IGF-I, 94%; 10 nmol/L IGF-I, 94%; 1 nmol/L IGF-I, 91%), whereas IGF-II increased somatostatin secretion with a maximal effect at 1 nmol/L (controls, 100%; 1 nmol/L IGF-II, 164%, $P < .05$; Table 4).

IGF-I and IGF-II Regulate DNA Synthesis of Clonal Insulin-, Glucagon-, and Somatostatin-Secreting Cells

IGF-I and IGF-II increased ^{3}H-thymidine incorporation into the DNA of all three cell types. In HIT cells, IGF-II stimulated DNA synthesis more potently than IGF-I. The effect of both hormones was maximal at 10 nmol/L (controls, 100%; 10 nmol/L IGF-I, 125%; 10 nmol/L IGF-II, 146%; Table 5). DNA synthesis of INR1G9 cells was stimulated maximally by 100 nmol/L IGF-I (controls, 100%; 100 nmol/L IGF-II, 168%). ^{3}H-thymidine incorporation into DNA of RIN 1027 B2 cells was also increased by IGF-I and IGF-II, and the maximal effect occurred at 100 nmol/L (controls, 100%; 100 nmol/L, IGF-I, 226%; 1 nmol/L IGF-II, 133%; 10 nmol IGF-II, 130%).

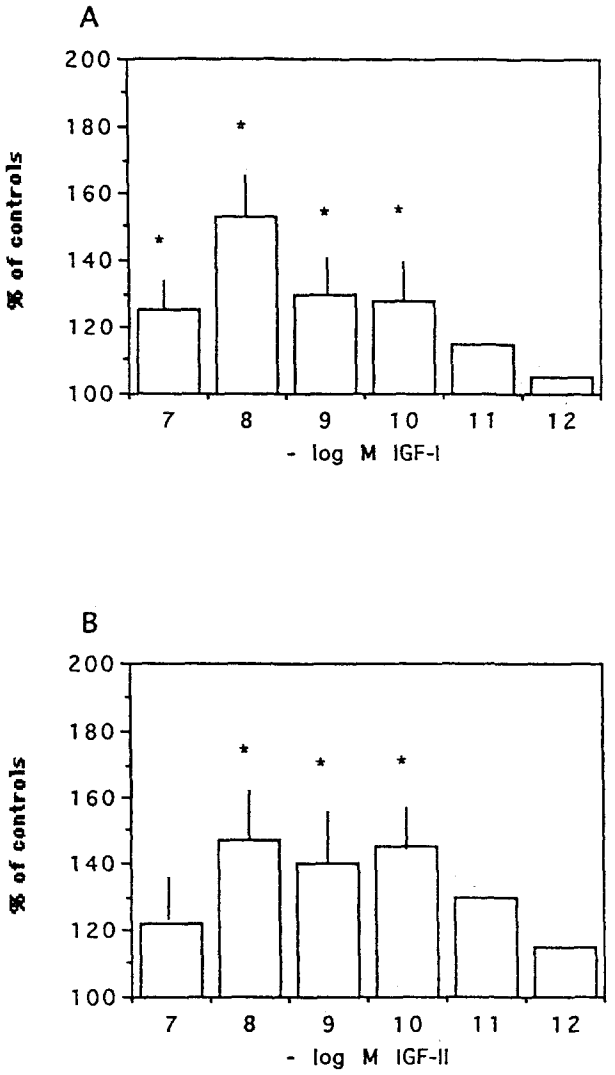

Fig 6. Stimulation of prosomatostatin gene transcription by IGF-I (A) and IGF-II (B) in somatostatin-producing RIN 1027 B2 cells. Cells were transfected with a plasmid containing 900 bp of the rat prosomatostatin promotor. Treatment with IGF-I and IGF-II was performed for 24 hours. *$P < .05$. n = 6 experiments.

DISCUSSION

This study demonstrates that clonal endocrine pancreatic cell lines express specific receptors for IGF-I and IGF-II. IGFs have contrasting effects on insulin-, glucagon-, and somatostatin-producing cell function. In our opinion, these data are important to better understand the function of the endocrine pancreas. Therefore, several aspects of this study should be discussed in greater detail.

