

Insulin-induced Loss of Insulin-like Growth Factor-I Receptors on IM-9 Lymphocytes

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SUMMARY

Preexposure of IM-9 lymphocytes to the somatomedin peptide insulin-like growth factor-I (IGF-I) results in a time- and concentration-dependent reduction in specific receptors for IGF-I. Since insulin and proinsulin are structurally homologous to IGF-I, we investigated the ability of insulin analogues to compete for occupancy and to directly modulate IGF-I receptor concentrations.

IGF-I binds rapidly and reversibly to IM-9 cells at 15°C, with half-maximal displacement of ^{125}I -IGF-I at IGF-I concentrations of 3.6×10^{-9} M and insulin concentrations of 5×10^{-7} M. Preexposure of cells at 37°C to either IGF-I or insulin produced a concentration-dependent reduction in binding of ^{125}I -IGF-I. A 50% decrease in binding was observed following preincubation of cells with IGF-I at 2.5×10^{-9} M and insulin at 2×10^{-7} M. At higher insulin concentrations (10^{-6} – 10^{-5} M), up to 70% reduction in ^{125}I -IGF-I binding occurred. Bovine proinsulin and guinea pig insulin competed less potently than porcine insulin for the IGF-I receptor, and produced receptor loss in proportion to their ability to occupy the IGF-I receptor. Scatchard analysis indicated that at all insulin concentrations, the decrease in binding was secondary to loss of available IGF-I receptors, with no change in affinity. Receptor loss was evident following 1–2 h preexposure to insulin, with a $t_{1/2}$ of 4 h and maximal receptor loss within 10 h. Similarly, IGF-I and IGF-II competed for occupancy of the IM-9 insulin receptor, with 50% displacement of ^{125}I -insulin occurring at peptide concentrations of 3.5×10^{-9} M (insulin), 3.5×10^{-8} M (IGF-II), and 3×10^{-7} M (IGF-I). Preexposure of cells to these peptides at 37°C for 20 h resulted in a concentration-dependent reduction in binding of ^{125}I -insulin, with the order of analogue effectiveness being insulin > IGF-II > IGF-I.

These data emphasize the structural and functional homology of insulin and the somatomedin peptides, IGF-I and II, as well as their respective receptors. Additionally, the data support the conclusion that the insulin and somatomedin peptides not only bind to both receptors, but downregulate each receptor in proportion to their ability to occupy that receptor. **DIABETES** 31:375–381, May 1982.

The ability of insulin and similar hormones to directly modulate homologous receptor concentrations has been proposed as a fundamental mechanism of cellular homeostasis.^{1–3} Gavin et al.¹ demonstrated that preincubation of IM-9 lymphocytes with high concentrations of insulin resulted in a time- and concentration-dependent reduction of binding of ^{125}I -insulin, and that this decrease could be explained by a loss of specific membrane receptors for insulin. These findings have been extended to a variety of cell systems, including other cultured lymphocyte lines,⁴ human diploid fibroblasts,⁵ adipocytes,⁶ and circulating monocytes.⁷

We have recently characterized a specific receptor for somatomedin-C (SM-C) on IM-9 cells and have demonstrated that SM-C, like insulin, is capable of inducing a dose-dependent loss of homologous receptors.⁸ The affinities of purified SM-C and insulin-like growth factor I (IGF-I) for this receptor are identical, thus adding to the growing body of data suggesting that IGF-I and SM-C are functionally, and perhaps structurally, identical molecules.^{9,10} Rinderknecht and Humbel¹¹ have sequenced IGF-I and demonstrated a major structural similarity to human insulin, with homologous regions in both the B and A chains. Furthermore, insulin competes for occupancy of the SM-C/IGF-I receptor,^{8,12} and King et al.¹³ have proposed that the major mitogenic actions of insulin result from binding to this receptor rather than to the specific insulin receptor. In the present study, we have evaluated the interaction of insulin with the SM-C/IGF-I receptor by assessing the relative ability of insulin analogues to bind to this receptor and directly modulate SM-C/IGF-I receptor concentrations. Similarly, we have studied

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the ability of IGF-I and the related somatomedin peptide, IGF-II, to bind to the IM-9 insulin receptor and regulate insulin receptor concentrations.

MATERIALS AND METHODS

Cultured lymphocytes. IM-9 cells, an established line of human lymphocytes,¹⁴ were grown in continuous culture in RPMI-1640 medium with 25 mM Hepes buffer (Grand Island Biologicals Company, Grand Island, New York), supplemented with 10% fetal bovine serum (GIBCO), L-glutamine (4 mM) (GIBCO), penicillin (100 U/ml), and streptomycin (100 µg/ml). The cells were grown in 250-ml sterile plastic culture flasks (Falcon) at 37°C. At 72 h, the cells were divided 1 to 4, and fresh medium added to each flask. Cell viability was ascertained by trypan blue exclusion, and exceeded 90% in all experiments.

