Characterization of Insulinlike Growth Factor I Produced by Fetal Rat Pancreatic Islets

RAPHAËL SCHARFMANN, MAITÉ CORVOL, AND PAUL CZERNICHOW

Pancreatic islets were prepared from 22-day-old rat fetuses. After 5 days of culture in dishes allowing cell attachment, neoformed islets were kept free floating in RPMI-1640 medium (16.5 mM glucose, 1% fetal calf serum). The islets were then pulsed with [^{3}H]leucine and [^{35}S]methionine for 24 h. The conditioned medium was acidified with acetic acid (final pH 2.7), desalted, concentrated, and gel filtered on Bio-Gel P100 in acid conditions. The radioactive material that comigrated with immunoreactive insulinlike growth factor I (IGF-I) produced by the islets was pooled, concentrated, and further characterized by reverse-phase high-performance liquid chromatography on a C_{18} Bondapak column with a linear gradient of acetonitrile (20–80%). The radioactive material that eluted as pure IGF-I (40% acetonitrile) was further studied by chromatofocusing on a Pharmacia PBE 94 column. A sharp radioactive peak containing [^{3}H]leucine and [^{35}S]methionine was eluted at pH 8.55. This material was immunoprecipitated with an antiserum to IGF-I. This study demonstrated that fetal islet cells synthesize molecules that are, by several criteria, equivalent to native IGF-I. *Diabetes* 38:686–90, 1989

The somatomedins are growth-promoting factors, which include somatomedin C insulinlike growth factors I (IGF-I) and II (IGF-II) (1). These peptides, first thought to exert mitogenic effects in an endocrine fashion (2), have been shown to originate from many different tissues and to act in a paracrine or autocrine fashion (3). Indeed, peptides that cross-react with anti-IGF-I antibodies are secreted by many types of cells in tissue culture (4–8) and are involved in the regulation of DNA synthesis in those cells (9). Some of the peptides have been partially purified and biochemically characterized (7,10,11).

Several factors are involved in the regulation of proliferation of the pancreatic β-cell (12). Two of them, IGF-I and IGF-II, have mitogenic effects on rat neonatal islet β-cell in culture (13,14). Similarly, specific radioreceptor assays and radioimmunoassays (RIAs) have shown that IGF accumulates in the conditioned medium of these cells (14). More recently, Swenne et al. (15) confirmed the secretion of immunoreactive IGF-I (IR-IGF-I) by the fetal rat islets in tissue culture and demonstrated the regulation of IR-IGF-I secretion by growth hormone.

In this article, we report the partial purification and biochemical characterization of IGF-I produced and secreted by fetal pancreatic rat islets in culture. We show that these cells synthesize molecules that are, according to several criteria, equivalent to native IGF-I.

MATERIALS AND METHODS

Preparation and culture of fetal islets. Fetuses were removed from pregnant Wistar rats (Iffa Credo, l'Arbresle, France) at 21 days of gestation. The day of mating was considered day 0. Fetal islet cells were prepared according to the method of Hellerström et al. (16) as previously described (17). Briefly, pregnant rats were killed by cervical dislocation, and the fetuses were removed and decapitated. The pancreases were removed aseptically, placed in cold Hanks' balanced salt solution (HBSS) supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin (Flow, Rockville, MD), and minced. Four milliliters of HBSS containing 6 mg/ml collagenase (Boehringer Mannheim, Mannheim, FRG) was added to each of four centrifuge tubes containing ~10 pancreases each. Digestion was performed in a shaking water bath at 37°C for 4–5 min. The resulting tissue digests were washed three times with cold HBSS, and four pellets were pooled and resuspended in 500 μl HBSS. Aliquots (100 μl) of this suspension were distributed among 50-mm plastic culture dishes (model 3002, Falcon, Los Angeles, CA). The islets were cultured for 5 days in 5 ml RPMI-

From the Institut National de la Santé et de la Recherche Médicale (Unit 30), Hôpital des Enfants Malades, Paris, France.

Address correspondence and reprint requests to Paul Czernichow, MD, Hôpital Robert Debzé 48 Boulevard Serurier Paris 19, France.

Received for publication 15 August 1988 and accepted in revised form 22 November 1988.

