The Human Placenta Contains Two Distinct Binding and Immunoreactive Species of Insulin-like Growth Factor-I Receptors*

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Helen A. Jonas and Len C. Harrison

From the Endocrine Laboratory, University of Melbourne Department of Medicine, The Royal Melbourne Hospital, Parkville, Victoria, 3050, Australia

Two species of insulin-like growth factor-I (IGF-I) receptors in human placenta have been delineated on the basis of their immunoreactivity with an autoantiserum (B-2) to the insulin receptor. When all the IGF-I binding sites in solubilized human placenta were assayed by polyethylene glycol precipitation, a curvilinear Scatchard plot was obtained which could be resolved into two single classes of binding sites: one immunoprecipitable by B-2 IgG and the other, nonimmunoprecipitable. The B-2 reactive sites bound IGF-I with lower affinity ($K_d = 7.1 \times 10^{-10}$ M) than the B-2 nonreactive sites ($K_d = 2.1 \times 10^{-10}$ M) and cross-reacted more readily with insulin, the IGF-I/insulin-binding potencies being $\sim 120$ and $\sim 1100$, respectively. Both receptor subtypes bound IGF-I with $\sim 30$-fold higher affinity than multiplication-stimulating activity, and, after affinity cross-linking with $^{125}$I-IGF-I, migrated as specific reduced bands of $M_r = 138,000$ during sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The subunit sizes of the B-2 reactive IGF-I receptor were similar to those of the insulin receptor. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of $^{125}$I-labeled receptors immunoprecipitated by autoantiserum B-2 or autoantiserum B-10 (which recognizes only insulin receptors) revealed, in both cases, specific reduced bands of $M_r = 130,000$ and 90,000; the same bands were also seen after sequential precipitation with B-10 and B-2 antiserum to enrich the proportion of IGF-I receptors recovered.

The presence of two distinct binding and immunoreactive species of IGF-I receptors in human placenta raises the possibility that cell- or tissue-specific isoforms of the IGF-I receptor could mediate the different biological actions of IGF-I.

Insulin-like growth factor-I is a growth hormone-dependent polypeptide which shares with insulin a high degree of amino acid sequence homology (1) and biological effects ranging from acute stimulation of metabolism to nucleic acid and protein synthesis and mitogenesis (2). Receptors for IGF-I and insulin are also structurally related in that they react with antibodies to the insulin receptor (3–5) and are composed of similarly sized disulfide-linked subunits (6–10).

Each hormone cross-reacts, albeit with considerably lower affinity, with the other's receptors (9). IGF-I also binds with moderate affinity to the structurally distinct Type II IGF receptor which binds insulin-like growth factor II and multiplication-stimulating activity with high affinity and has little or no affinity for insulin (9). Because IGF-I, at low concentrations, promotes growth far more readily than insulin, and because, in tissues containing no IGF-I receptors (e.g., adipocytes (9, 11)), 100-fold higher concentrations of IGF-I are required to elicit insulin's metabolic actions, it has generally been considered that insulin and IGF receptors mediate, respectively, the metabolic and mitogenic functions of these hormones (2). On the other hand, in cells and tissues containing IGF-I receptors (e.g., human fibroblasts (12), BChI muscle cells (13), and soleus/cardiac muscle (2, 14)) low concentrations of IGF-I and IGF-II can stimulate glucose and amino acid transport, suggesting that these acute metabolic responses may also be mediated by IGF receptors.

It is not known whether the metabolic and mitogenic effects of IGF-I are mediated via IGF-I binding to the same site or to separate sites on its own or related (e.g. IGF-II) receptors. There has been no evidence to date suggesting the existence of IGF-I receptor subtypes or isoforms. In this paper, we present evidence that the human placenta contains two types of IGF-I receptors with different binding affinities for IGF-I and different cross-reactivities with insulin, which can be delineated on the basis of their immunoreactivity with a human autoantiserum to the insulin receptor.