Peptides. Pure insulin-like growth factor I and II (IGF-I and IGF-II) were gifts from Dr. R. E. Humbel and were purified by previously described methods.¹⁵ ¹²⁵I-IGF-I was prepared by a modification of the method of Hunter and Greenwood¹⁶ to an average of less than 1 atom ¹²⁵I-iodine/molecule IGF-I. Subsequently, the iodinated peptide was purified by cellulose chromatography. Prior to use in binding assays, the iodinated peptide was repurified by gel filtration over a Sephadex G-50 column (1.0 × 120 cm) at 4°C and eluted with 100 mM Hepes buffer (pH 7.4) with 1% bovine serum albumin, 120 mM NaCl, 1.2 mM MgSO₄, 5 mM KCl, 15 mM Na acetate, 10 mM dextrose, and 1 mM EDTA. The specific binding of this purified peptide to a standard placental membrane preparation was always greater than 30%,¹⁷ and trichloroacetic acid precipitability of the iodinated peptide always exceeded 95%. The rechromatographed ¹²⁵I-IGF-I was stored at -20°C and always used within 7 days of gel filtration.

Because receptor regulation studies require relatively large amounts of peptide, additional IGF-I and IGF-II were purified by a modification of our previously published methods.¹⁸ The acid-ethanol extract of human Cohn fraction IV-I paste was chromatographed on Sephadex G-50 columns with 0.25 M formic acid as the solvent. The active fractions were pooled, lyophilized, and rechromatographed on Sephadex G-50 in 0.05 M NH₄HCO₃. After lyophilization, the fractions were further purified by thin layer isoelectric focusing, followed by G-50 chromatography in 0.05 M NH₄HCO₃ to remove the ampholytes. One-milliliter fractions were collected and assayed by SM-C/IGF-I radioimmunoassay¹⁹ and by a radioimmunoassay developed against the 8-amino acid C-peptide segment of IGF-II.²⁰ A basic peptide, with a pI of 8.0, was isolated, and found to have a SM-C/IGF-I concentration of 6615 ng/ml, with no detectable IGF-II. A second peptide with a pI of 6.0 was also isolated, and found to have an IGF-II concentration of 2057 ng/ml and an IGF-I concentration of 800 ng/ml.

Additionally, a partially purified SM-C/IGF-I preparation was employed for several of the standard curves. This peptide was the Cohn fraction IV-I extract, purified just short of isoelectric focusing, and had a potency of 36 U/mg weight, as determined by placental membrane radioreceptor-assay.¹⁷ For purpose of this assay, a pool of human plasma from 20 normal adult males was used as the standard, and arbitrarily assigned a potency of 1 U/ml. When assayed by radioimmunoassay, the SM-C/IGF-I content of the peptide

was calculated to be 12.9 µg/mg weight.¹⁹ Crystalline porcine insulin was obtained from Elanco Products Company (Lot 3NM99A). Bovine proinsulin (Lot 615-07J-91-1), porcine proinsulin (Lot 615-70N-247), and guinea pig insulin (Lot 615-53-228-3) were the generous gifts of Dr. R. Chance and Lilly Research Laboratories. ¹²⁵I-insulin, with a specific activity of 100-120 µCi/µg, was prepared by a modification of the method of Hunter and Greenwood.¹⁶ Trypsin and soybean trypsin inhibitor were purchased from Worthington Biochemical Corp. (Freehold, New Jersey).

IGF-I Binding assays. IM-9 cells in late log or early stationary phase were sedimented by centrifugation and resuspended in Hepes buffer. Incubation mixtures had a final volume of 0.5 ml, and consisted of IM-9 cells at a final concentration of 10-20 × 10⁶ cells/ml, ¹²⁵I-IGF-I (10,000 cpm), and unlabeled peptides (IGF-I, porcine insulin, guinea pig insulin, and bovine proinsulin) at final concentrations of 0-10,000 ng/ml. The cells were incubated for 100 min at 15°C in a gently shaking waterbath, as previously described.⁸

Preincubation experiments. IM-9 cells in late log phase were pooled, sedimented, and resuspended in serum-free medium in 75-ml or 250-ml sterile plastic culture flasks, at concentrations of 10⁶ cells/ml. Buffer or various concentrations of peptide hormones (IGF-I, porcine insulin, bovine proinsulin, guinea pig insulin) were added to the flasks, which were incubated for specified times at 37°C. At the end of the preincubation the cells were sedimented by centrifugation at 1400 × g at 22°C for 5 min. After aspiration of the supernatant, the cell pellets were suspended in 5.0 ml of Hepes buffer for 20 min and then again sedimented by centrifugation. After three sequential suspension-centrifugation steps, the washed pellets were resuspended in equal volumes of buffer and counted in a hemocytometer. Preincubation of IM-9 cells with IGF-I, insulin, or proinsulin under these conditions had no demonstrable effect on cell counts, determined by hemocytometer, or cell viability, determined by trypan blue exclusion.

