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IGF-II BINDING ON HUMAN LYMPHOID CELLS: DEMONSTRATION OF A COMMON HIGH AFFINITY RECEPTOR FOR INSULIN LIKE PEPTIDES

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SUMMARY. We have studied the binding of ^{125}I-IGF-II to the IM-9 human lymphoid cell line, and to human placental membranes. All of IGF-II radioligand binding to IM-9 cells, and half of the binding to human placental membranes is to a previously unrecognized common (Type-III) high affinity receptor site for insulin-like peptides, in which IGF-I and IGF-II are equipotent and insulin only slightly less potent. This common receptor represents another mechanism by which insulin, and the somatomedins can exert biological action.

The somatomedins are a family of growth hormone dependent, growth promoting polypeptides, that have been purified from serum (1,2). Two major human somatomedins have been characterized, insulin-like growth factor I (IGF-I), somatomedin-C (SM-C) and insulin-like growth factor II (IGF-II) (3,4,5). SM-A now appears to be identical, or nearly so, with IGF-I/SM-C (6). The sequence of these somatomedins is strongly homologous to proinsulin, and it is clear that insulin, SM-C/IGF-I, and IGF-II all belong to a family of peptide hormones with shared structure, biological action, and evolutionary history. Receptors for somatomedins IGF-I/SM-C, SM-A, IGF-II and multiplication stimulation activity (MSA) have been demonstrated and studied in many tissues of the body, both in whole cells and cell membrane preparations (7).

Complementary to the two major SM-peptides found, there are two types of distinct binding sites for somatomedins so far identified. Type-I receptor has been identified with IGF-I, SM-C or SM-A as radioligand. IGF-I is more potent than IGF-II in displacing the labelled hormone from the Type-I receptor, and insulin interacts at high concentrations. Type-II receptor has been identified with MSA or IGF-II radioligand. This receptor has higher affinity

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for IGF-II compared to IGF-I, but no interaction with insulin. Type-I and Type-II receptors have been studied further, using the photoaffinity coupling technique in several tissues. By this technique the Type-I receptors have a subunit structure very similar to the insulin receptor, and are predominant in human placental membranes. IM-9 cells have only Type-I receptors. Type-II receptors have a larger molecular weight under reducing conditions than Type-I, and are predominant on rat liver and rat placental membranes (8).

The IM-9 cell line is a human transformed lymphoid line that has been extensively used for a variety of hormone receptor sites.

In this cell line, Werther et al recently demonstrated that IGF-II caused an increase of IGF-I receptor sites (9). This biological action of IGF-II on cells apparently without a Type-II receptor prompted us to study the IGF-II binding to IM-9 cells in detail, and to compare this to IGF-II binding by human placental membranes. These studies establish the presence of a common receptor for insulin-like peptides which has not been previously defined.

MATERIAL AND METHODS

Cells

IM-9 cells, an established line of human lymphoid cells, were grown in continuous culture in RPMA-1640 medium with 22 mM Hepes buffer (Grand Island, NY), supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamide (4 mM).

The cells were grown in 500 ml flasks (Falkner Plastics, Los Angeles, CA) at 37°C. The cells were used for binding studies at 72 hours after division. The specific binding of ^{125}I-insulin to these IM-9 cells was 22.6% per 20×10^6 cells ml^{-1}.

Membranes

The human placental membranes were prepared according to the method of Cuatrecasas (10), from term placentae and stored at -20°C. Placental membrane protein content was determined by the method of Lowry et al (11).

Peptides

Pure IGF-I and IGF-II were a generous gift from Dr René Humbel and were used for ^{125}I-labelling. In the displacement studies pure preparations of IGF-I and IGF-II were used, prepared by the method of Enberg et al (6). SMA/IGF-I and IGF-II were iodinated by the lactoperoxidase method (12), and purified by ion exchange chromatography.

Binding technique

IM-9 cells: IM-9 cells in late log phase were sedimented by centrifugation and washed x 2 and resuspended in Hepes buffer. Cells were counted in a hemocytometer and viability was determined by trypan blue exclusion. In all experiments the viability exceeded 90%. Unless otherwise stated the cells ($15\text{-}30 \times 10^6$/ml) were incubated for 90 minutes with ^{125}I-IGF-II 0.06 ng/ml and increasing concentrations of unlabelled peptides at 15°C in 0.5 ml Hepes buffer (50 mM Hepes, 10 mM dextrose, 1 mM EDTA, 1.4 mM sodium acetate, 5 mM

potassium chloride, 120 mM sodium chloride, 1.2 mM magnesium sulfate, 1% human serum albumin).