1640 medium (Flow) containing 11 mM glucose, 10% heat-inactivated fetal calf serum (FCS), 50 U/ml penicillin, and 50 μg/ml streptomycin. The culture dishes were kept at 37°C in a humidified atmosphere of 5% CO_2/95% air. The complete growth medium was changed daily. After the culture period, β-cell–rich islets, devoid of nonendocrine tissue, could be removed with a sterile pipette. The isolated islets were transferred to petri dishes (300 islets/dish) and cultured free floating in 5 ml RPMI-1640 containing 16.5 mM glucose. The culture medium was supplemented with 1% FCS and antibiotics.

Labeling. After 2 days in culture, the medium was changed to the same medium without leucine and methionine. One hundred fifty microcuries each of L-[4-5-^{3}H]leucine and L-[^{35}S]methionine (sp act 171 and 1400 Ci/mmol, respectively; Amersham, Les Ulis, France) was added to each dish. The conditioned medium was collected 24 h later, rapidly acidified with acetic acid (final pH 2.7), desalted on PD10 columns (Pharmacia, Bois d'Arcy, France) in 1 M acetic acid, and dried in polypropylene tubes.

Gel-filtration chromatography. Gel-filtration chromatography was carried out on Bio-Gel P100 columns (1.6 × 100 cm; Bio-Rad, Richmond, CA) in 1 M acetic acid (pH 2.7). The column, previously calibrated with standard proteins, was run at room temperature (6 ml/h), and 4-ml fractions were collected. Aliquots of each fraction were assayed for IGF-I and insulin by specific RIAs. The radioactivity of each fraction was measured in a liquid-scintillation counter with 4 ml of Biofluor (Du Pont de Nemours, Paris, France).

Reverse-phase high-performance liquid chromatography (HPLC). Filtered water and HPLC-grade solvents (Baker, Deventer, The Netherlands) were used. The samples, reconstituted in 500 μl of 0.1% trifluoracetic acid (TFA) were subjected to HPLC (Waters, Millipore, Bedford, MA) on a microBondapak C_{18} column (7.6 × 300 mm; Waters). Elution was carried out with a linear gradient of acetonitrile in 0.1% TFA in water. One-milliliter fractions were collected (flow rate 1.0 ml/min). The radioactivity in each fraction was counted. The fractions were then concentrated and kept at 4°C for further analysis.

C_{18} Sep Pak experiments. Sep Pak C_{18} cartridges (Waters) were prewashed with 5 ml isopropyl alcohol, 5 ml methanol, and 10 ml 0.1% TFA. Samples were applied to the prewet cartridges, washed with 6 ml of 20% acetonitrile, 0.1% TFA, and 80% water and eluted with 5 ml of 50% acetonitrile, 0.1% TFA, and 50% water.

Chromatofocusing. A column (1 × 30 cm) of polybuffer exchanger (PBE 94; Pharmacia, Uppsala, Sweden) was equilibrated with 0.025 M ethanolamine HCl (pH 9.5). The dried samples were diluted in 200 μl of polybuffer 96 (PB 96, Pharmacia), applied on the column, and eluted with 10% (vol/vol) PB 96 (pH 5.8, flow rate 25 ml/h). After the gradient, the column was washed with two volumes of 1 M NaCl. The pH, radioactivity, IR-IGF-I, and immunoreactive insulin (IRI) were measured for each fraction.

RIAs. The IGF-I RIA was performed with purified human IGF-I (Humbel, Zurich, Switzerland) as standard and a rabbit antiserum (lot UBK 487, supplied by L.E. Underwood and J.J. Van Wyk, Univ. of North Carolina, via the National Hormone and Pituitary Program) directed against IGF-I somatomedin C (18). The antiserum has 0.5% cross-reactivity with IGF-II and cross-reacts minimally with insulin at 10^{-6} M. Recombinant IGF-I was iodinated by the chloramine-T method (19) and purified by chromatography on Sephadex G50 (Pharmacia) in phosphate-buffered saline (PBS; pH 7). The insulin RIA was performed with insulin antiserum (no. 8309; Novo, Copenhagen) and porcine insulin (Novo) as standard.