Following the wash procedure, the cell suspensions were diluted with Hepes buffer to a final cell concentration of 10-20 × 10⁶ cells/ml and assayed for their ability to bind ¹²⁵I-IGF-I. A 400-µl aliquot of cells was placed in a fresh 12 × 75-mm polypropylene tube containing 50 µl of ¹²⁵I-IGF-I (10,000 cpm) and 50 µl of unlabeled, partially purified SM-C/IGF-I, at final concentrations of 0-10 µg/ml. Incubations were at 15°C for 100 min, and were performed as previously described.⁸

The efficacy of the wash procedure was confirmed by comparing the serial 5-ml wash steps with 15-ml and 40-ml washes. Under all wash conditions, similar changes in ¹²⁵I-IGF-I binding were observed following preincubation of cells with insulin or IGF-I. Furthermore, radioimmunoassay of the third wash supernatant following 20-h preincubation with 10⁻⁶ M insulin revealed strikingly low levels of insulin (3 ng/ml following 5-ml washes and <1 ng/ml following 40-ml washes). These insulin levels are far below the concentrations required to compete for occupancy of the IGF-I receptor and would not have contributed to the observed decrease in binding.

Insulin binding assays and preincubation experiment. ¹²⁵I-insulin binding to IM-9 cells was performed by the methods described above for ¹²⁵I-IGF-I binding. Competi-

tion for ¹²⁵I-insulin binding sites was evaluated for porcine insulin, porcine proinsulin, IGF-I, and IGF-II. Preincubation experiments were performed as described above, using porcine insulin and proinsulin, IGF-I, and the IGF-II-enriched preparation.

Effects of trypsinization and alteration of pH on the insulin and SM-C/IGF-I receptors. Binding assays for both ¹²⁵I-insulin and ¹²⁵I-IGF-I were performed by the methods described above at a pH range of 5.5–8.5. In a separate series of experiments, IM-9 cells were incubated with trypsin at final concentrations of 0.01–0.5% for 10 min at 22°C, followed by the addition of a twofold excess of soybean trypsin inhibitor. After 5 min, cells were sedimented, washed twice in 5-ml HEPES buffer, resuspended in HEPES buffer, and assayed for their ability to bind ¹²⁵I-insulin and ¹²⁵I-IGF-I.

Binding assay calculations. The data from competitive binding assays were subjected to Scatchard analysis.²¹ As stated above, a partially purified SM-C/IGF-I preparation was employed for some studies. This preparation had a potency of 36 U/mg weight by placental membrane RRA, and a SM-C/IGF-I content of 12.9 μg/mg weight by RIA. Where this preparation was employed, SM-C/IGF-I content is expressed as both mU/ml and moles/L.

RESULTS

Association of IGF-I and insulin with IM-9 receptors. The binding of ¹²⁵I-IGF-I to IM-9 cells at 15°C is rapid, with over 90% of specific binding achieved by 90–120 min. At cell concentrations of 20 × 10⁶/ml, total binding averaged 25.05%, and nonspecific binding 1.45%. Binding of ¹²⁵I-insulin to 20 × 10⁶ cells/ml, averaged 57.2%, of which 1.06% was nonspecific. Figures 1A and B depict the effect of altered pH and trypsinization of IM-9 cells on binding of ¹²⁵I-insulin and ¹²⁵I-IGF-I. As can be seen, under all conditions a synchronous reduction in binding of both radioligands was observed. By lowering the pH of the assay to 6.5, ¹²⁵I-insulin binding was decreased by 67% and ¹²⁵I-IGF-I binding by 59%. Similarly, both insulin and IGF- binding were reduced by greater than 50% by pretreatment of IM-9 cells with 0.05% trypsin.

Figure 2 demonstrates that unlabeled IGF-I effectively inhibits binding of ¹²⁵I-IGF-I to IM-9 receptors, with 50% occupancy observed at IGF-I concentrations of 30 ng/ml (4 × 10⁻⁹ M). Compared to IGF-I, the relative potencies of the insulin analogues tested are: porcine insulin > bovine proinsulin > guinea pig insulin. Fifty percent displacement of ¹²⁵I-IGF-I is achieved at insulin concentrations of 2–3 μg/ml (5 × 10⁻⁷ M), approximately 100-fold greater levels than required for IGF-I.