At the end of incubation, duplicate 200 µl aliquots of the suspension were layered over 200 µl of ice cold 2% human serum albumin Hepes buffer in plastic microtubes. The tubes were centrifugated, the supernatant discarded and the radioactivity in each pellet determined.

Placental membrane: Placental membranes (130 µg protein) were incubated with ^{125}I-IGF-I or IGF-II (0.05 ng) and increasing concentrations of unlabelled peptides in a final volume of 50 mM Tris buffer 1% human serum albumin pH 7.4. Nonspecific binding was defined as the radioactivity that remained bound in the presence of 10 µg/ml of a partially purified preparation of somatomedin. At the final concentration this equalled 300 ng/ml of IGF-I and 200 ng/ml IGF-II. The nonspecific binding has been subtracted from all data points. The data from the competitive inhibition studies were subjected to Scatchard analysis (13). Bound radioactivity was used as a measure of the concentrations of bound IGF peptides (B) and the total IGF concentration minus B as the free concentration (F). A plot of B/F to B was then constructed.

RESULTS

The binding of ^{125}I-IGF-II to IM-9 cells at 15^{0}C showed a time course comparable to the binding of insulin or IGF-I/SM-C. An apparent plateau in specific binding was reached by 90 minutes. There was a linear relationship between the amount of ^{125}I-IGF-II specifically bound, and the concentrations of IM-9 cells over the range of 6×10^6 to 50×10^6 cells/ml.

The binding of ^{125}I-IGF-II showed a marked dependence on pH (Fig. 1). Specific binding of ^{125}I-IGF-II increased rapidly with increasing pH, reaching a plateau at pH 8.2 - 8.4 that was 5-fold above the specific binding at physiological pH (Fig. 1).

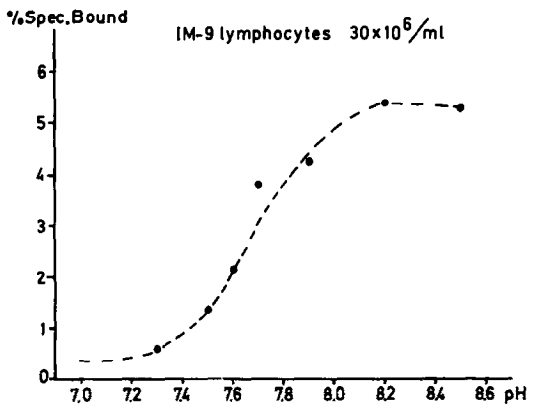

Figure 1. pH dependence of ^{125}I-IGF-II binding of IM-9 cells. IM-9 cells were incubated with 10.000 CPM ^{125}I-IGF-II for 120 min at 15^{0}C in buffers of different pH's. Nonspecific binding was determined in the presence of 10 µg/ml partially purified SM and was subtracted to obtain specific binding. Specific % B is plotted against the final pH of the incubation.

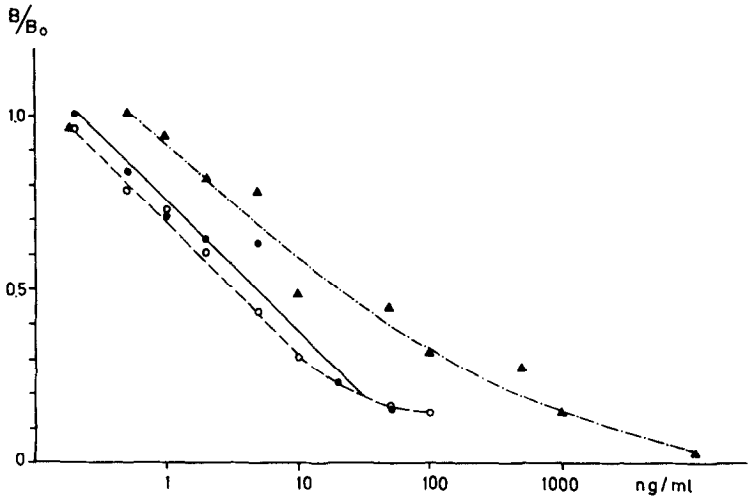

Figure 2. Displacement of ^{125}I-IGF-II from IM-9 cells. IM-9 cells (30×10^6 cells/ml) were incubated in the presence of known concentrations of IGF-I (o), IGF-II (●) and insulin (▲). Nonspecific binding in the presence of 10 µg/ml partially purified somatomedin is subtracted from each point. Results represent the mean of four separate experiments, and are plotted as B/B_0.