Immunoprecipitation. Immunoprecipitation was performed as described for insulin with minor modifications (20). Briefly, 10 μl IGF-I antiserum (same lot as used for IGF-I RIA) was diluted 1:10 and incubated overnight at 4°C with the sample previously concentrated and then diluted in 0.03 M PBS/bovine serum albumin (BSA) buffer (0.5% vol/vol; pH adjusted to 7.5 with HCl). The total incubation volume was 100 μl of PBS/BSA buffer. Antibody-antigen complex was then precipitated by adding 5 mg of protein A Sepharose (Pharmacia) in 100 μl of PBS/BSA buffer. The contents of the tubes were mixed at room temperature for 15 min, and the tubes were centrifuged. The pellet was washed twice with 200 μl of PBS/BSA buffer, suspended in 400 μl of 1 M acetic acid and 2.5 mg/ml of BSA, and centrifuged. Radioactivity in the supernatant was measured. Nonspecific binding of radioactive material to either the antiserum or the protein A Sepharose was determined in parallel incubations by use of nonimmune serum in place of anti-IGF-I serum. Nonspecific binding was <5% of the total bound radioactivity and was subtracted for the expression of the results.

RESULTS

CHROMATOGRAPHY ON Bio-Gel P-100 AND RIA OF IGF-I

Two IR-IGF-I peaks were detected when the conditioned medium (24-h incubation) was chromatographed on Bio-Gel P-100 (Fig. 1*A*). The low-molecular-weight peak eluted with iodinated IGF-I. RIA of this material was used to quantify the IGF-I secreted into the medium by islet cells. In our experimental conditions, the islets secreted 585 ± 94 pg IR-IGF-I/μg of islet DNA in 24 h (mean ± SE of 6 experiments). The other immunoreactive peak eluted with high-molecular-weight proteins could correspond partly to binding proteins (14,21).

IN VITRO LABELING EXPERIMENTS AND IGF-I CHARACTERIZATION

Bio-Gel P-100. Four major radioactive peaks were detected when conditioned medium of islets incubated in the presence of radioactive amino acids was analyzed on Bio-Gel P-100 in acidic conditions (Fig. 1*B*). The largest peak, eluted near the void volume of the column, represented all the newly synthesized labeled proteins excluded from the gel (M_r >50,000). Nonincorporated labeled amino acids or radioactive peptides of M_r <3000 were eluted in the salt volume of the column.

A large radioactive peak eluted at the same volume as labeled insulin and IRI. A small percentage (5%) of the total radioactivity eluted from the column migrated as ^{125}I-labeled IGF-I. It comigrated with IGF-I produced by the islets as measured by RIA (Fig. 1*A*).

Reverse-phase HPLC. Fractions 23 and 24 collected from the gel-filtration column were pooled, concentrated, and diluted in 500 μl of 0.1% TFA. This material was then subjected to HPLC on a C_{18} reverse-phase column and eluted with a