Decrease in ¹²⁵I-IGF-I binding following preincubation with insulin. IM-9 cells in late log phase were incubated for 20 h at 37°C in the presence and absence of various concentrations of porcine insulin. After extensive washing for 2 h, aliquots of IM-9 cells were assessed for their ability to bind ¹²⁵I-IGF-I (Figure 3). A 17% reduction in cellular binding of ¹²⁵I-IGF-I was observed following preincubation with insulin at concentrations of 0.3 μg/ml (5 × 10⁻⁸ M). Increasing insulin concentrations in the preincubation resulted in a dose-dependent decrease in subsequent cellular binding of ¹²⁵I-IGF-I, with a 50% decrease in binding following preincubation with 1.0 μg/ml (2 × 10⁻⁷ M) and a 60% decrease at 10 μg/ml (2 × 10⁻⁶ M). Further increases in insulin concen-

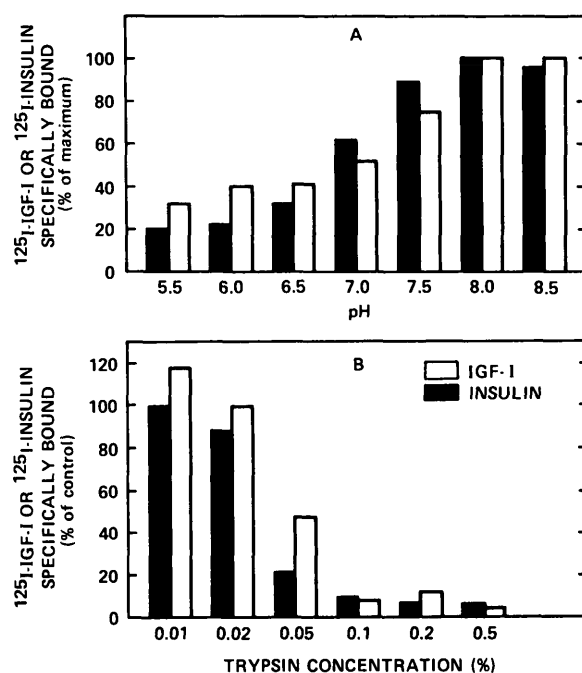
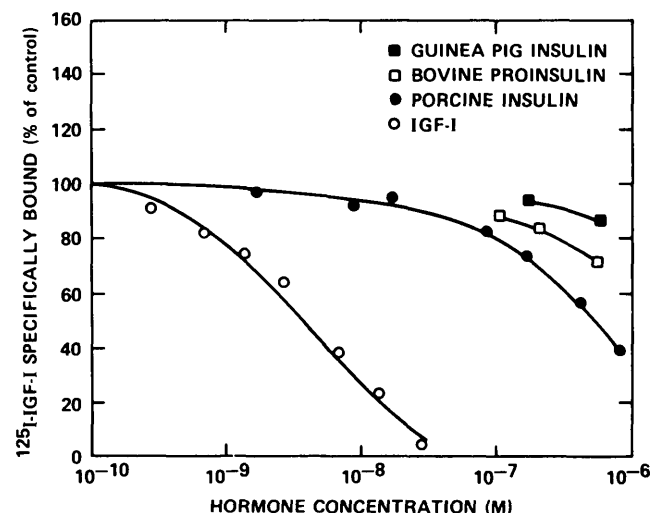


FIGURE 1. Effect of assay conditions upon binding of insulin and IGF-I to IM-9 cells. (A) Effect of pH. 20 × 10⁶ cells/ml were incubated with ¹²⁵I-insulin or ¹²⁵I-IGF-I in the presence or absence of 10 μg/ml of unlabeled insulin or SM-C/IGF-I, respectively. Incubations were for 100 min at 15°C, and were performed at a pH ranging from 5.5 to 8.5. Data are expressed as percent of maximum specific binding, which was at pH 8.0 for both peptides, and averaged 56.1% for ¹²⁵I-insulin and 21.7% for ¹²⁵I-IGF-I. Data represent the means of duplicate experiments. (B) Effect of trypsinization. IM-9 cells at concentrations of 20 × 10⁶ cells/ml in HEPES buffer without BSA were incubated for 10 min with trypsin at final concentrations ranging from 0.01–0.5%. After the addition of a twofold excess of trypsin inhibitor for 5 min, cells were sedimented, washed twice, resuspended in buffer and assayed for their ability to specifically bind ¹²⁵I-insulin and ¹²⁵I-IGF-I. Data are expressed as a percent of maximum specific binding, which averaged 46.8% for ¹²⁵I-insulin and 17.3% for ¹²⁵I-IGF-I. Data represent the means of duplicate experiments.

FIGURE 2. Competition for occupancy of the IGF-I receptor on IM-9 cells. IM-9 cells, at a final concentration of 20 × 10⁶ cells/ml, were incubated with ¹²⁵I-IGF-I and unlabeled hormones, at final concentrations of 0–10,000 ng/ml: ○, IGF-I; ●, porcine insulin; □, bovine proinsulin; ■, guinea pig insulin. Incubations were for 100 min at 15°C, as described in MATERIALS AND METHODS. The level of specific binding at each point is expressed as a percent of the specific binding of ¹²⁵I-IGF-I observed in the absence of unlabeled hormones (B₀ = 19.44% of radioactivity).