Table 3. Effect of IGF-I and IGF-II on Glucagon Release From INR1G9 Cells (n = 6 experiments)

Peptide Concentration (-log M)	IGF-I	IGF-II
Control	100%	100%
10	82% ± 1%	93% ± 5%
9	50% ± 2%*	ND
8	56% ± 12%*	74% ± 3%*
7	35% ± 7%*	111% ± 10%

Abbreviation: ND, not determined.
*$P < .05$.

Table 4. Effect of IGF-I and IGF-II on Somatostatin Release From RIN 1027 B2 Cells (n = 4 experiments)

Peptide Concentration (-log M)	IGF-I	IGF-II
Control	100%	100%
9	91% ± 10%	164% ± 18%*
8	94% ± 3%*	137% ± 21%*
6	94% ± 6%	147% ± 15%*

*$P < .05$.

With receptor-binding assays, we have shown that insulin-producing HIT cells and somatostatin-producing RIN 1027 B2 cells express classic type I and type II IGF receptors. The type I IGF receptor binds IGF-I with a slightly higher affinity than IGF-II, but insulin is only a weak ligand at this receptor. Glucagon-producing INR1G9 cells express IGF-I receptors with the same affinity for IGF-I and insulin. Whether this is caused by hybrid receptors of the type I IGF receptors and insulin receptors remains to be established. Rat pancreatic A cells purified from rat pancreatic islets express classic type I receptors.[12] The type II IGF receptor binds only IGF-II, not insulin or IGF-I. Our data are in good agreement with a recent study suggesting the presence of type I IGF receptors on B and A cells.[12] In this study, ^{125}I-IGF-I bound to purified A cells and B cells with a K_d of 2×10^{-9} mol/L, which is similar to our data obtained with cultured cell lines (A cells, EC_{50} 6×10^{-10} mol/L; B cells, 5×10^{-10} mol/L). In addition, we show that both cell types also possess type I and type II IGF receptors. We found a much higher number of IGF-I binding sites on somatostatin-secreting RIN 1027 B2 cells than on the other cell types, but IGF-I stimulated DNA synthesis at equal potency in INR1G9 and RIN 1027 B2 cells.

IGF-I and IGF-II are expressed in endocrine pancreatic cells during embryonic development. In fetal rat and human pancreas, IGF-I immunoreactivity and proinsulin mRNA

Table 5. Effects of IGF-I and IGF-II on ^{3}H-Thymidine Incorporation Into DNA of HIT Cells, INR1G9 Cells, and RIN 1027 B2 Cells (n = 6 experiments)

Peptide Concentration (-log M)	IGF-I	IGF-II
HIT cells		
Control	100%	100%
9	93% ± 12%	144% ± 8%*
8	125% ± 15%*	146% ± 9%*
7	94% ± 13%	117% ± 3%
INR1G9 cells		
Control	100%	100%
9	114% ± 13%	119% ± 11%
8	104% ± 5%	127% ± 19%*
7	205% ± 26%*	168% ± 23%*
RIN 1027 B2 cells		
Control	100%	100%
9	101% ± 19%	133% ± 15%*
8	126% ± 12%*	130% ± 16%*
7	226% ± 5%*	117% ± 22%

*$P < .05$.

were localized in B cells.[1-3,5] In adult pancreas, IGF-II was detected in A and D cells, but not in B cells.[19] In contrast, IGF-I is colocalized in adult pancreas with insulin, but it is absent in A and D cells.[19,20] These data indicate the synthesis of both peptides in these cells, rather than peptide sequestration. Furthermore, both peptides are secreted from isolated fetal islets in response to various stimuli.[3,6] mRNA levels for IGF-II were much higher than those for IGF-I.[5] They decline in islets during neonatal life, as reported in other tissues.[5] Based on these data, it can be anticipated that IGFs may play a role as important regulators of endocrine pancreatic function during development and probably also during postnatal life. Both IGF-I and -II increase the DNA synthetic rate in fetal rat islets.[4,13,14] This study demonstrates that both growth factors stimulate DNA synthesis in all three major islet cell types.

