Neonatal Rat Islet Cell Cultures Synthesize Insulin-like Growth Factor I

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SUMMARY

Monolayer cultures of islet B-cells were established from neonatal rat pancreas. Serum-free media conditioned by these cultures for 72 h were concentrated and fractionated on Sephadex G-50 at acid pH into a high-molecular-weight pool containing binding protein for insulin-like growth factors (IGFs) and a low-molecular-weight pool containing IGFs. IGF activity in the IGF pool was demonstrated by a specific radioreceptor assay using rat liver plasma membranes and ^{125}I-labeled rat IGF-II. The IGF in islet cell media was characterized further by radioimmunoassays specific for human IGF-I and for rat IGF-II. Islet cell IGF was identified as predominantly IGF-I or a closely related species and not IGF-II. Levels of ~15–50 ng IGF-I (based on human IGF-I standard)/10^6 islet cells accumulated in media after 72 h, and presumably represented synthesis by the islet cells. Concentrations of IGF-I attained in culture media, ~0.1 ng/ml, were sufficient to stimulate [^{3}H]thymidine incorporation into B-cells. Growth hormone did not consistently increase IGF-I synthesis, suggesting that the previously described effects of growth hormone on islet cell replication do not result from stimulation of IGF-I synthesis by islet cells. Thus, although the IGF-I synthesized by islet cells may be a physiologically relevant growth factor for these cells, the mitogenic effects of growth hormone in islet cells appear to be independent and not mediated by IGF-I. DIABETES 1985; 34:696–702.

The hormonal regulation of islet B-cell replication is poorly understood. Insulin and multiplication-stimulating activity (MSA), the rat homologue of insulin-like growth factor II (IGF-II), stimulated B-cell replication in monolayer cultures established from neonatal rat pancreas.[1] Rat IGF-II was 100 times more potent than insulin, and the effect of combined addition of both hormones was not additive,[1] strongly suggesting that both peptides acted via an IGF receptor.[2] In a subsequent study, it was observed that ovine growth hormone also stimulated B-cell replication.[3] Since growth hormone stimulates IGF synthesis in vivo[4,5] in perfused rat liver[6] and in cultured fibroblasts,[7,8] and since IGF synthesis has been reported in short-term explants of rat liver[9] and multiple fetal mouse organs,[10] the possibility was considered that growth hormone acted on B-cells by stimulating the synthesis of the proximate mitogen, IGF. In the present study, we have used specific radioreceptor and radioimmunoassays to demonstrate the presence of IGFs in media conditioned by islet cell cultures. Evidence is presented that this IGF is immunologically related to human IGF-I and may represent rat IGF-I. The levels of IGF-I in islet cell media are not consistently increased by growth hormone, suggesting that the mitogenic effects of growth hormone are direct and not mediated by the IGFs.

MATERIALS AND METHODS

Preparation of islet cell cultures. Monolayer cultures of neonatal rat pancreatic islets cells were prepared as previously described.[3] In brief, pancreata from 3–4-day-old Sprague-Dawley rats were isolated and digested with trypsin and collagenase. Dissociated cells were resuspended in medium 199 containing 10% (vol/vol) fetal calf serum and 16.7 mM glucose, plated in 35-mm culture dishes (1.5–2 ml media/dish), and incubated in a humidified 37°C incubator in a 95% air-5% CO_2 atmosphere. After 3 days, cultures were treated with iodoacetic acid (2 μg/ml for 5–6 h) to eliminate fibroblasts. Cultures were fed with growth media 3 times/wk for a total of 7–10 days. At this time, cell preparations consisted of clusters of islet cells (~80% B-cells) without detectable contamination by fibroblasts, endothelial cells, or pancreatic acinar cells.