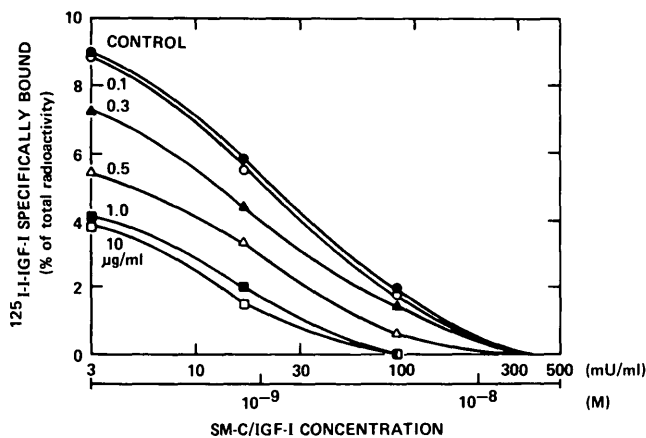


FIGURE 3. Effect of preincubation with porcine insulin on IGF-1 binding to IM-9 cells. IM-9 cells were preincubated in the absence or presence of various concentrations of porcine insulin at 37°C for 20 h. Subsequently, the cells were washed extensively for 2 h, resuspended in HEPES buffer, and incubated with ¹²⁵I-IGF-1 for 100 min at 15°C, in the presence and absence of increasing concentrations of unlabeled SM-C/IGF-1, as described in MATERIALS AND METHODS. The concentration of insulin during the preincubation is noted adjacent to each binding curve. The concentration of partially purified SM-C/IGF-1 during the incubation with ¹²⁵I-IGF-1 is recorded on the abscissa, both in molar concentrations and in mU/ml, as described in MATERIALS AND METHODS.

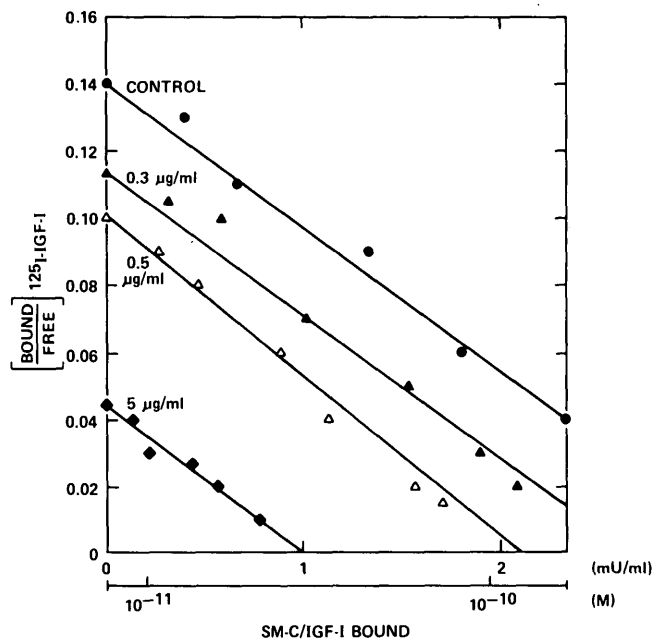


FIGURE 4. Scatchard plot of data from Figure 3 and similar experiments. The ratio of bound:free ¹²⁵I-IGF-1 is plotted as a function of the total concentration of bound SM-C/IGF-1. The concentration of insulin during the preincubation is noted adjacent to each binding curve.

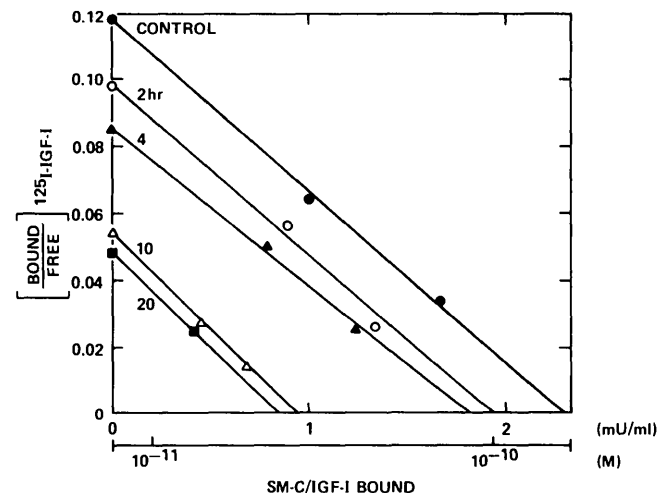
trations resulted in little further changes in ¹²⁵I-IGF-1 binding, with a 63–71% decrease in binding observed following preincubation with 100 µg/ml insulin (2×10^{-5} M). Scatchard analysis of these binding experiments exhibited parallel slopes under all preincubation conditions (Figure 4), demonstrating that the quantitative change in ¹²⁵I-IGF-1 binding can be accounted for by a proportional decrease in the number of available IGF-1 receptor sites, with no alteration in receptor affinity.