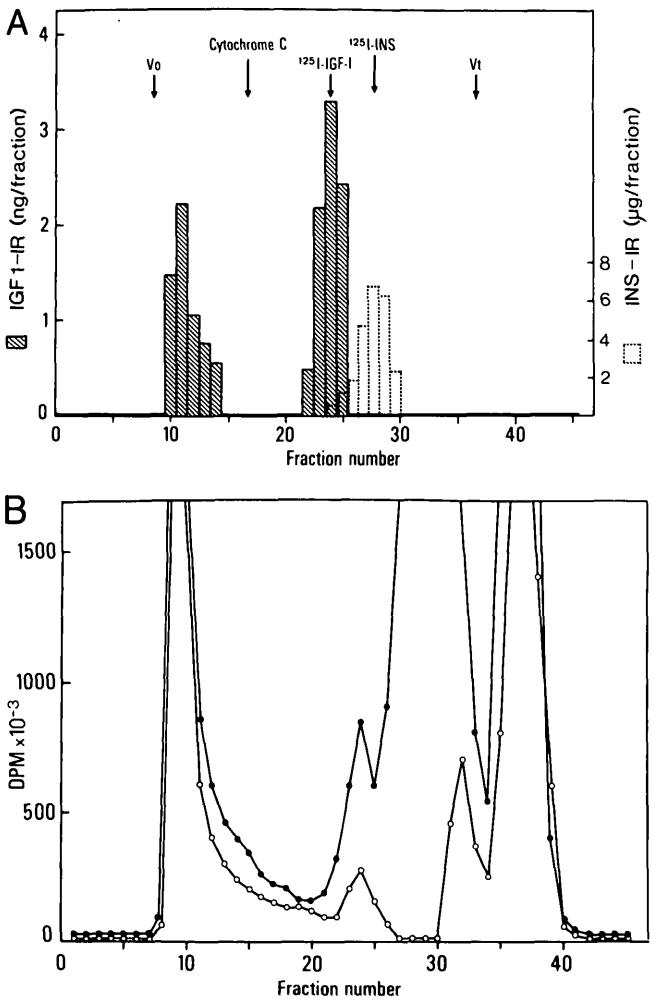

FIG. 1. Bio-Gel P-100 gel filtration of fetal islet-conditioned medium. Culture medium was acidified, desalted on PD-10 columns, and evaporated to dryness. Material was reconstituted in 1 M acetic acid and placed on Bio-Gel P-100 column (100 × 1.5 cm) previously equilibrated with 1 M acetic acid. Column was eluted with same buffer at flow rate of 6 ml/h. Four-milliliter fractions were collected. Immunoreactive insulinlike growth factor I (IGF1-IR, *hatched bars*) and insulin (INS-IR, *open bars*) (*A*) and radioactivity of [^{3}H]leucine (●) and [^{35}S]methionine (○) (*B*) in each fraction were quantified. Column was previously calibrated with standard proteins of known molecular weight. V_o, void volume of column; V_t, total volume of column.

linear gradient from 20 to 80% acetonitrile in 0.1% TFA. A representative profile (Fig. 2) showed two peaks. A large peak (80% of total radioactivity) was eluted with 42% acetonitrile; a smaller peak eluted with 40% acetonitrile. The latter comigrated with pure IGF-I and was therefore submitted to further purification.

Chromatofocusing. The apparent IGF-I fraction that was eluted from HPLC was concentrated, diluted in PB 96 buffer (pH 5.8), and subjected to chromatofocusing on a Pharmacia PBE 94 column (1 × 30 cm) with a pH gradient from 9.6 to 5.8. Fraction 22 from HPLC (40% acetonitrile in 0.1% TFA) produced only two radioactive peaks (Fig. 3). Part of the radioactivity (60%) eluted with 1 M NaCl after the gradient (pI <5.8). A second sharp radioactive peak was eluted at pH 8.55. This peak contained peptide(s) that had incorporated [^{3}H]leucine and [^{35}S]methionine. As a control, fraction 24 from HPLC (42% acetonitrile in 0.1% TFA) was chromatographed under the same conditions. All the radioactive material in that fraction eluted at a pH of <5.8, a pH different from the known isoelectric point of IGF-I (results not shown).

IMMUNOPRECIPITATION

The fraction eluted at pH 8.55 from the chromatofocusing column was concentrated and diluted in 0.03 M PBS/BSA buffer, and the pH was adjusted to 7.5 with HCl. It was then immunoprecipitated as described in MATERIALS AND METHODS. In our experimental conditions, 30% of the radioactivity was immunoprecipitated by an anti-IGF-I antibody after subtraction of the nonspecific binding.

CHROMATOFOCUSING AFTER C_{18} Sep Pak CHROMATOGRAPHY

To simplify the procedure and to gain more information on other insulinlike peptides labeled during the incubation procedure, we chromatofocused the fraction eluted from the Sep Pak cartridge with 50% acetonitrile. Most of the radioactivity (70%) was eluted after the gradient by NaCl elution (Fig. 4). This peak comigrated with IRI.

IGF-I, as measured by RIA, was eluted at pH 8.5 and was readily separated from IRI. IR-IGF-I coeluted with a sharp radioactive peak that contained [^{3}H]leucine and [^{35}S]methionine. Other radioactive peaks were detected, and their characterization is in progress.

DISCUSSION

Studies have shown that neonatal rat islet cells (14) and fetal rat islets in culture (15) synthesize IGF-I. This production has been demonstrated by RIA but has not been characterized physicochemically. We used in vitro labeling of proteins and characterization of the IGF(s) produced to confirm and extend these results. The rationale for using this technique was based on several arguments. First, the culture of fetal islets requires FCS because no defined medium is suitable for this purpose. The fact that the serum contains IGF-I makes demonstrating and quantifying IGF-I synthesis by RIA difficult. In vitro labeling provided unequivocal confirmation of the synthesis of the growth factor by the fetal material. Such con-