For experiments, cells were incubated for 48 h in a serum-free, 1:1 mixture of Ham's F-12 and Dulbecco's modified

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Eagle's Medium containing 10 μg/ml charcoal-extracted bovine serum albumin (BSA) and 16.7 mM glucose. This medium was discarded, and fresh serum-free medium was added for 72 h. When indicated, ovine growth hormone, 3 μg/ml, was added before the final media change. Media were harvested and pooled separately from cells cultured in the absence and presence of growth hormone, frozen, and lyophilized. Cells were washed with phosphate-buffered saline (PBS), removed from the dishes by scraping, pooled as for media collections, and DNA measured by a fluorometric method[11] as modified for pancreatic islets.[12]

Hormones and growth factors. Rat IGF-II (multiplication-stimulating activity) was purified as previously described.[13] MSA II (M_r 8700) was used as reference standard in MSA receptor and radioimmunoassays. MSA II-1 (M_r 8700, 80–200 Ci/g) and MSA III-2 (M_r 7100, 290 Ci/g [HPLC-purified]) were labeled with ^{125}I and used as radioligands in the receptor and immunoassays, respectively. MSA preparations obtained from Collaborative Research were used for DNA synthesis experiments.

IGF-I (lot 16SPII) was purified and provided by Prof. René Humbel (Zürich), and iodinated (50–150 Ci/g) as previously described.[14] (The chloramine-T concentration in ref. 14 was inadvertently given as 333 μg/10 μl instead of 333 ng/10 μl.)

Ovine growth hormone (oGH, NIH-GH-S11, 0.56 IU/mg) was obtained from the National Hormone and Pituitary Program (Baltimore, Maryland). Crystalline bovine insulin was kindly supplied by Dr. R. Chance, Lilly Research Laboratories (Eli Lilly and Company, Indianapolis, Indiana).

Partial purification of IGFs from islet cell media. Lyophilized pools of media (~50–150 ml) were resuspended in ~2.0 ml of 1 M acetic acid, and the pH adjusted to ~3.5 with glacial acetic acid. After removal of insoluble material by centrifugation, the sample was applied to a column (Pharmacia, 1.5 × 85 cm, ~130 ml bed volume) of Sephadex G-50 fine (Pharmacia) equilibrated with 1 M acetic acid. The column was run at room temperature; 1.2-ml fractions were collected. Before sample application, the column had been calibrated with ^{125}I-MSA II and ^{125}I-MSA III-2. Aliquots of alternate fractions in the void and post-void regions were dried, resuspended, and assayed for their ability to bind ^{125}I-MSA (see below). On the basis of the calibration and the binding protein assay, fractions corresponding to binding protein and IGF regions were separately pooled and lyophilized. (For example, in the column shown in Figure 1, fractions 44–52 were considered the binding protein peak, and fractions 55–90 the IGF pool.) Recovery of ^{125}I-labeled MSA in the IGF pool was >60%. Results are presented from three separate islet cell cultures.

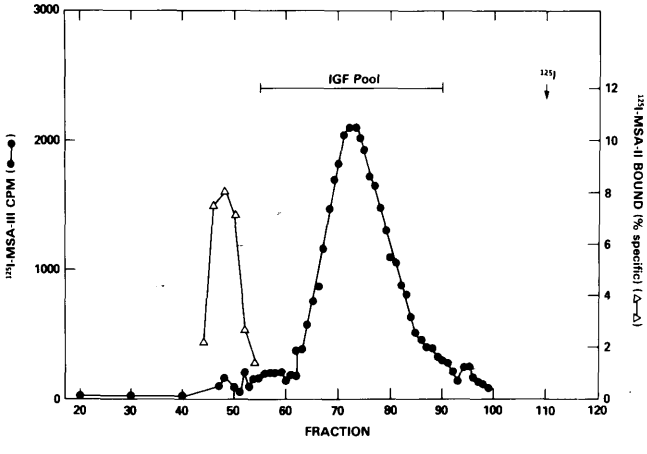

FIGURE 1. Sephadex G-50 gel filtration of neonatal rat islet cell media. Islet cell media were concentrated by lyophilization, resuspended in 1 M acetic acid, and gel filtered on a 130-ml column of Sephadex G-50 (fine) as described in MATERIALS AND METHODS. Fractions containing IGF binding protein were identified by their ability to specifically bind ^{125}I-MSA II (△) as determined in a charcoal separation assay. The location of the IGF pool was identified by separate calibrations with ^{125}I-MSA-III (●) and ^{125}I-MSA-II (not shown).