EXPERIMENTAL PROCEDURES

Materials—Porcine monocomponent insulin was purchased from Novo Research Institute. IGF-I (preparation 1/4) was kindly donated by Dr. R. Humbel, Zurich. MSA (rat IGF-II), purified from the conditioned media of BRL-3A rat liver cells (15) was kindly donated by Dr. M. Czech, Worcester. For nonspecific binding measurements, a 1% pure insulin-free preparation of somatomedin-C/IGF-I isolated from human serum (16) was kindly donated by Dr. R. Baxter, Sydney. Porcine insulin, used to estimate nonspecific binding, human $\gamma$-globulin (Cohn fraction II), and Staphylococcus aureus (strain Cowan I), supplemented as a formalin-fixed heat-killed 10% (w/v) suspension, were purchased from the Commonwealth Serum Laboratories, Melbourne Glucose oxidase (Type VII from Aspergillus niger), N-acetyl-d-glucosamine, -cellulose, bovine albumin, leupeptin, phenylmethylsulfonyl fluoride, and bacitracin were purchased from Sigma, IDOGEN and disuccinimidyl suberate from Pierce Chemical Co., Trasylol (aprotinin) from Bayer AG, Germany, lactoperoxidase from Calbiochem, agarose-bound wheat germ agglutinin from Vector Laboratories Inc., bovine albumin ("Fentex") from Miles Laboratories, Inc., and polyethylene glycol 6000 from the Merck Institute. All reagents for SDS-polyacrylamide gel electrophoresis were from Bio-Rad.
other materials were of reagent grade. ²⁵¹-Insulin was prepared to a specific activity of 100-150 µCi/µg (17); ²⁵¹-somatomedin-C/IGF-I, by the IODO-GEN method (18) to a specific activity of approximately 350 pCi/µg. Triton X-100, were prepared as previously reported (17).

To analyze the subunit composition of the ²⁵¹-labeled receptors, the nonspecific binding of radioactivity in the presence of unlabeled insulin (20 µg/ml) or unlabeled IGF-I (1% pure; 100 µg/ml) was subtracted from total binding. Data from the competition binding studies were analyzed by the method of Scatchard (22). 

Immunoprecipitation of Receptors—Solubilized placental membranes, incubated with tracer concentrations of ²⁵¹-insulin or ²⁵¹-IGF-I under the conditions described above, were further incubated with antiserum B-2 or a control nonimmune serum (0.5 ml, final assay dilution, 1/200). After 2 h at 22 °C or 20 °C for 4 h, antibody-bound receptors were precipitated by S. aureus (0.05 ml of 10% suspension) as previously described (3).

To assess specific binding of ²⁵¹-insulin or ²⁵¹-IGF-I to their receptors, the nonspecific binding of radioactivity in the presence of unlabeled insulin (20 µg/ml) or unlabeled IGF-I (1% pure; 100 µg/ml) was subtracted from total binding. Data from the competition binding studies were analyzed by the method of Scatchard (22).

RESULTS

Immunodepletion of Insulin and IGF-I Receptors by Autoantiserum B-2—To assess the ability of autoantiserum B-2 to immunodeplete solubilized human placental membranes of insulin and IGF-I receptors, increasing concentrations of B-2 IgG, prebound to S. aureus, were incubated with the solubilized membranes, and the supernatants were analyzed for residual ²⁵¹-insulin or ²⁵¹-IGF-I binding activities (Fig. 1). All PEG-precipitable and immunoprecipitable insulin binding activities, as described under “Binding Assays,” were assayed for residual ²⁵¹-insulin or ²⁵¹-IGF-I binding activities, as described under “Binding Assays.”

Electrophoresis was performed according to Laemmli (24) in 7.5% polyacrylamide slab gels. The following molecular weight standards (Bio-Rad) were used: myosin (M, = 200,000), β-galactosidase (116,000), phosphorylase b (94,000), bovine serum albumin (68,000), and ovalbumin (45,000). Gels were stained overnight in a solution containing 0.1% Coomassie Blue (B-250), 50% methanol, and 10% acetic acid. Autoradiographs were scanned using a LKB 2202 Ultroscan laser densitometer.