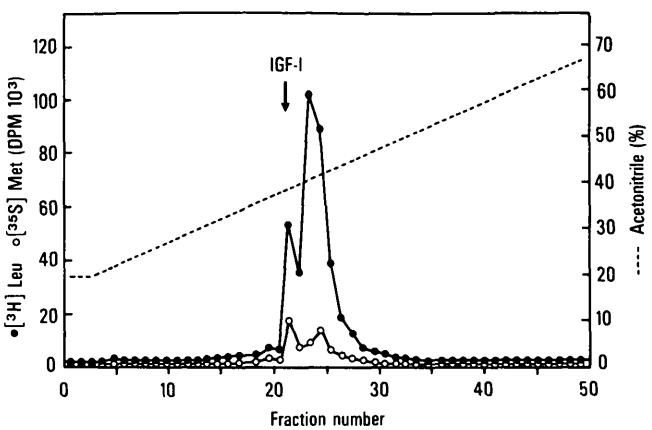

FIG. 2. High-performance liquid chromatography. Fractions 23 and 24 from gel-filtration column were pooled, dried, and reconstituted in 100 μl of 80% water, 0.1% trifluoracetic acid, and 20% acetonitrile. Material was injected into reverse-phase C_{18} column and eluted with linear gradient of acetonitrile (20–80%) for 60 min (flow rate 1 ml/min). Pure insulinlike growth factor I (IGF-I) was chromatographed in parallel as standard.

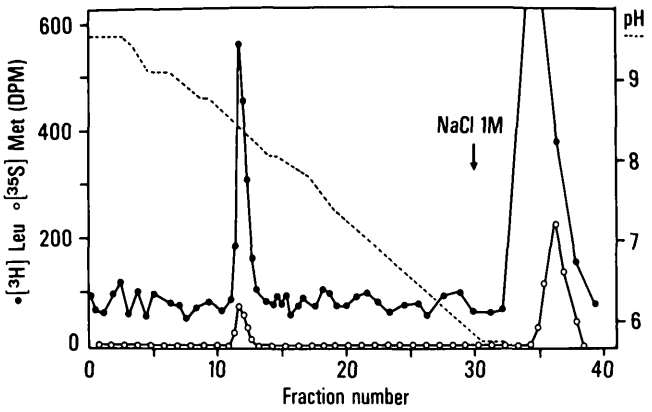

FIG. 3. Chromatofocusing of fraction coeluting with pure insulinlike growth factor I after high-performance liquid chromatography. PBE 94 column (30 × 1 cm) was equilibrated with 0.025 M ethanolamine-HCl (pH 9.5). Column was eluted with 10% (vol/vol) PB 96 (pH 5.7) at flow rate of 25 ml/h, and 4-ml fractions were collected. After gradient, column was rinsed with 2 volumes of 1 M NaCl. pH and radioactivity of each fraction were measured.

firmation is not provided on the use of cycloheximide inhibition of protein biosynthesis, because the reagent does not totally abolish the release of IGF-I by fetal rat myoblasts cultivated in the presence of FCS (10). Second, with in vitro labeling, the characterization of the IGF(s) is possible, even when only a small number of cells are used. Studies of islet growth-factor production are particularly difficult. The starting material is not abundant, even when cultured fetal islets are used; if an islet is assumed to contain 2000–3000 cells (16), a petri dish with 300 islets contains 6 to 9 × 10^5 cells, which is much less material than in other systems (7,10). However, this technique can be used to study the physicochemical properties of the minute amounts of peptide produced by the in vitro system.

Gel chromatography of islet-conditioned medium on Bio-Gel P-100 in acid conditions revealed two immunoreactive peaks, as shown previously (14,21). The high-molecular-weight peak bound ^{125}I-IGF-I, suggesting that this peak contained IGF-I–binding proteins. Moreover, IGF-I precursors may elute in this high-molecular-weight peak. The second peak migrated as pure IGF-I, did not bind IGF-I, and corresponded to IGF (21).

When the islets were pulsed with [^{3}H]leucine and [^{35}S]methionine, part of the radioactivity (3%) was incorporated into the proteins secreted into the medium. Forty-five percent of the incorporated radioactivity migrated like insulin on Bio-Gel P-100, 5% like IGF-I. The latter radioactivity was chromatographed on a microBondapak C_{18} column, and the 40% acetonitrile fraction was chromatofocused. A major peak eluted at pH 8.55. This material was partly immunoprecipitated with an anti-IGF-I antibody.