No consensus exists concerning the action of IGF-I on insulin release and biosynthesis, since inhibition and stimulation have been described, as well as no effect.[13,15-17] But this is probably due to the use of different experimental models, since isolated fetal and adult islets and the isolated perfused rat pancreas were used. We found no effect of IGF-I or IGF-II on insulin release from HIT cells. Furthermore, IGF-I did not influence proinsulin gene transcription. On the other hand, we observed a weak induction of proinsulin gene transcription by IGF-II. In agreement with previous data, we found a stimulation of DNA synthesis in these insulin-producing cells by IGF-I and IGF-II.[4,13,15] Therefore, the peptides differently influence the function of pancreatic B cells.

In A cells (INR1G9 cells), a stimulation of the DNA synthetic rate was shown in response to IGF-I and IGF-II. A strong inhibition of proglucagon gene transcription and glucagon release was induced by IGF-I, but not by IGF-II. In D cells (RIN 1027 B2 cells), both IGFs increased DNA synthesis and somatostatin gene expression, but only IGF-II stimulated somatostatin release. Therefore, IGF-I and IGF-II also show contrasting effects on the different functions of endocrine pancreatic cells. Thus, peptide hormone gene transcription and peptide hormone release are differentially regulated by IGF-I and IGF-II in these cells. As mentioned earlier, during development IGF-I and IGF-II are located in endocrine pancreatic cells. Fetal pancreatic islets contain mostly B cells. Therefore, it can be speculated that IGFs play a role as autocrine and paracrine mediators (growth factor?) within the islet homeostasis. Such an effect could be mediated by paracrine mechanisms, since blood flow in the islets first reaches the B cells and then the A and D cells.[21] This hypothesis has to be addressed in future studies; it could be verified by immunoneutralization or gene knock-out experiments.

IGF-I has several effects on glucose metabolism. In particular, IGF-I exerts several insulinomimetic effects when infused into humans.[8-11,22] It suppresses plasma levels of insulin, C-peptide, glucagon, and free fatty acids.[22] Glucose uptake and oxidation are stimulated similarly by both hormones.[22] During insulin-induced hypoglycemia, the increase in glucagon secretion was inhibited by intravenous infusion of IGF-I.[23] This IGF-I effect was even more potent than insulin-induced suppression of glucagon release. From these data, it was concluded that IGF-I mediates its effects on A cells via specific receptors.[23] In the present study, we provide evidence that A cells indeed express functional, active IGF-I receptors. IGF-I directly inhibits glucagon secretion and proglucagon gene expression and stimulates DNA synthesis in these cells. Thus, IGF-I influences several functions of A cells.

Insulin has been well characterized as a potent inhibitor of glucagon release and proglucagon gene transcription.[24,25] In INR1G9 cells, IGF-I inhibits glucagon release more potently than insulin[24] (and this study). Interestingly, a similar effect of IGF-I on glucagon secretion has been demonstrated in man.[23] On the other hand, both peptides inhibit proglucagon gene transcription with similar potency[24] (and this study). Recently, an "insulin-response element" was shown to be located in the proglucagon promotor.[25] Further studies will show whether this DNA sequence also mediates the IGF-I effect on proglucagon gene transcription. This is possible, since both peptides use similar second-messenger pathways (see below).

Several studies were performed to characterize plasma levels of IGFs in patients with non–insulin-dependent and insulin-dependent diabetes mellitus. In most studies, decreased levels of IGF-I were found.[26] These data, together with the results of other studies, indicate a relationship between IGF-I and glycemic control, especially since higher IGF-I plasma levels were seen after improvement of glucose metabolism. It was therefore speculated that the decreased IGF-I plasma levels contribute to the elevated plasma glucagon concentrations typically found in diabetic patients. Consequently, patients with type II diabetes were experimentally treated with IGF-I.[11,27] It was clearly shown that short-term administration of IGF-I improved glycemic control in these patients.[28,29] But the risk to benefit ratio, especially of a long-term treatment, should be assessed carefully.

Much less is known about changes of IGF-II plasma concentrations during deterioration of glucose metabolism. Here, increased, decreased, and unchanged IGF-II levels have been determined.[26] Nonislet tumor-associated hypoglycemia is often caused by IGF-II secretion from the tumor itself.[30] Several mechanisms account for this hypoglycemia: in some cases, "big IGF-II" is secreted that does not form the 150-kd complex with these binding proteins. This allows more IGF-II to interact with IGF-I, IGF-II, and insulin receptors. Furthermore, growth hormone and insulin secretion are impaired.[31] In addition, IGF-II may suppress glucagon secretion both directly and indirectly by a paracrine effect mediated via type I IGF receptors, and probably also by increased somatostatin secretion (this study).