Competition for binding of ^{125}I-IGF-II to receptors on IM-9 cells was shown by low concentrations of not only pure IGF-II, but by low concentrations of IGF-I and insulin as well (Fig. 2). Fifty per cent displacement of ^{125}I-IGF-II was achieved by concentrations of 3.5 ng/ml IGF-I, 5.0 ng/ml of IGF-II and 20 ng/ml of insulin. All of the specific binding of ^{125}I-IGF-II was displaceable in the presence of excess (10 µg/ml). When the displacement data was analyzed by the method of Scatchard, IGF-I and IGF-II were indistinguisable (Fig. 3a). The Scatchard plot was curvelinear, suggesting either two binding sites or negative cooperativity. Analysis of the initial high affinity component of the receptor showed an apparent K_a of approximately 4×10^{-10}M with a calculated receptor site concentration of 150.000/cell. The second component of the curve had a calculated apparent affinity of 2×10^{-8}M. Scatchard analysis of insulin competition for IGF-II binding was also curvelinear (Fig. 3b) and the high affinity component was similar to that found with IGF-I and IGF-II. However, there appeared to be a larger proportion of the low affinity component seen with insulin.

When the characteristics of ^{125}I-IGF-II binding to IM-9 cells are compared to human placental membranes, a marked difference is seen (Fig. 4a). Both

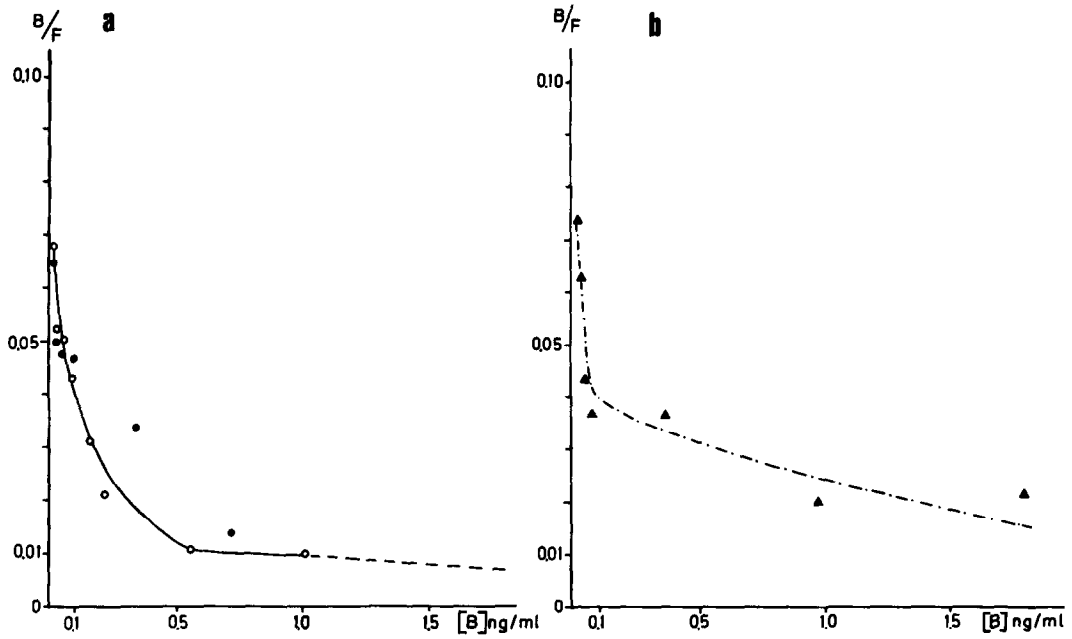

Figure 3. Scatchard analysis of ^{125}I-IGF-II binding to IM-9 cells.
a) IGF-I and IGF-II displacement of ^{125}I-IGF-II. o = IGF-I, ● = IGF-II.
b) Insulin (▲) displacement of ^{125}I-IGF-II.