IGF-I produced by fetal islets has an estimated M_r of 7000, which is similar to that of pure IGF-I. This value is in the same range as values for the IGF-I produced by fetal rat myoblasts (10), hepatocytes (6), Sertoli cells (7), and fetal calvariae (8). It differs from that of skin fibroblast IGF-I (11), which has an M_r of 21,500 and can be a precursor of IGF-I. These results indicate that IGF-I, which is derived from a large precursor (22), is secreted by fetal islets as a mature molecule.

The chromatofocusing experiments showed that IGF-I is eluted at pH 8.55. This basic value is identical to results on IGF-I from fetal rat myoblasts (10), human serum IGF-I (23), and rat hepatocytes (6) but is less basic than that described for adult rat Sertoli cells (7) and rat serum (24). The isoelectric point is a useful marker for differentiating between the insulinlike peptides synthesized in the islets. Only IGF-I has a basic isoelectric point, the isoelectric point of IGF-II is neutral, and that of insulin and proinsulin is acidic.

The use of two labeled amino acids, leucine and methionine, allowed further separation of IGF-I from IGF-II. IGF-I contains both amino acids (24), whereas IGF-II does not contain methionine (25). The basic molecule characterized after chromatofocusing contains leucine and methionine, as does IGF-I. Because IGF-I and IGF-II have similar molecular weights and hydrophobic properties (1), the two peptides cannot be separated on the basis of these physicochemical properties. On the other hand, their different isoelectric points are useful.

Gel filtration and chromatography on a C_{18} column indicated that no neutral molecule having the same molecular

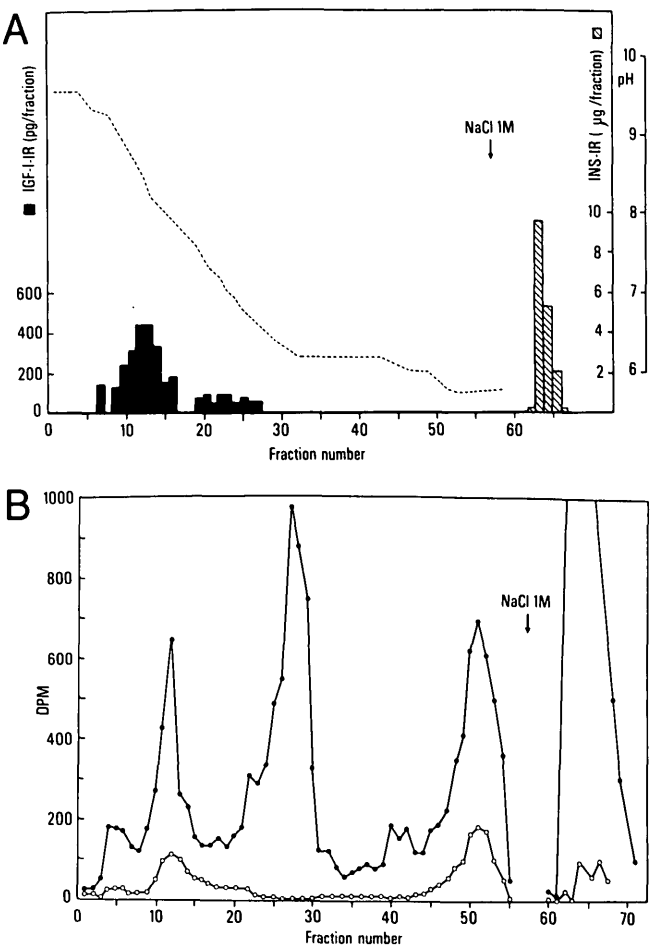

FIG. 4. Chromatofocusing of crude conditioned medium after C_{18} Sep Pak chromatography. PBE 94 column (5 × 0.8 cm) was equilibrated with 0.025 M ethanolamine-HCl (pH 9.5). Column was eluted with 10% (vol/vol) PB 96 (pH 5.7) at flow rate of 25 ml/h, and 0.5-ml fractions were collected. pH was measured in each fraction. *A*: immunoreactive insulinlike growth factor I (IGF-I-IR, *solid bars*) and insulin (INS-IR, *hatched bars*) eluted in each fraction. *B*: radioactivity of [^{3}H]leucine (●) and [^{35}S]methionine (○) in each eluate.

weight and hydrophobic property as IGF-II was synthesized by the islets (results not shown). Using an RIA method, Romanus et al. (14) were unable to demonstrate the presence of IGF-II in the conditioned medium of neonatal islet cells. Our indirect approach produced similar results.