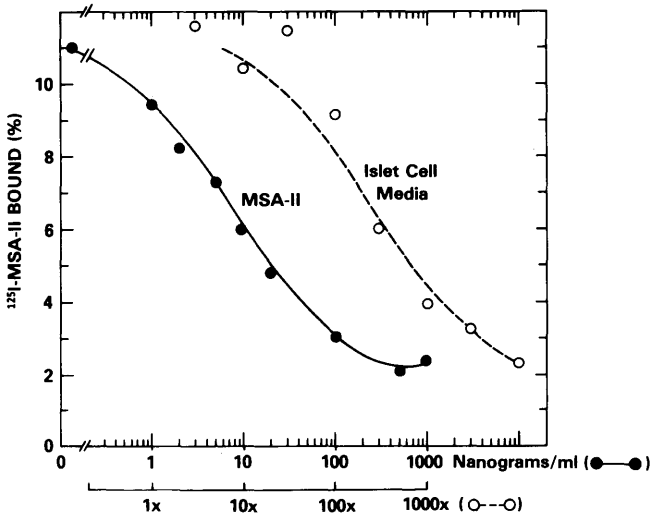

FIGURE 2. Rat liver membrane radioreceptor assay of neonatal rat islet cell media. Dilutions of MSA-II (●) and of the IGF pool prepared from islet cell media (○) were examined for their ability to inhibit the binding of ^{125}I-MSA-II to its receptor on rat liver plasma membranes. ^{125}I-MSA-II bound (expressed as percent of input radioactivity) is plotted against ng/ml of standard or concentration of unknown (on an arbitrary scale, 1× to 1000×). The islet cell media used are from experiment 1, plus growth hormone, Table 2.

Charcoal separation assay for binding protein activity. Samples were incubated with ^{125}I-MSA II (~20,000 cpm) in phosphate-buffered saline (without Ca^{2+} and Mg^{2+}) containing 2 mg/ml bovine serum albumin (Sigma, fatty acid free), pH 7.4, in a total volume of 0.4 ml overnight at 4°C. Unbound ^{125}I-MSA II was removed by adsorption at 0°C to activated charcoal (0.5 ml of 50 mg/ml suspension in PBS containing 20 mg/ml fatty acid-free BSA) and centrifugation as previously described.[9] Complexes of ^{125}I-MSA II and binding protein remained in the supernate. Bound radioactivity was quantitated in a gamma counter. Specifically bound radioactivity was calculated by subtraction of a reagent blank (tracer plus charcoal without added binding protein), since this blank is equivalent to "nonspecific binding" determined in the presence of excess unlabeled MSA.

Rat liver membrane radioreceptor assay. ^{125}I-MSA II (~15,000 cpm), rat liver plasma membranes, and aliquots of MSA-II standard or unknown samples were incubated in 0.15 ml Krebs-Ringer phosphate buffer, pH 7.5, containing 1.1 mg/ml BSA (Sigma RIA grade) for 90 min at 22°C as previously described.[15] Membrane-bound radioactivity was determined after centrifugation (Beckman Microfuge B).

Radioimmunoassays. The MSA radioimmunoassay using ^{125}I-MSA III-2, rabbit antiserum 422,[16] and MSA II standard in an incubation volume of 200 μl was performed as previously described.[17] Results are expressed as B/B_0, where B_0 is the maximum specific binding and B is the specific binding in the presence of the indicated additions, and plotted on a logit-log scale. IGF-I shows <1% cross-reactivity with this antiserum.[16] Partially purified rat IGF-I is unreactive.[18]

The IGF-I radioimmunoassay using the somatomedin-C antiserum[4] provided by the National Hormone and Pituitary Program (Baltimore, Maryland), ^{125}I-IGF-I, and unlabeled IGF-I in an incubation volume of 200 μl was performed as previously described.[17] Somatomedin C and IGF-I have identical amino acid sequences[19] and are equipotent in this assay.[20] MSA III and MSA II have ~1% and <1% cross-reactivity, respectively.[20] Rat IGF-I, chemically homologous to human IGF-I, shows one-sixth cross-reactivity.[21]

Islet B-cell DNA synthesis. Cultures were incubated for 18 h with serum-free control or test media and [methyl-^{3}H]thymidine as previously described.[3] Autoradiographs were prepared, and the percentage of labeled B-cells determined as previously described.[3]