The IGF-I receptor belongs to the family of growth factor receptors with the common feature of a tyrosine kinase in the cytoplasmic part of the B-chain.[26] After binding of the ligand to the extracellular binding domain, the receptor is activated by autophosphorylation. Then it phosphorylates itself and several effector proteins necessary for further

signal transduction. It was suggested that receptors for insulin and IGF-I use similar or even identical signal transduction pathways.[26] Future studies will show whether this is also true for endocrine pancreatic cells. Of special interest will be the question of whether the same signal transduction pathways and mediators are used in these cells to trigger the described stimulatory and inhibitory effects.

Taken together, this study demonstrates the presence of functional, active type I and type II IGF receptors on clonal insulin-producing (HIT), glucagon-producing (INR1G9), and somatostatin-producing (RIN 1027 B2) cells. In HIT cells, IGF-I and IGF-II stimulate ^{3}H-thymidine incorporation, but proinsulin gene transcription was stimulated only by IGF-II and insulin release was not influenced. Proglucagon gene transcription and glucagon secretion were potently inhibited by IGF-I and weakly by IGF-II. DNA synthesis of glucagon-producing cells was increased by both peptides. In somatostatin-producing RIN 1027 B2 cells, ^{3}H-thymidine incorporation was stimulated by IGF-I and IGF-II. Somatostatin release was not influenced. Proglucagon gene transcription and glucagon secretion were potently inhibited by IGF-I and weakly by IGF-II. DNA synthesis of glucagon-producing cells was increased by both peptides. In somatostatin-producing RIN 1027 B2 cells, ^{3}H-thymidine incorporation was stimulated by IGF-I and IGF-II. Somatostatin release was increased by IGF-II, but was not influenced by IGF-I. Both peptides stimulated somatostatin gene transcription in RIN 1027 B2 cells. IGF-I and IGF-II have distinct biological actions in A, B, and D cells.

ACKNOWLEDGMENT

This study is part of the doctoral thesis of U.M.

REFERENCES

1. Han VKM, Hill DJ, Strain AJ, et al: Identification of somatomedin/insulin-like growth factor immunoreactive cells in the human fetus. Pediatr Res 22:245-249, 1987

2. Han VKM, Lund PK, Lee DC, et al: Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: Identification, characterization and tissue distribution. J Clin Endocrinol Metab 66:422-429, 1988

3. Hill DJ, Frazer A, Swenne I, et al: Somatomedin-C in human fetal pancreas. Cellular localization and release during organ culture. Diabetes 36:465-471, 1987

4. Hogg J, Han VKM, Clemmons Dr, et al: Interactions of nutrients, insulin-like growth factors (IGFs) and IGF-binding proteins in the regulation of DNA synthesis by isolated fetal rat islets. J Endocrinol 138:401-412, 1993

5. Lund PK, Moats-Staats BM, Hynes MA, et al: Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in fetal and adult tissues. J Biol Chem 261:14539-14544, 1986

6. Scharmann R, Corvol M, Czernichow P: Characterization of insulin-like growth factor I produced by fetal rat pancreatic islets. Diabetes 38:686-690, 1989

7. Smith FE, Rosen KM, Villa-Komaroff L, et al: Enhanced insulin-like growth factor I gene expression in regenerating rat pancreas. Proc Natl Acad Sci USA 88:6152-6156, 1991

8. Boulware SD, Tamborlane WV, Matthews LS, et al: Diverse effects of insulin-like growth factor I on glucose, lipid, and amino acid metabolism. Am J Physiol 262:E130-E133, 1992

9. Boulware SD, Tamborlane WV, Rennert NJ, et al: Comparison of the metabolic effects of recombinant human insulin-like growth factor-I and insulin. J Clin Invest 93:1131-1139, 1994

10. Guler HP, Zapf J, Froesch ER: Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. N Engl J Med 317:137-140, 1987

11. Shaich DS, Turman NJ, Marcsisin VS, et al: Short-term effects of recombinant human insulin-like growth factor I on metabolic control of patients with type II diabetes mellitus. J Clin Endocrinol Metab 77:1563-1568, 1993