IGF-I and IGF-II compete for ^{125}I-IGF-II binding to human placental membranes, with 50% displacement of ^{125}I-IGF-II achieved by 8 ng/ml of somatomedin peptide. However, the ability of insulin to displace IGF-II radioligand is markedly different. Although some displacement of ^{125}I-IGF-II is seen by low concentrations of insulin, more than 50% of ^{125}I-IGF-II specific binding is still present even in the face on an insulin concentration of 10 μg/ml. This suggested that there are at least two components of ^{125}I-IGF-II binding to human placental membranes; insulin sensitive and insulin insensitive. When the insulin sensitive binding of ^{125}I-IGF-II was blocked by the presence of 10 μg/ml insulin in the buffers, there was a shift in the apparent potency of IGF-I and IGF-II (Fig. 4b). Under these conditions, IGF-II is more potent than IGF-I in competing for ^{125}I-IGF-II binding. This is accounted for by a shift in the IGF-I displacement curve, with little or no change in the IGF-II displacement curve. These data suggest that the insulin sensitive component of ^{125}I-IGF-II binding to human placental membranes has higher affinity for IGF-I than the insulin insensitive component, and also that insulin in nanogram quantities competes for this IGF-II receptor. Thus, the insulin sensiti-

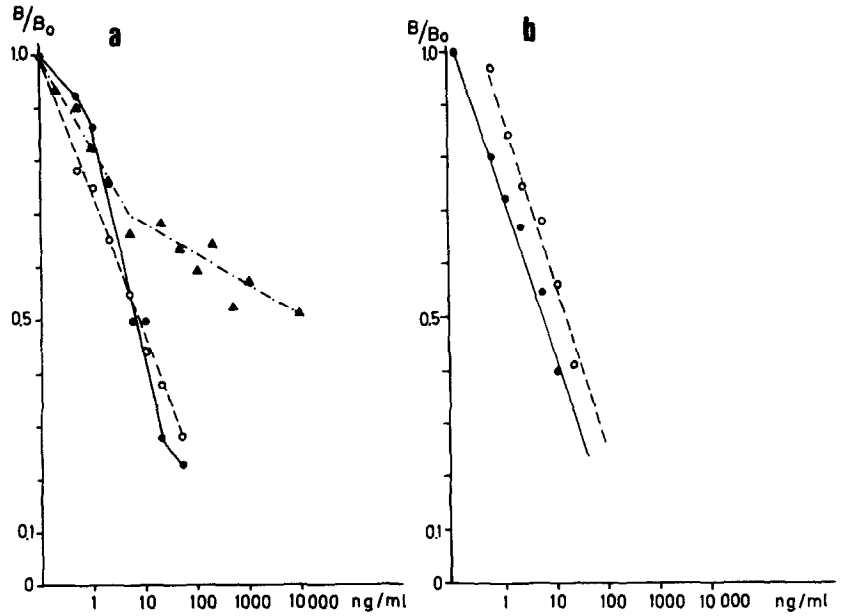

Figure 4.
a) Displacement of ^{125}I-IGF-II from human placental membranes. Human placental membranes were incubated with known concentrations of IGF-I (o), IGF-II (●) and insulin (▲). Nonspecific binding in the presence of 10 µg/ml partially purified somatomedin is subtracted from each point. Results are the mean of duplicate determinations.
b) Displacement of ^{125}I-IGF-II from human placental membranes in the presence of insulin. Human placental membranes were incubated with known concentrations of IGF-I (o) and IGF-II (●). Insulin was included in all the buffers so that the final concentration of insulin in the final incubation volumes was 10 µg/ml. Nonspecific binding in the presence of 10 µg/ml partially purified somatomedin is subtracted from each point. Results are the mean of duplicate determinations.

ve component of IGF-II binding to human placental membranes is strikingly similar to the IM-9 binding site for ^{125}I-IGF-II that we have demonstrated.