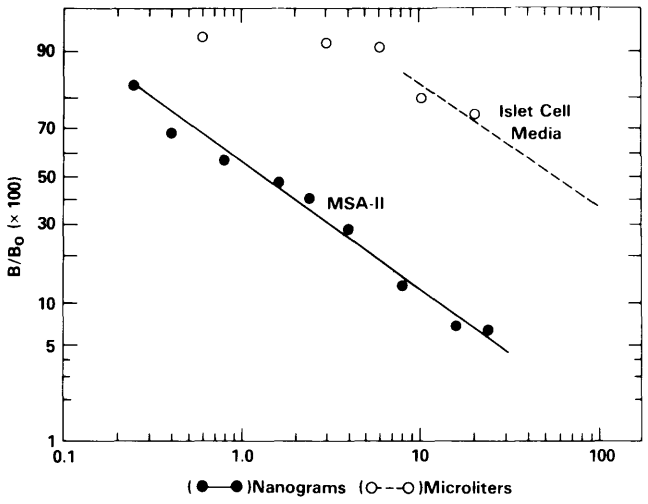

FIGURE 4. MSA radioimmunoassay of islet cell media. Aliquots of MSA-II (●) or the IGF pool of islet cell media used in Figure 2 (○) were incubated with rabbit antiserum to MSA and ^{125}I-MSA-III as described in MATERIALS AND METHODS. B/B_0 (×100) is plotted against concentration of the addition.

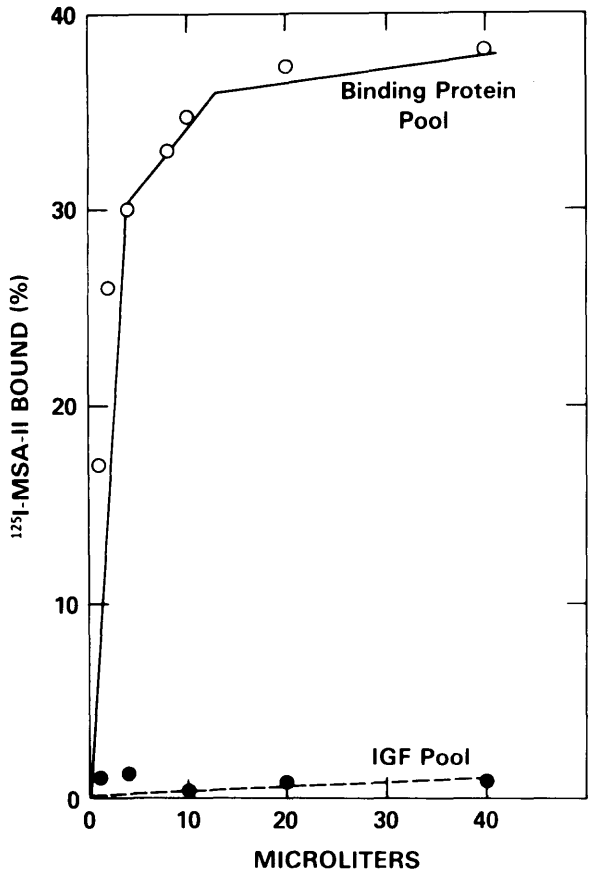

FIGURE 3. Direct assay of binding protein activity. Aliquots of the IGF pool (●) and binding protein pool (○) resolved by acid gel filtration were incubated with ^{125}I-MSA-II. Tracer bound to binding protein was determined as radioactivity in the supernate after adsorption of free ^{125}I-MSA to activated charcoal. ^{125}I-MSA bound specifically (expressed as percent of input radioactivity) is shown for different volumes (μl) of resuspended sample. Note that 50 μl of this IGF pool gave full displacement (10,000×) in the rat liver membrane assay (Figure 2).

RESULTS

Presence of IGF and IGF carrier protein in islet cell conditioned media. Islet cell monolayers containing ~80% B-cells were incubated in serum-free medium for 72 h. The conditioned media were lyophilized, reconstituted at 50-fold higher concentrations, and fractionated on a Sephadex G-50 column equilibrated with 1 M acetic acid. IGF carrier protein was demonstrated in aliquots of fractions from the void volume by its ability to specifically bind ^{125}I-MSA-II in a direct binding assay (Figure 1).