Affinity Labeling of Placental Membranes—Crude placental membranes (1.3 mg protein) in 0.1 M sodium phosphate buffer, pH 7.5, containing bovine serum albumin (1 g/100 ml) were incubated at 4 °C for 18 h with ²⁵¹-IGF-I (8 × 10⁵ cpm/ml) in the presence or absence of unlabeled IGF-I (1% pure, 100 µg/ml). The membranes were washed twice with ice-cold phosphate buffer and then resuspended in the original incubation volume in phosphate buffer containing no albumin. Disuccinimidyl suberate, freshly dissolved in dimethyl sulfoxide, was added to a final concentration of 0.03 mM. After 15 min at 0 °C, the reaction was quenched by the addition of 5 volumes of ice-cold 20 mM Tris-HCl, 1 mM EDTA, pH 7.4, and the mixture centrifuged (20 min, 3000 × g). A portion of each pellet (0.4 mg of membrane protein) was boiled for 5 min in SDS-polyacrylamide gel electrophoresis sample buffer. The remainder (1.4 mg of membrane protein) was solubilized in 1 ml of 1% Triton X-100 in 0.1 M sodium phosphate buffer, pH 7.5, for 1 h at 22 °C and then centrifuged at 100,000 × g for 90 min at 4 °C. Solubilized receptors were immunoprecipitated by adding 10-20 µl of SDS-polyacrylamide gel electrophoresis sample buffer to 1/200 dilution of antiserum B-2 and incubation 1/200, 12 h at 4 °C, and S. aureus (0.25 ml of 10% suspension), and the pellet was boiled with SDS-polyacrylamide gel electrophoresis sample buffer. Samples were subjected to the same conditions of electrophoresis, autoradiography, and densitometry described in the preceding section of “Experimental Procedures.”
sites were depleted equally, reaching undetectable levels at a serum dilution of 1/250 (Fig. 1A). This was not the case for the IGF-I binding sites. Although the IGF-I binding sites were depleted progressively with increasing concentrations of B-2 IgG, PEG-precipitable 125I-IGF-I binding remaining in the supernatants always exceeded immunoprecipitable 125I-IGF-I binding. At serum B-2 dilutions of 1/250 or less there was no further decrease in the PEG-precipitable 125I-IGF-I binding activity, and this residual binding activity (usually 50–80% of original binding activity) could not be further precipitated using serum B-2 (Fig. 1B). Thus serum B-2, while capable of immunoprecipitating all the insulin receptors in the solubilized membranes, could only recognize a fragment of the IGF-I binding sites. After the immunodepleted receptor preparation was equilibrated with 125I-IGF-I, the proportion of 125I-IGF-I specifically bound and precipitated by PEG (18%) was comparable to that recovered in the void volume after gel filtration on a Sephadex G-50 column (20%, Fig. 2). IGF binding proteins in B-2 serum (2) could not account for the precipitation was effected by adding washed B-2 IgG-S. aureus complexes.

**Binding Characteristics of Immunoprecipitable and Nonimmunoprecipitable IGF-I Receptors**—When solubilized human placental membranes, preincubated with 125I-IGF-I and increasing concentrations of IGF-I or insulin, were precipitated by PEG and the binding data (Fig. 3B) subjected to Scatchard analysis, a curvilinear plot was obtained (Fig. 3C). However, the 125I-IGF-I binding precipitated with serum B-2 and S. aureus (Fig. 3A) yielded a linear Scatchard plot, consistent with a single class of lower affinity binding sites ($K_d = 7.1 \times 10^{-10} M, R_0 = 0.13 \times 10^{-12} mol/mg protein$; Fig. 3C). In contrast, the residual nonimmunoprecipitable 125I-IGF-I binding, assayed by PEG precipitation (Fig. 4A) and subjected to Scatchard analysis, yielded a single class of higher affinity binding sites ($K_d = 2.1 \times 10^{-9} M; R_0 = 0.077 \times 10^{-12} mol/mg protein$; Fig. 4C). Curvilinear Scatchard plots were obtained when normal nonimmune globulins prebound to S. aureus were incubated with solubilized placental membranes, and the supernatants were analyzed for PEG-precipitable 125I-IGF-I binding (Fig. 4, B and C).