Finally, labeled material was precipitated by an excess of polyclonal IGF-I antibody. Only 30% of the radioactivity was immunoprecipitated. We cannot explain this low percentage of precipitation. The conditions of the experiment might not have been optimal, but more work is needed to obtain information on this point. Another possibility is that the tertiary structure of the peptide differs from that of the IGF-I used to generate the antibody. This hypothesis is not favored in view of the physicochemical characterization of the molecule.

The technique for preparation and culture of fetal islets has been studied extensively over the past few years (16–26). These fetal islets are devoid of exocrine tissue contamination, and electron-microscopic examination indicates that no fibroblastlike or acinar elements are present (16). The islets are rich in β-cells (>90%) but also contain glucagon, somatostatin (27), and IR-TRH cells (17).

The cell type that produces IGF-I in islets remains unknown. IR-IGF-I has been visualized in adult islets (28) and is localized to the same cell type that insulin is in the human fetal pancreas (29). In situ hybridization and immunocytochemical studies are in progress in our laboratory to determine which cells synthesize IGF-I in fetal and adult islets.

The presence of IGF-I receptors has recently been demonstrated on islet β- and α-cells (30). This observation, coupled with the demonstration of an inhibition of islet DNA replication by an anti-IGF-I antibody (15) and with synthesis of IGF-I by islets in vitro, suggests that insular IGF-I has an autocrine role in islet cell replication.

ACKNOWLEDGMENTS

We thank Dr. Bernard Desbuquois for the gift of iodinated insulin. The skillful technical assistance of Annie Basmaciogullari is gratefully acknowledged.

This work was presented in part at the 27th Annual Meeting of the European Society for Pediatric Endocrinology, Copenhagen, 1988.