The low-molecular-weight fractions corresponding to IGF were identified by prior calibration of the column with ^{125}I-MSA-II (not shown) and ^{125}I-MSA-III. A pool of the fractions corresponding to the elution of the radiolabeled MSA standards was made, lyophilized, and reconstituted in neutral buffer. Aliquots of the IGF pool were examined for their ability to competitively inhibit ^{125}I-MSA-II binding to IGF receptors of rat liver plasma membranes (Figure 2). Unlabeled MSA-II gave dose-dependent inhibition, with ED_{50} = 7 ng/ml. Dilutions of islet cell media gave parallel and complete inhibition. If all of the inhibition observed were attributable to MSA-II, this would correspond to 2.8 ng MSA-II/ml of initial conditioned media.

The rat liver membrane receptor assay is highly specific for IGFs,[15,22] and strongly suggests that islet cell conditioned media contain IGFs. Although this receptor preferentially binds IGF-II, IGF-I exhibits extensive cross-reactivity. Insulin, although chemically similar, does not bind to this site. It is possible, however, that unsaturated IGF carrier proteins may give falsely positive inhibition by forming complexes with radioligand that do not co-sediment with the membranes. This possibility is excluded by the results presented in Figure 3. Dilutions of the IGF pool did not contain excess free carrier protein sufficient to bind ^{125}I-MSA-II in the charcoal separation assay. The concentrations tested are comparable to those that gave inhibition of binding in the receptor assay. For comparison, binding by dilutions of the carrier protein pool are presented.

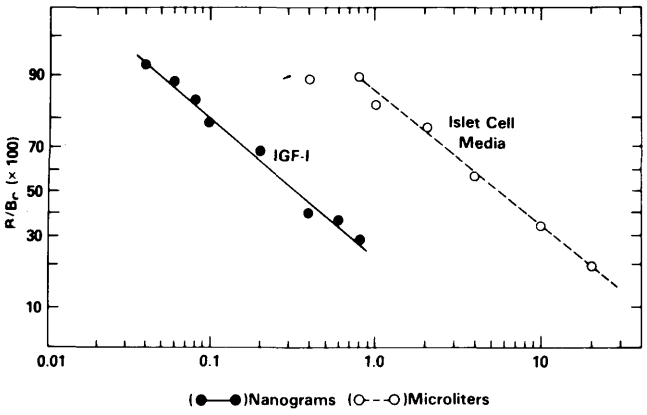

FIGURE 5. IGF-I radioimmunoassay of islet cell media. Aliquots of the same IGF pool shown in Figures 2 and 4, or human IGF-I, were incubated with ^{125}I-IGF-I and rabbit antiserum to somatomedin C/IGF-I as described in MATERIALS AND METHODS. B/B_0 (× 100) is plotted against nanograms of IGF-I or microliters of islet cell IGF pool.

Conditioned islet cell media contain predominantly IGF-I. The previous results strongly suggested that conditioned islet cell media contained an IGF, but did not distinguish whether this was related to IGF-I or IGF-II.[23-25] Specific radioimmunoassays were performed to discriminate between the two related polypeptides. First, media samples were examined in an MSA radioimmunoassay using an antiserum that is highly specific for MSA (rat IGF-II), and reacts weakly with human[16] and rat[18] IGF-I. The assay detects as little as 0.3 ng of MSA-II (Figure 4). Islet cell media exhibited poor reactivity, with only a 30% decrease in tracer binding at the highest concentration tested. This corresponds to ~0.08 ng of MSA-II/ml of islet cell media.

Next, dilutions of the islet cell IGF pool were examined in a heterologous radioimmunoassay using rabbit antiserum to human IGF-I, and human IGF-I as labeled and unlabeled ligand (Figure 5). This antiserum is highly specific for IGF-I: rat IGF-I shows one-sixth cross-reactivity,[21] and MSA III-2 ~1.2% cross-reactivity.[20] The assay detects as little as 0.1 ng of IGF-I. The IGF pool from islet cell media gave complete inhibition of tracer binding with a dose-response curve parallel to that seen with the human IGF-I standard. This corresponds to 0.18 ng/ml human IGF-I equivalents.