The B-2-immunoreactive low affinity IGF binding sites cross-reacted more readily with insulin (Fig. 3A) than did the nonimmunoreactive high affinity sites (Fig. 4A). Porcine insulin was $\approx 120$ times less potent than IGF-I in competing with 125I-IGF-I for its low affinity site and $\approx 1100$ times less potent in competing for the high affinity site. In another experiment, both IGF-I binding sites reacted similarly with MSA, their relative IGF-I/MAA potencies being $>30$ (Table 1).

To determine the size of the IGF-I binding sites, 125I-IGF-I was covalently coupled to placental membranes with the bifunctional reagent disuccinyl suberate. When the membranes were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions, 125I-IGF-I appeared in $M_r = 138,000$ and $M_r > 300,000$ complexes (Fig. 5). Only the $M_r = 138,000$ band could be detected when membranes were solubilized with Triton X-100 and immunoprecipitated with serum B-2 prior to electrophoresis (Fig. 5). The $M_r > 300,000$ complex may represent IGF-I receptor cross-linked to its own subunits or to other proteins, because this band could also be immunoprecipitated by serum B-2 when higher concentrations (0.2 mM) of cross-linking agent were used.

**Immunoprecipitation of 125I-IGF-I from Human Placental Membranes**—Autoantiserum B-10, which recognizes only insulin receptors and autoantiserum B-2, which recognizes both insulin and IGF-I receptors (3), both precipitated specific 125I-labeled proteins of reduced molecular weights 130,000 and 90,000 (Fig. 6). The immunoprecipitation procedures were monitored in parallel experiments using 125I-labeled wheat germ eluates. PEG precipitation of 125I-insulin binding activities remaining after the first and second precipitations step; 125I-IGF-I binding activities remaining after immunodepletion with autoantiserum B-10 and serum B-2 was 6 and 0.5%, respectively, compared to 30% for control serum. IGF-I receptors were not removed by serum B-10 but were fractionally removed by serum B-2; specific 125I-IGF-I binding after immunodepletion by serum B-10 and serum B-2 was 6 and 0.5%, respectively, compared to 22% for control serum.

To enrich the proportion of IGF-I receptors immunoprecipitated, iodinated proteins were immunoprecipitated sequentially with B-10 and then B-2 antiseraum (Fig. 7). 125I-insulin binding activities remaining after the first and second precipitation steps were 2.8 and 0.3% respectively, compared to 18% with control serum. On the other hand, residual 125I-IGF-I binding activities were diminished only after the second precipitation step; 125I-IGF-I binding activities remaining after the first and second steps were 12 and 7%, respectively, compared to 11% after control serum. Analysis of the immunoprecipitate, depleted of 85% of 125I-insulin binding activity, again revealed specific 125I-labeled proteins of molecular weights 130,000 and 90,000 (Fig. 7).

**DISCUSSION**

We have shown that human placenta contains two types of IGF-I receptors that can be delineated on the basis of their immunoreactivity with an autoantiserum to the insulin receptor (serum B-2). The IGF-I receptor recognized by serum B-2 ($K_d = 7.1 \times 10^{-10} M$) is more related to the insulin receptor than the nonimmunoprecipitable IGF-I receptor ($K_d = 2.1 \times 10^{-8} M$), in terms of its antigenicity and cross-reactivity with insulin, and its subunits appear to have the same size as those of the insulin receptor. Neither receptor can be defined as a...
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Fig. 3. Immunoprecipitation versus PEG precipitation of 125I-IGF-I-labeled receptors. Solubilized placental membranes (115 µg/tube) were incubated with 125I-IGF-I and increasing concentrations of unlabeled IGF-I or insulin for 24 h at 4 °C as described under "Experimental Procedures"; "Binding Assays." After a further incubation (20 h at 4 °C) with serum B-2 (50 µl/tube; final dilution, 1/1000) or buffer (0.1% Triton-X; 50 µl/tube), 125I-IGF-I-labeled receptors were precipitated with S. aureus (A; see "Experimental Procedures"; "Immunoprecipitation of Receptors") or PEG (B; see "Experimental Procedures"; "Binding Assays"), respectively. Under the assay conditions employed, serum B-2 did not alter the binding of 125I-IGF-I to its receptors because PEG precipitation in the presence or absence of serum B-2 was identical (C). The binding data obtained with unlabeled IGF-I were subjected to Scatchard analysis (C).