Figure 5 depicts the time course for regulation of SM-C/IGF-1 receptors following preincubation with insulin at a concentration of 10 µg/ml (2×10^{-6} M). A clear time dependency is demonstrable, with 25% of the total receptor loss observed following 2-h preincubation, and almost 50% of maximal receptor loss occurring after 4-h preincubation. This is indistinguishable from the time course previously described for SM-induced loss of homologous receptors.⁹ The Scatchard plots of these binding data indicate that the time-dependent reduction in ¹²⁵I-IGF-1 binding, like the dose-dependent reduction, is entirely explained by a decrease in the number of specific SM-C/IGF-1 receptors, with no observable alteration in binding affinity.

Effect of insulin analogues on IGF-1 receptor loss. The ability of 4 insulin-like peptides to induce SM-C/IGF-1 receptor loss is depicted in Figure 6. Porcine insulin was approximately 1/100th as potent as IGF-1 in inducing loss of IGF-1 receptors, with 50% receptor loss occurring following preincubation with IGF-1 at concentrations of $2-3 \times 10^{-9}$ M and insulin at concentrations of $1.5-2 \times 10^{-7}$ M. Bovine proinsulin and guinea pig insulin were respectively, only 10% and 4% as active as porcine insulin in inducing loss of SM-C/IGF-1 receptors. When Figures 6 and 2 are compared, it is apparent that the order of analogue effectiveness for occupancy and regulation of the IGF-1 receptor are identical. Furthermore, a stoichiometric relationship exists between the molar quantities of peptides required for receptor occu-

pancy and for induction of SM-C/IGF-1 receptor loss. Fifty percent occupancy of the SM-C/IGF-1 receptor occurs at IGF-1 concentrations of 3.6×10^{-9} M, while 50% receptor loss follows 20-h preincubation with IGF-1 at a concentration of 2.5×10^{-9} M. Similarly, 50% occupancy of the SM-C/IGF-

FIGURE 5. Effect of duration of preincubation with porcine insulin on IGF-1 binding to IM-9 cells. IM-9 cells in late log phase were pooled and resuspended in serum-free medium at a final concentration of 10^6 cells/ml for 20 h at 37°C. At specified times prior to harvesting, porcine insulin was added, at a final concentration of 10 µg/ml. At the end of the preincubation, the cells were washed extensively for 2 h, resuspended in HEPES buffer, and incubated with ¹²⁵I-IGF-1 for 100 min at 15°C, in the presence and absence of increasing concentrations of unlabeled SM-C/IGF-1. The duration of preincubation with insulin is noted next to each binding curve. The concentration of partially purified SM-C/IGF-1 during the incubation with ¹²⁵I-IGF-1 is recorded on the abscissa both as molar concentrations and as mU/ml. The data are expressed as the ratio of bound:free ¹²⁵I-IGF-1 plotted as a function of the total concentration of bound SM-C/IGF-1.



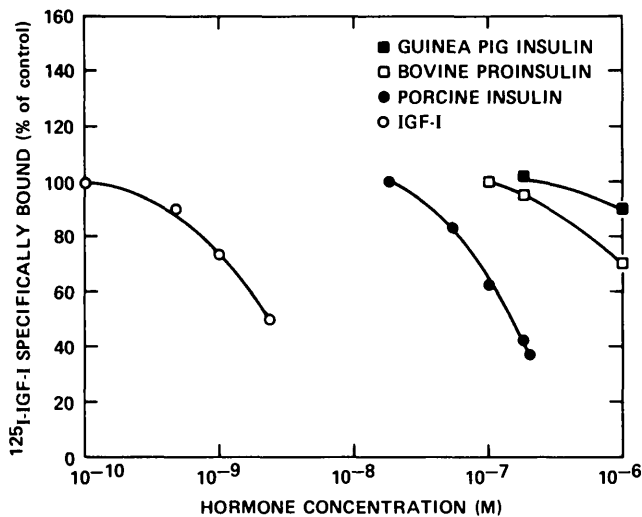


FIGURE 6. Effect of preincubation with insulin analogues on IGF-I binding to IM-9 cells. IM-9 cells were preincubated at 37°C for 20 h with various concentrations of hormones: ○, IGF-I; ●, porcine insulin; □, bovine proinsulin; ■, guinea pig insulin. After preincubation, cells were assayed for their ability to bind ¹²⁵I-IGF-I, as described in MATERIALS AND METHODS, and the legend of Figure 3. Data are expressed as a percentage of the counts bound to cells preincubated with buffer.