The results of the MSA and IGF-I radioimmunoassays are compared with the results predicted if all of the IGF reactivity determined in the rat liver membrane receptor assay represented rat IGF-II (Table 1). The results of both assays make this hypothesis untenable: observed reactivity in the MSA radioimmunoassay is ~30-fold too low, whereas observed reactivity in the IGF-I radioimmunoassay is more than sixfold greater than predicted. These results are most compatible with the interpretation that the IGF reactivity in islet cell media represents predominantly rat IGF-I or a closely related peptide.

The IGF-I content in islet cell culture media in three separate experiments was ~2–7 ng/μg cell DNA/72 h (based on human IGF-I standard), or ~12–42 ng of rat IGF-I equivalents/μg DNA/72 h. Since ~150,000 islet cells contain 1 ng DNA, these values correspond to ~15–50 ng human IGF-I (~90–300 ng rat IGF-I)/10^6 cells/72 h.

Effect of growth hormone on islet cell IGF-I levels. The levels of IGF-I were compared in islet cell cultures that had been incubated with or without ovine growth hormone. The results of three experiments are summarized in Table 2. Although a possible twofold increase in IGF-I levels was observed in growth hormone-treated cultures in experiment 1, no increase in IGF-I content was observed in experiments 2 or 3. Since the effect of growth hormone is small and variable, we do not think it likely that the mitogenic effects of growth hormone on B-cells are mediated by stimulation of islet cell synthesis of IGF-I.

Mitogenic effect of IGF-I on neonatal islet B-cells. Although islet cell IGF-I synthesis does not appear to mediate the mitogenic effects of growth hormone on these cells, it remained to be demonstrated that islet cells respond to IGF-I. The relative abilities of IGF-I, MSA, and insulin to stimulate DNA synthesis (measured as B-cell nuclei labeled by [^{3}H]thymidine) are compared in Figure 6. IGF-I and MSA stimulated B-cell labeling to the same extent (~200% of control). At low concentrations (0.1–1 ng/ml), IGF-I was significantly more potent than MSA. Interestingly, levels of IGF-I in islet cell media (0.04–0.18 ng/ml) are potentially capable of stimulating DNA synthesis in these cells.

DISCUSSION

Previous studies have shown that rat IGF-II is a potent mitogen for neonatal islet B-cells in monolayer culture.[1] The present study demonstrates the presence of IGFs in serum-free media conditioned by islet cell cultures for 72 h. After acid-gel filtration to obtain a low-molecular-weight IGF pool free of IGF binding protein, IGF reactivity was demonstrated in a specific radioreceptor assay in which both IGF-I and IGF-II react, but other peptides including insulin do not cross-react. The low reactivity of islet cell IGF in an IGF-II-specific radioimmunoassay and its high reactivity in an IGF-I-specific radioimmunoassay strongly suggest that the predominant IGF in islet cell media is immunologically related to IGF-I. We

TABLE 1
Relative reactivity of islet cell IGF in the rat liver membrane receptor assay and in MSA and IGF-I radioimmunoassays

Assay	Predicted* (ng/ml)	Observed (ng/ml)	Observed/Predicted
(1) MSA radioimmunoassay†	2.8	0.08	0.028
(2) IGF-I radioimmunoassay‡	<0.03	0.18	>6.0

*Predicted value if all IGF activity present in the IGF pool of gel-filtered islet cell media as measured by the rat liver membrane receptor assay (2.8 ng/ml) represents IGF-II.
†MSA-II standard.
‡Human IGF-I standard.

TABLE 2
Quantitation of IGF-I in islet cell media

Exp.	Growth hormone	Medium volume (ml)	Cell DNA (μg)	IGF-I (ng/ml)	IGF-I (ng/μg cell DNA)
1	–	142	3.38	0.07	2.9
	+	144	3.92	0.18	6.6
2	–	101	2.01	0.044	2.2
	+	99	2.21	0.046	2.1
3	–	47	0.86	0.13	7.1
	+	48	0.80	0.09	5.4

Islet cell cultures were incubated ± ovine growth hormone for 72 h. Medium was collected, concentrated, and acid-gel filtered as described in MATERIALS AND METHODS. The low-molecular-weight IGF pool was assayed in an IGF-I radioimmunoassay using human IGF-I standard. Results are expressed per ml culture medium and per μg cell DNA (~150,000 cells/μg DNA).