Fig. 4. Specific binding of 125I-IGF-I after immunodepletion of unlabeled IGF-I receptors by serum B-2. Solubilized placental membranes (115 µg/0.1 ml) were swirled for 19 h at 4 °C with serum B-2 or control nonimmune serum IgG prebound to S. aureus (final serum dilution, 1/250), as described in the legend to Fig. 1). The supernatants (0.1-ml aliquots) were incubated with 125I-IGF-I and increasing concentrations of unlabeled IGF-I or insulin for 21 h at 4 °C. 125I-IGF-I binding was measured by PEG precipitation, as described under "Experimental Procedures"; "Binding Assays" (A and B). The binding data obtained with unlabeled IGF-I were subjected to Scatchard analysis (C).

classical IGF-II receptor, because affinity-labeling studies with 125I-IGF-I and whole membranes have only identified $M_r = 138,000$ (not $M_r = 128,000$) cross-linked proteins on reduced SDS-polyacrylamide gels (9, 11). Furthermore, both receptors demonstrate high affinity for IGF-I, moderate affinity for MSA, and cross-reactivity with insulin (9, 11).

Our finding, that antiserum B-2 can delineate two types of placental IGF-I receptors, is supported indirectly by Rechler et al. (25) who showed that B-2 IgG inhibited nearly all (90%) of the binding of 125I-IGF-I to IM-9 lymphoblasts, but, even at high concentrations (>100 µg/ml) only partially inhibited IGF-I binding to human placenta (40%). This inhibitory effect of B-2 IgG on IGF-I binding was absent from our binding assays (Fig. 3C) under the incubation conditions employed.

The binding of insulin to its receptor exhibits, universally, a curvilinear Scatchard plot. The experiments of De Meyts and colleagues (26) support the idea that the curvilinear
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TABLE I

| IGF-I receptor                      | Hormone concentration at B/B₀ = 0.5 | Relative potency (IGF-I/MSA) |
|-------------------------------------|-------------------------------------|-----------------------------|
|                                     | % ng/ml                             |
| B-2 immunoreactive                  | 3.9 3.7 113 31                      |
| B-2 nonimmunoreactive               | 11.2 3.7 100 27                     |

The experimental format was similar to that described in the legends to Figs. 3 and 4 except that MSA was substituted for insulin and another preparation of solubilized placental membranes (34 μg/tube) was used. B, and B₀ refer to specific [¹²⁵I]-IGF-I binding (%) in the absence and presence of unlabeled hormones.

Fig. 5. Affinity labeling of [¹²⁵I]-IGF-I to human placental membranes. [¹²⁵I]-IGF-I was cross-linked to human placental membranes using disuccinimidyl suberate. A portion of the cross-linked membranes was solubilized in Triton X-100 and precipitated with serum B-2 and S. aureus. Membranes (top panel) and immunoprecipitate (bottom panel) were solubilized by boiling in SDS-polyacrylamide gel electrophoresis sample buffer and then analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography (see “Experimental Procedures”; “Affinity Labeling of Placental Membranes”). Densitometric tracings were performed on each autoradiogram.

Fig. 6. Precipitation of insulin and IGF-I receptors from iodinated human placental membranes using antisera B-10 and B-2. Human placental membranes were iodinated with Na[¹²⁵I] using lactoperoxidase, solubilized in Triton X-100, and partially purified by chromatography on wheat germ agglutinin-agarose (see “Experimental Procedures”; “Iodination of Placental Membranes”). After immunoprecipitation with serum B-10, B-2, or control nonimmune serum, [¹²⁵I]-labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography (see “Experimental Procedures”; “Immunoprecipitation of Iodinated Receptors and SDS-Polyacrylamide Gel Electrophoresis”). Densitometric tracings were performed on each autoradiogram.