I receptor requires 5×10^{-7} M insulin, while 50% receptor loss results from preincubation with 2×10^{-7} M insulin. A similar relationship exists for both bovine proinsulin and guinea pig insulin.

Competition for and regulation of the insulin receptor site. Figure 7 depicts the competition of ¹²⁵I-insulin with porcine insulin, IGF-I and IGF-II for occupancy of IM-9 receptor sites. Fifty percent occupancy was observed at peptide concentrations of 3.7×10^{-9} M (insulin) and 3.7×10^{-8} M (IGF-II). It is of note that IGF-II is approximately ten times more potent than IGF-I in its ability to compete for the insulin receptor, while IGF-I is ten times more potent than IGF-II in its ability to compete for occupancy of the SM-C/IGF-I receptor.⁸

Figure 8 illustrates the effect on ¹²⁵I-insulin binding of preincubation of IM-9 cells with various peptides for 20 h at

FIGURE 7. Competition for occupancy of the insulin receptor on IM-9 cells. IM-9 cells, at a final concentration of 20×10^6 cells/ml, were incubated for 100 min at 15°C with ¹²⁵I-insulin and unlabeled hormones at final concentrations of 0–10,000 ng/ml: ○, IGF-I; ●, porcine insulin; ▲, IGF-II. The level of specific binding at each point is expressed as a percent of the specific binding of ¹²⁵I-insulin observed in the absence of unlabeled hormones ($B_0 = 58.2\%$ of total radioactivity).

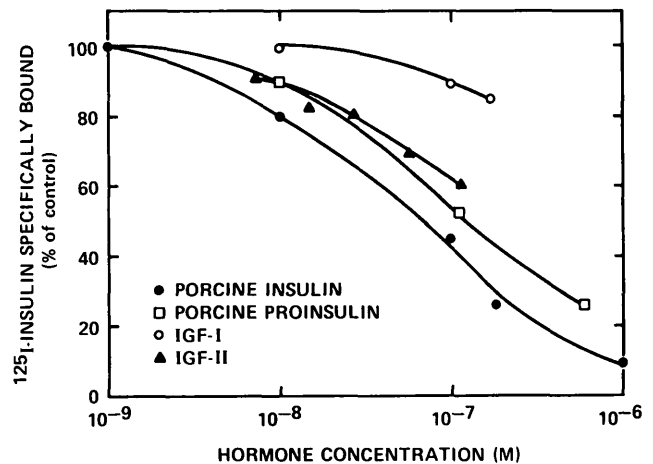
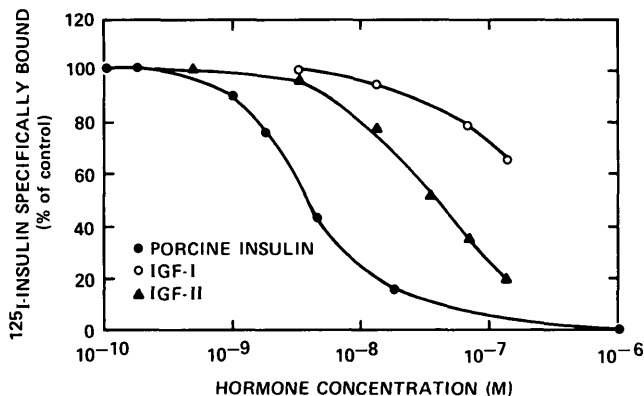


FIGURE 8. Effect of preincubation with insulin and SM analogues on insulin binding to IM-9 cells. IM-9 cells were preincubated at 37°C for 20 h with various concentrations of hormones: ○, IGF-I; ▲, IGF-II; ●, porcine insulin; □, porcine proinsulin. After preincubation, cells were assayed for their ability to bind ¹²⁵I-insulin, as described in MATERIALS AND METHODS, and the legend of Figure 7. Data are expressed as a percentage of the counts bound to cells preincubated with buffer.

37°C. Following preincubation with 10^{-7} M peptide, the following reduction in ¹²⁵I-insulin binding was observed: 55% decrease (porcine insulin), 46% decrease (porcine proinsulin), 39% decrease (IGF-II), and 10% decrease (IGF-I). As observed in similar experiments with the SM-C/IGF-I receptor, the order of analogue effectiveness for receptor occupancy was similar to that observed for receptor regulation: porcine insulin > IGF-II > IGF-I. However, it is important to note that the ranking of peptide potency is different for each receptor.