cannot, however, exclude the possibility that small amounts of IGF-II also are synthesized.*

We believe that the IGF activity observed in islet cell media after 72-h incubation represents time-dependent accumulation and most likely synthesis by islet cells. After 24-h incubation, no IGF-I was detectable by radioimmunoassay in islet cell media (i.e., <<0.005 ng/ml) (results not shown). This indicates that the measured IGF-I did not pre-exist in the incubation media, and that its accumulation was time dependent.† It is most probable that islet cells themselves are the source of the observed IGF-I. Electron microscopic evaluation of our islet cell cultures reveals 80% B-cells, 10% A-cells, and 10% D-cells,[26] without detectable fibroblasts or endothelial cells.[3] Moreover, the observed rate of IGF-I accumulation in the islet cell cultures (~2–7 ng/μg cell DNA/72 h, ~15–50 ng/10^6 cells/72 h) is similar to the highest synthetic rate of IGF-I observed by Adams et al.[27] for fibroblast cultures established from the skin of 50-day-old rats (73 ng/10^6 cells/72 h). Thus, it would be most unlikely that a minor contamination of the islet cell cultures by fibroblasts, if it occurred, could account for the observed levels of IGF-I.

Although it has been proposed that the liver is a major site of IGF synthesis,[28] recent evidence strongly suggests that other tissues also may synthesize IGFs. These include: (1) synthesis of immunoreactive IGF-I by explants of multiple organs of fetal mice,[10] (2) synthesis of IGF-I[7,8,29] and IGF-II[17,27] by cultures of human and rat fibroblasts, and (3) an increase in extractable immunoreactive IGF-I from multiple tissues after the administration of growth hormone to hypophysectomized rats.[30] The present study is the first report of the synthesis of IGFs by islet B-cells.

It has been proposed[10,30] that IGFs produced at local sites may act at the same or adjacent cells by autocrine or paracrine mechanisms to exert their mitogenic effects. The fact that exogenous IGF-I and IGF-II are potent mitogens for islet B-cells is certainly consistent with this hypothesis. Indeed, the levels of IGF-I observed in islet cell media (0.04–0.18 ng/ml) are comparable to the concentrations of IGF-I required for significant stimulation of islet cell DNA synthesis (≧0.1 ng/ml, Figure 6). It is of interest that rat islet tumor cells could be cultured in serum-free, defined medium in which insulin was replaced by MSA.[31]

Although islet cell IGF-I is immunologically related to circulating human and presumably rat IGF-I, it remains to be determined whether it is chemically identical to rat IGF-I purified from serum.[24] For example, whereas IGF-I isolated from human plasma has a M_r ~7500,[23] a M_r ~20,000 species of immunoreactive and biologically active IGF-I has been purified from human fibroblast conditioned media.[32] It is not presently known whether these represent distinct proteins from different genes, or whether they represent precursor and product. Rat IGF-II is synthesized in BRL-3A rat liver cells as a M_r ~22,000 pre-prohormone and converted to mature M_r ~7500 IGF-II.[33,34]

The predominant IGF species present in rat plasma varies

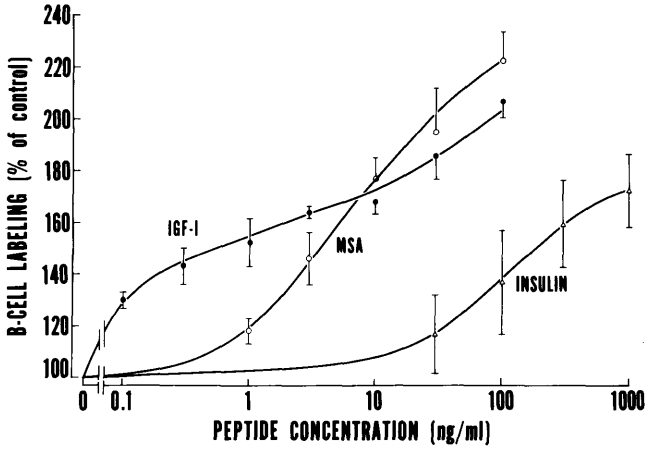

FIGURE 6. Dose-dependent effects of IGF-I, MSA, and insulin on [^{3}H]thymidine incorporation in islet B-cells. Mean values ± SEM for three dishes cultured for 18 h in medium supplemented with a peptide are shown as percentages of the mean value in the corresponding control cultures in peptide-free medium (3.8% of B-cells labeled).