DISCUSSION

The receptor site we have delineated by ^{125}I-IGF-II binding to the IM-9 cells in our study is markedly different in its characteristics than the Type-I receptor, the Type-II receptor or the insulin receptor. The Type-I receptor has a 5- to 10-fold preference for IGF-I when compared to IGF-II and insulin crossreacts at approximately 1/500 ± the potency of IGF-I. On the Type-II receptor, IGF-II is 5- to 10-fold more potent than IGF-I, and insulin does not compete even at high concentrations. The insulin receptor shows cross-reaction with both IGF-I and IGF-II at approximately 1/100 potency of insulin itself. The receptor site on human IM-9 cells we have demonstrated has essen-

tially equal affinity for IGF-I and IGF-II, and insulin is only slightly less potent. Thus, it is probably best to view this as a "common" receptor for insulin-like peptides, rather than an IGF-II receptor. We would propose that it should be regarded as a third type IGF receptor. Despite the relative unspecificity of this receptor, it does show high apparent affinity, and IGF-I, IGF-II and insulin can bind to this receptor site at concentrations well within the physiologic range of these hormones. This receptor is best seen in the IM-9 cells with ^{125}I-IGF-II because radiolabelled insulin or IGF-I preferentially bind to their own receptor sites. The IM-9 cells has little or no Type-II receptor sites, so that ^{125}I-IGF-II binds preferentially to the "common" receptor. The work of Werther et al demonstrating an up-regulation of the IGF-I receptor on IM-9 cells by low concentrations of IGF-II most likely is a biological effect mediated by this common insulin-like peptide receptor (9).

Our work with human placental membrane binding of ^{125}I-IGF-II strongly suggests that this "common" insulin-like peptide receptor exist on other human cells in addition to the IM-9 lymphoid cell line. The complex nature of ^{125}I-IGF-II binding to human placental membranes has been previously studied by Daughaday and his coworkers (14). In agreement with their work, we demonstrate that even high levels of insulin will not compete for all of IGF-II binding. In addition, our work demonstrates that insulin at concentrations in the range of 0.5 to 10 μg/ml will compete with ^{125}I-IGF-II binding. There is no combination of Type-I, Type-II and/or insulin receptor binding that can readily explain these findings. We have used the approach of using high (10 μg/ml) concentrations of insulin in the buffer while studying ^{125}I-IGF-II binding to placental membranes. Under these conditions, all of the binding to the insulin receptor and the vast majority of potential binding to the Type-I receptor is blockaded. Under these circumstances the ^{125}I-IGF-II binding resembles the classical Type-II receptor binding described in other studies. The insulin sensitive component of ^{125}I-IGF-II binding represents about half of the total, and by comparison we conclude that it has a higher affinity for IGF-I and is sensitive to low concentrations of insulin. We propose that

this is a Type-III IGF receptor and the same as the common receptor for insulin-like peptides defined on the IM-9 cells.

There are tissues other than human placenta which have been shown to have IGF-II binding which is partially inhibited by insulin. These include embryonic chick fibroblasts (15), human fetal brain membranes (16) and immature human erythrocytes (17). We predict that in these tissues the Type-III receptor will be found. It is interesting to note that three of these examples (fetal brain, placenta and chick embryo) are of fetal origin, one of the others is rich in immature erythrocyte percursors and the other is a transformed cell line. On the other hand, other tissues (rat placenta (14), chondrosarcoma (18) and rat liver (17)) appear to have pure Type-II receptor binding of ^{125}I-IGF-II. Clearly a broad investigation of cell receptors including fetal, transformed and malignant cells, will be necessary before a clear picture of the distribution of Type-III receptors can be developed.

The physical nature of this proposed Type-III receptor is an unsettled question and a subject for further study. The data of Massague and Czech demonstrated little or no binding to a Type-II receptor and this makes it likely that the Type-III receptor does not have the molecular characteristics of the Type-II receptor (8). The IM-9 cell should provide a convenient model for the characterization and purification of the Type-III receptor since all of ^{125}I-IGF-II binding is to this receptor site.

The biological role of this Type-III receptor is at this point unclear. Because of its common nature it could conceivably mediate some of the growth promoting effects of insulin in some tissues. It is also interesting that it is present in at least one fetal tissue. The presence of a phylogenetically primitive common receptor site for insulin-like peptides could explain the proposed role of insulin and the somatomedins as growth factors in fetal life.

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