Scatchard plot of insulin binding is due to negative cooperativity, although this explanation is disputed (27). The present findings clearly demonstrate that the curvilinear Scatchard plot of IGF-I binding to placenta is explained by two orders of binding affinity related to structurally distinct receptor subtypes. Curvilinear Scatchard plots have been obtained by other workers for IGF-I binding to particulate placental cell membranes (28, 29) and to human placental explants in organ culture (30). However, Scatchard analyses of IGF-I binding to cultured cell lines (e.g. human fibroblasts (31), IM-9 lymphocytes (32), rat chondrocytes (33), and mouse muscle BC₃H-1 cells (34)) reveal only single classes of IGF-I receptors. Thus, the curvilinear plots obtained for human placental membranes could represent binding to two (or more) types of IGF-I receptors from different cell populations present in the placenta. Sara et al. (35) have observed two species of IGF-I receptors at different stages in the development of the human fetal brain, a lower affinity receptor present in high concentrations before 17 weeks gestation and a higher affinity receptor present after 25 weeks gestation. As well, the IGF-I receptor in chicken embryo fibroblasts exhibits markedly higher affinity for IGF-II than does the IGF-I receptor in Erlich-Lettre strain E cells (11). Alternatively, since the “mi-
the notion that different IGF-I receptors could exist within the same cell type. They showed that the solubilized iodinated membranes (0.68 × 10^6 cpm) were immunoprecipitated sequentially with serum B-10, then serum B-2 (middle panel), or control nonimmune serum (bottom panel); see "Experimental Procedures", "Immunoprecipitation of Iodinated Receptors and SDS-Polyacrylamide Gel Electrophoresis"). For comparison, 1.0 × 10^6 cpm of solubilized iodinated membranes were immunoprecipitated sequentially with control nonimmune serum, then serum B-2 (top panel).

crosomal" placental preparations consist of both endoplasmic reticulum and plasma membranes, curvilinear binding plots could also result from binding to both internal and external IGF-I receptor subtypes within the one cell. The findings of Kull et al. (10), using monoclonal antibodies to immunoprecipitate the human IGF-I receptor, provide some support for the notion that different IGF-I receptors could exist within the same cell type. They showed that the Mᵣ = 95,000 or β subunit of the IGF-I receptor from biosynthetically labeled or lactoperoxidase-iodinated IM-9 lymphoblasts appeared as a broad band on SDS-polyacrylamide gels, and sometimes as a doublet, the faint lower component having a mobility similar to that of the corresponding subunit of the insulin receptor (10). Similar gel patterns were noted for lactoperoxidase-labeled human placental membranes (10).

A number of questions arise. Could the different IGF-I receptors be derived from each other by post-translational processing or are they synthesized by different types of placental cells? Regardless, what biological roles might they play? Although insulin and IGF receptors have clearly distinct hormonal binding sites, their physiological roles are less sharply defined, in that both receptors can mediate metabolic or mitogenic effects, depending on the cell type (25). Are the varying bioeffects mediated by one receptor due to subtypes of that receptor or to different postbinding mechanisms in different cells? Are similar "effector" regions on the insulin and IGF receptors responsible for mediating the metabolic actions of the hormones? Could the IGF-I receptor most closely related to the insulin receptor mediate the acute metabolic actions of IGF-I, and the nonimmunoreactive IGF-I receptor, the growth-promoting effects of IGF-I? Studies with other cell types may resolve this question.