12. Van Schravendijk CFH, Foriers A, Brande JLV, et al: Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. Endocrinology 121:1784-1788, 1987

13. Swenne I, Heldin CH, Hill DJ, et al: Effects of platelet-derived growth factor and somatostatin-C/insulin-like growth factor I on the DNA replication of fetal rat islets of Langerhans in tissue culture. Endocrinology 122:214-218, 1988

14. Swenne I, Hill DJ, Hellerstrom C: Growth hormone regulation of DNA synthesis, but not insulin production, is mediated by somatostatin-C/insulin-like growth factor I in isolated pancreatic islets. Diabetologia 32:191-197, 1988

15. Sieradzki J, Fleck H, Chatterjee AK, et al: Stimulatory effect of insulin-like growth factor-I on (^{3}H) thymidine incorporation, DNA synthesis and insulin biosynthesis, and secretion of isolated pancreatic rat islets. J Endocrinol 117:59-62, 1988

16. Leahy JL, Vanderkerkhove KM: Insulin-like growth factor-I at physiological concentrations is a potent inhibitor of insulin secretion. Endocrinology 126:1593-1598, 1990

17. Van Schravendijk CFH, Heylen L, Van den Brande JL, et al: Direct effect of insulin and insulin-like growth factor-I on the secretory activity of rat pancreatic beta cells. Diabetologia 33:649-653, 1990

18. Rabinovitch A, Quigley C, Russel T, et al: Insulin and multiplication activity (an insulin-like growth factor) stimulate islet β-cell replication in neonatal rat pancreatic monolayer culture. Diabetes 31:160-164, 1982

19. Maake C, Reinecke M: Immunohistochemical localization of insulin-like growth factor I and II in the endocrine pancreas of rat, dog and man and their coexistence with classical islet hormones. Cell Tissue Res 273:249-259, 1993

20. Höög A, Grimelius L, Falkmer S, et al: A high molecular weight IGF-II immunoreactive peptide (pro-IGF-II?) in the insulin cells of the islets of Langerhans in pancreas of man and rat. Regul Pept 47:275-282, 1993

21. Weir GC, Bonner-Weir S: Islets of Langerhans: The puzzle of intraislet interactions and their relevance to diabetes. J Clin Invest 85:983-987, 1990

22. Hussain MA, Schmitz O, Mengel A, et al: Insulin-like growth factor I stimulates lipid oxidation, reduces protein oxidation, and enhances insulin sensitivity in humans. J Clin Invest 92:2249-2256, 1993

23. Kerr D, Tamborlane WV, Rife F, et al: Effect of insulin-like growth factor-I on the responses to and recognition of hypoglycemia in humans. A comparison with insulin. J Clin Invest 91:141-147, 1993

24. Fehmann HC, Strowski M, Lankat-Buttgereit B, et al:

Molecular and functional characterization of insulin receptors present on hamster glucagonoma cells. Digestion 55:214-220, 1994

25. Philipps J: Insulin regulation of the glucagon gene is mediated by an insulin-responsive DNA element. Proc Natl Acad Sci USA 88:7224-7227, 1991

26. Bach LA, Rechler MM: Insulin-like growth factors and diabetes. Diabetes Metab Rev 8:229-257, 1992

27. Zenobi PD, Jaeggi-Groisman SE, Riesen WF, et al: Insulin-like growth factor-I improves glucose and lipid metabolism in type 2 diabetes mellitus. J Clin Invest 90:2234-2242, 1992

28. Kuzuya H, Matsuura N, Sakamoto H, et al: Trial of insulin-like growth factor I therapy for patients with extreme insulin resistance syndrome. Diabetes 42:696-705, 1993

29. Schoenle EJ, Zenobi PD, Torresani T, et al: Recombinant human insulin-like growth factor I (rhIGF I) reduces hyperglycemia in patients with extreme insulin resistance. Diabetologia 34:675-679, 1991

30. Lowe WL Jr, Roberts CT Jr, LeRoith D, et al: Insulin-like growth factor-II in non–islet cell tumors associated with hypoglycemia: Increased levels of messenger ribonucleic acid. J Clin Endocrinol Metab 69:1153-1159, 1989

31. LeRoith D: Insulin-like growth factors in health and disease. Ann Intern Med 116:854-862, 1992