*Although neonatal B-cell cultures synthesize ~50 ng insulin/18 h^1 and insulin synthesis is linear with time (unpublished observations), the projected concentrations at 72 h (~200 ng/ml) could not account for the observed reactivity in radioreceptor and radioimmunoassays. Insulin is < 10^{-4} as potent as MSA II in the rat liver membrane receptor assay, and could not account for the observed levels of 2.8 ng/ml. In the IGF-I radioimmunoassay, pork insulin is ~10^{-5} as potent as IGF-I (and gives a nonparallel dose-response curve).[4] Since pork insulin is chemically more similar to human insulin than to rat insulin, and since the antiserum to human IGF-I shows only ~15% cross-reactivity with rat IGF-I, this potency should overestimate the possible interference by rat insulin in the radioimmunoassay. At most, insulin might contribute ~0.002 ng/ml of apparent IGF-I reactivity, substantially lower than the observed levels of 0.044–0.18 ng/ml (Table 2).

†Although this result suggests the possibility of a lag before IGF-I accumulates in the media, the possibility of incomplete recovery of IGF-I in samples containing low amount of protein has not been excluded.

with the developmental stage. In the rat fetus, IGF-II levels are high and quantitatively account for all of the total IGF.[35] From 5 to 20 days after birth, plasma IGF-II levels precipitously decrease to the low levels seen in adult rats.[35] Conversely, immunoreactive IGF-I is low in the rat fetus, shows a gradual increase during the first 15 days of life, and a dramatic increase after the 15th day.[36] Adams et al.[27] have demonstrated a similar developmental switch in IGF synthesis in fibroblast cultures established from rat skin and lung at different ages. IGF-I levels were low in fetal fibroblasts and increased in fibroblasts cultured from 3-day or older rats. The present results showing predominant IGF-I synthesis by islet cell cultures from 3-day-old rats are similar to the time course of its appearance in fibroblast cultures. The earlier appearance of IGF-I in fibroblast and islet cell cultures relative to serum may be explained by the fact that circulating IGF-I appears to arise predominantly from liver,[6,30] and the possibility that the switch to IGF-I synthesis occurs later in liver.

Ovine growth hormone is a potent mitogen for neonatal islet B-cell cultures.[3] Growth hormone stimulates IGF-I synthesis in perfused rat liver,[6] and in human and rat fibroblast cultures.[7,8,27] We were unable to demonstrate a consistent stimulation of IGF-I synthesis by islet cell cultures, suggesting that growth hormone may act directly as a mitogen in islet cells and not via IGF-I. A similar conclusion was reached from the results of growth experiments in which it was observed: (1) that the effects of growth hormone and MSA were additive, and (2) that stimulation by the two agents occurred with the same kinetics.[3] Actions of growth hormone that are not mediated by IGFs also have been recognized in other systems: anti-insulin effects and induction of refractoriness to the insulin-like effects of growth hormone,[37] conversion of preadipocytes to adipocytes,[38] stimulation of longitudinal bone growth,[39] and the appearance of M_r ~150,000 IGF binding protein.[40] The binding protein is synthesized by most cells in culture, including those that do not synthesize IGFs (reviewed in ref. 41). Two size forms of carrier protein have been identified in rat serum: M_r ~150,000 in normal adult rat plasma[42] and M_r ~40,000 in fetal rat plasma.[43] The transition to the adult profile occurs at approximately day 20 after birth.[43] The size of the carrier protein in islet cell media remains to be determined.

In summary, islet cell cultures established from 3-day-old neonatal rats and containing predominantly B-cells appear to synthesize an IGF immunologically related to IGF-I. Endogenous IGF-I may play a role in B-cell growth, since exogenous IGF-I is active at concentrations attained in culture media. Synthesis of IGF-I by islet cell cultures does not appear to be regulated by growth hormone, and does not appear to mediate the mitogenic effects of growth hormone in these cells.

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