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REFERENCES
1. Rinderknecht, E., and Humbel, R. E. (1978) J. Biol. Chem. 253, 2769-2776
2. Zapf, J., Rinderknecht, E., Humbel, R. E., and Froesch, E. R. (1978) Metabolism 27, 1803-1827
3. Jonas, H. A., Baxter, R. C., and Harrison, L. C. (1982) Biochem. Biophys. Res. Commun. 109, 463-470
4. Kull, F. C., Jr., Jacobs, S., Su, Y.-F., Svoboda, M. E., and Cuatrecasas, P. (1982) in Program and Abstracts of the 64th Annual Meeting, p. 261, The Endocrine Society, Bethesda, MD
5. Kasuga, M., Sasaki, N., Kain, C. R., Nissley, S. P., and Rechler, M. M. (1983) J. Clin. Invest. 72, 1459-1469
6. Kasuga, M., Van Obberghen, E., Nissley, S. P., and Rechler, M. M. (1981) J. Biol. Chem. 256, 5305-5308
7. Chernausek, S. D., Jacobs, S., and Van Wyk, J. J. (1981) Biochemistry 20, 7345-7350
8. Bhaumick, R., Bala, R. M., and Hollenberg, M. D. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4279-4283
9. Massagüé, J., and Czech, M. P. (1982) J. Biol. Chem. 257, 5038-5045
10. Kull, F. C., Jr., Jacobs, S., Su, Y.-F., Svoboda, M. E., Van Wyk, J. J., and Cuatrecasas, P. (1983) J. Biol. Chem. 258, 6561-6566
11. Czech, M. P. (1982) Cell 31, 8-10
12. Berhanu, P., and Olefsky, J. M. (1981) Diabetes 30, 523-529
13. De Vroede, M. A., Rechler, M. M., Nissley, S. P., Standaert, M. L., and Pollet, R. J. (1982) Diabetes 31, Suppl. 2, 123A
14. Poggi, C., Le Marchand-Brustel, Y., Zapf, J., Froesch, E. R., and Freychet, R. (1979) Endocrinology 105, 723-730
15. Oppenheimer, C. L., Pessin, J. E., Massague, J., Gitomer, W., and Czech, M. P. (1983) J. Biol. Chem. 258, 4824-4830
16. Baxter, R. C., and Brown, A. S. (1982) Clin. Chem. 29, 486-487
17. Harrison, L. C., Billington, T., East, I. J., Nichols, R. J., and Clark, S. (1978) Endocrinology 102, 1485-1495
18. Markwell, M. A., and Fox, C. F. (1978) Biochemistry 17, 4807-4817
19. Roth, J. (1975) Methods Enzymol. 37, 223-233
20. Kain, C. R., and Harrison, L. C. (1981) in Carbohydrate Metabolism and Its Disorders (Randle, P. R., Steiner, D. P., and Whelan, W. J., eds) Vol. 3, pp. 279-330, Academic Press, New York
21. Taylor, S. J., Dons, R. F., Hernandez, E., Roth, J., and Gorden, P. (1982) Ann. Intern. Med. 97, 851-855
22. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672
23. Harrison, L. C., and Itin, A. (1980) J. Biol. Chem. 255, 12066-12072
24. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685
Two Species of Human Placental IGF-I Receptors

25. Rechler, M. M., Kasuga, M., Sasaki, N., de Vroede, M. A., Romanus, J. A., and Nissley, S. P. (1983) in Insulin-like Growth Factors/Somatotropins (Spencer, E. M., ed) pp. 459–490, Walter de Gruyter & Co., Berlin

26. De Meyts, P., Bianco, A. R., and Roth, J. (1976) J. Biol. Chem. 251, 1877–1888

27. Corin, R. E., and Donner, D. B. (1982) J. Biol. Chem. 257, 104–110

28. Bala, R. M., Baumick, B., Armstrong, G. H., and Hollenberg, M. D. (1983) in Insulin-like Growth Factors/Somatotropins (Spencer, E. M., ed) pp. 491–507, Walter de Gruyter & Co., Berlin

29. Baxter, R. C., and Williams, P. F. (1983) Biochem. Biophys. Res. Commun. 116, 62–67

30. Bhaumick, B., and Bala, R. M. (1983) in Program and Abstracts of the 65th Annual Meeting, p. 98, The Endocrine Society, Bethesda, MD

31. Rosenfeld, R. G., and Dollar, L. A. (1982) J. Clin. Endocrinol. Metab. 55, 434–440

32. Rosenfeld, R. G., Hintz, R. L., and Dollar, L. A. (1982) Diabetes 31, 375–381

33. Masakawa, A., and Schalch, D. S. (1982) in Program and Abstracts of the 64th Annual Meeting, p. 382, The Endocrine Society, Bethesda, MD

34. de Vroede, M. A., Rechler, M. M., Standaert, M. L., and Pollet, R. J. (1982) in Program and Abstracts of the 64th Annual Meeting, p. 161, The Endocrine Society, Bethesda, MD

35. Sara, V. R., Hall, K., Misaki, M., Fryklund, L., Christensen, N., and Wetterberg, L. (1983) J. Clin. Invest. 71, 1084–1094