DISCUSSION

Although insulin and the insulin-like growth factors are immunologically distinct polypeptides,^{9,10,20,22} they are characterized by striking similarities in their primary and tertiary structures.^{11,12} Indeed, the somatomedins, by definition, possess insulin-like activity,²³ and insulin can, at high concentrations, mimic SM's in vitro growth-promoting, mitogenic, and cartilage-stimulatory actions.^{12,24} These common biologic activities are explained by the observation that even though most cells have distinct insulin and IGF receptors,^{25–27} both insulin and the IGF peptides have weak affinities for each other's receptors.^{8,12}

Studies of insulin-mediated loss in insulin receptors have demonstrated that the ability of an insulin analogue to regulate insulin receptors in a specific cell line can generally be predicted by the peptide's affinity for that receptor.^{5,28} Our data indicate that since insulin is, in a sense, a somatomedin-like peptide, with an affinity for the SM-C/IGF-I receptor, its ability to induce SM-C/IGF-I receptor loss is similarly proportional to its affinity for that receptor.

Preincubation of IM-9 cells with insulin resulted in a time- and concentration-dependent reduction in specific receptors for SM-C/IGF-I. Receptor loss was demonstrable following 20-h preincubation with insulin at concentrations as low as 5×10^{-8} M, but was not observed at physiologic concentrations. At higher insulin concentrations, progressive SM-C/IGF-I receptor loss was demonstrable, although it was not

possible to decrease receptor number below 30% of control values, even when cells were preincubated with 2×10^{-5} M insulin. This plateau effect has been previously observed during induction of homologous receptor loss by insulin,^{1,28} hGH,² and SM-C/IGF-I, itself.⁶ When cells were preincubated with 2×10^{-6} M insulin, maximal receptor loss was attained by 10 h, further suggesting that a new steady-state concentration of SM-C/IGF-I receptors had been achieved.

The hypothesis that induction of SM-C/IGF-I receptor loss is contingent upon specific binding to the IGF-I receptor is supported by the relative potencies of the insulin analogues tested. The order of effectiveness of these peptides was identical for both occupancy and regulation of the IGF-I receptor (IGF-I > porcine insulin > bovine proinsulin > guinea pig insulin). In fact, the ability of a specific analogue to induce IGF-I receptor loss was directly proportional to its affinity for the IGF-I receptor, with a striking stoichiometric relationship demonstrable. This observation is in sharp contrast to the interaction of insulin with its own receptor on the IM-9 cells, where 50% occupancy occurs at insulin concentrations 1/10–1/100th the levels required to induce 50% receptor loss.¹ However, a stoichiometric relationship similar to that observed by us has been recently reported for insulin regulation of insulin receptors on human diploid fibroblasts, where the high affinity insulin dissociation constant and the insulin concentration required for half-maximal downregulation were equivalent and in the normal physiologic range.⁵

The experiments addressing regulation of insulin receptor concentrations add further support to the concept that ligand-induced receptor loss requires occupancy of the involved receptor. IGF-II, which competes for the SM-C/IGF-I receptor with 1/10th the potency of IGF-I, was, nevertheless, 10 times more active than IGF-I in its ability to induce insulin receptor loss, commensurate with its relative potency in competition for the insulin receptor. In general, the data are consistent with the conclusion that the insulin and SM peptides not only bind to both receptors, but downregulate either receptor in proportion to their ability to occupy that receptor. The sole exception, which is as yet unexplained, is the relatively high concentrations of insulin required to induce insulin receptor loss.^{1,28} Thus, following preincubation of IM-9 cells with insulin at concentrations of 1.8×10^{-7} M, insulin receptor concentrations decreased by 75% and SM-C/IGF-I concentrations declined by 50%, even though insulin competes for occupancy of the latter receptor with only 1/50–1/100th the potency with which it competes for its own receptor.

The data presented serve to emphasize the structural and functional similarity between the somatomedin peptides and insulin. Zapf et al.¹² have suggested that the close structural homology between the IGF molecules and human proinsulin indicates a common phylogenetic ancestor from which these molecules diverged. It is conceivable that the insulin and somatomedin receptors are similarly derived from a common ancestor membrane receptor, and that during the course of time the somatomedin peptide-receptor complex evolved as a potent mitogenic and growth-promoting system, while insulin and its unique receptor developed to mediate specific cellular metabolic requirements. The structural homology between the two receptors is supported by their similar susceptibility to trypsinization and to alteration of pH. Similarly, their functional homology is indicated

by the observation that both the insulin and SM peptides have retained an affinity for both receptors, thus explaining the overlapping biologic activities of these peptides. The ability of insulin to both bind to and directly regulate the SM-C/IGF-I receptor is consistent with recent reports suggesting structural similarities between the insulin and IGF-I receptors, as demonstrated by affinity crosslinking and sodium dodecyl sulfate polyacrylamide gel electrophoresis.^{29–31} Similarly, we have recently reported simultaneous blockade of insulin and SM-C/IGF-I binding to IM-9 lymphocytes by naturally occurring antireceptor antibodies, again suggesting structural homology between the receptors.³² While these observations support the hypothesis that insulin and SM/IGF receptors derive from a common ancestor membrane protein, the physiologic significance of these findings must await further studies.

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