REFERENCES

1. Van Wyk JJ: The somatomedins: biological actions and physiological control mechanisms. In *Human Proteins and Peptides*. Vol. 12. Li CH, Ed. New York, Academic, 1984, p. 82–125
2. Salmon WD, Daughaday WH: A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J Lab Clin Med* 49:825–36, 1957
3. D'Ercole AJ, Stiles AD, Underwood LE: Tissue concentrations of somatomedin-C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci USA* 81:935–39, 1984
4. Clemmons DR, Underwood LE, Van Wyk JJ: Hormonal control of immunoreactive somatomedin production by cultured human fibroblasts. *J Clin Invest* 67:10–19, 1981
5. Hill DJ, Crace CJ, Fowler L, Holder AT, Milner RDG: Cultured fetal rat myoblasts release peptide growth factors which are immunologically and biologically similar to somatomedin. *J Cell Physiol* 119:349–58, 1984
6. Scott CD, Martin JL, Baxter RC: Production of insulin-like growth factor I and its binding protein by adult rat hepatocytes in primary culture. *Endocrinology* 116:1094–101, 1985
7. Smith EP, Svoboda ME, Van Wyk JJ, Kierszenbaum AL, Tres LL: Partial characterization of a somatomedin-like peptide from the medium of cultured rat Sertoli cells. *Endocrinology* 120:186–93, 1987
8. Canalis E, McCarthy T, Centrella M: Isolation and characterization of insulin-like growth factor I (somatomedin-C) from cultures of fetal rat calvariae. *Endocrinology* 122:22–27, 1988
9. Clemmons DR, Van Wyk JJ: Evidence for a functional role of endogenously produced somatomedinlike peptides in the regulation of DNA synthesis in cultured human fibroblasts and porcine smooth muscle cells. *J Clin Invest* 75:1914–18, 1985
10. Hill DJ, Crace CJ, Nissley SP, Morrell D, Holder AT, Milner RDG: Fetal rat myoblasts release both rat somatomedin-C (Sm-C)/insulin-like growth factor I (IGF I) and multiplication-stimulating activity in vitro: partial characterization and biological activity of myoblast-derived Sm-C/IGF I. *Endocrinology* 117:2061–72, 1985
11. Clemmons DR, Shaw DS: Purification and biologic properties of fibroblast somatomedin. *J Biol Chem* 261:10293–98, 1986
12. Hellerström C, Swenne I: Growth pattern of pancreatic islets in animals. In *The Diabetic Pancreas*. 2nd ed. Volk BW, Arquilla ER, Eds. New York, Plenum, 1985, p. 53–79
13. Rabinovitch A, Quigley C, Russell T, Patel Y, Mintz DH: Insulin and multiplication stimulating activity (an insulin-like growth factor) stimulate β-cell replication in neonatal rat pancreatic monolayer cultures. *Diabetes* 31:160–64, 1982
14. Romanus JA, Rabinovitch A, Rechler MM: Neonatal rat islet cell cultures synthesize insulin-like growth factor I. *Diabetes* 34:696–702, 1985
15. Swenne I, Hill DJ, Strain AJ, Milner RDG: Growth hormone regulation of somatomedin C/insulin-like growth factor I production and DNA replication in fetal rat islets in tissue culture. *Diabetes* 36:288–94, 1987
16. Hellerström C, Lewis NJ, Borg H, Johnson R, Freinkel N: Method for large-scale isolation of pancreatic islets by tissue culture of fetal rat pancreas. *Diabetes* 28:769–76, 1979
17. Scharfmann R, Leduque P, Aratan-Spire S, Dubois P, Basmaciogullari A, Czernichow P: Persistence of PAMase activity and elevated TRH concentrations in fetal rat islets in culture. *Endocrinology* 123:1329–34, 1988
18. Furlanetto RW, Underwood LE, Van Wyk JJ, D'Ercole AJ: Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. *J Clin Invest* 60:648–57, 1977
19. D'Ercole AJ, Underwood LE, Van Wyk JJ, Decedue CJ, Foushee DB: Specific topography and ontogeny of the somatomedin-C receptor in mammalian tissues. In *Growth Hormone and Related Peptides*. Pecile A, Muller E, Eds. Amsterdam, Excerpta Med., 1976, p. 190–201
20. Halban PA, Wollheim CB: Intracellular degradation on insulin stores by rat pancreatic islets in vitro. *J Biol Chem* 255:6003–6006, 1980
21. Czernichow P, Hellerström C: Insulin-like growth factor I In the rat pancreas and in fetal islets in culture (Abstract). *Pediatr Res* 11:1184, 1986
22. Jansen M, Van Schaik FMA, Ricker AT, Bullock B, Woods DE, Gabbay KH, Nussbaum AL, Sussenbach JS, Van den Brande JL: Sequence of cDNA encoding human insulin-like growth factor I precursor. *Nature* (Lond) 306:609–11, 1983
23. Svoboda ME, Van Wyk JJ, Klapper DG, Fellows RE, Grissom FE, Schlueter RJ: Purification of somatomedin C from human plasma: chemical and biological properties, partial sequence analysis, and relationship to other somatomedins. *Biochemistry* 19:790–97, 1980
24. Rubin JS, Mariz I, Jacobs JW, Daughaday WH, Bradshaw RA: Isolation and partial sequence analysis of rat basic somatomedin. *Endocrinology* 110:734–40, 1982
25. Marquardt H, Todaro GJ, Henderson LE, Oroszlan S: Purification and primary structure of a polypeptide with multiplication-stimulating activity from rat liver cell cultures: homology with human insulin-like growth factor II. *J Biol Chem* 256:6859–63, 1981
26. Mourmeaux JL, Remacle C, Henquin JC: Morphological and functional characteristics of islets neoformed during tissue culture of fetal rat pancreas. *Mol Cell Endocrinol* 39:237–46, 1985
27. McEvoy RC, Leung PE: Tissue culture of fetal rat islets: comparison of serum-supplemented and serum-free, defined medium on the maintenance, growth, and differentiation of A, B, and D cells. *Endocrinology* 111:1568–75, 1982
28. Andersson I, Billig H, Fryklund L, Hansson HA, Isaksson O, Isgaard J, Nilsson A, Rozell B, Skottner A, Stemme S: Localization of IGF-I in adult rats: immunocytochemical studies. *Acta Physiol Scand* 126:311–12, 1986
29. Hill DJ, Frazer A, Swenne I, Wirdnam PK, Milner RDG: Somatomedin-C in human fetal pancreas: cellular localization and release during organ culture. *Diabetes* 36:465–71, 1987
30. Van Schravendijk CFH, Foriers A, Van den Brande JL, Pipeleers DG: Evidence of the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. *Endocrinology* 121:1784–88, 1987