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Delineation of atypical insulin receptors from classical insulin and Type I insulin-like growth factor receptors in human placenta

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Insulin-like growth factor (IGF)-binding sites copurifying with human placental insulin receptors during insulin-affinity chromatography consist of two immunologically distinct populations. One reacts with monoclonal antibody αIR-3, but not with antibodies to the insulin receptor, and represents Type I IGF receptors; the other reacts only with antibodies to the insulin receptor and is precipitated with a polyclonal receptor antibody (B-10) after labelling with ^{125}I-multiplication-stimulating activity (MSA, rat IGF-II). The latter is a unique sub-population of atypical insulin receptors which differ from classical insulin receptors by their unusually high affinity for MSA ($K_a = 2 \times 10^9$ M^{-1} compared with 5×10^7 M^{-1}) and relative potencies for insulin, MSA and IGF-I (40:5:1 compared with 150:4:1). They represent 10–20% of the total insulin receptor population and account for 25–50% of the ^{125}I-MSA binding activity in Triton-solubilized placental membranes. Although atypical and classical insulin receptors are distinct, their immunological properties are very similar, as are their binding properties in response to dithiothreitol, storage at −20 °C and neuraminidase digestion. We conclude that atypical insulin receptors with moderately high affinity for IGFs co-exist with classical insulin receptors and Type I IGF receptors in human placenta. They provide an explanation for the unusual IGF-II binding properties of human placental membranes and may have a specific role in placental growth and/or function.

INTRODUCTION

We have reported that insulin receptors purified to homogeneity from human placenta by sequential affinity chromatography on wheat-germ lectin–agarose and insulin coupled to 1,1′-carbonyl di-imidazole-activated agarose (insulin–CDI-agarose) bind significant amounts of ^{125}I-labelled insulin-like growth factors [IGF-I and multiplication stimulating activity (MSA), the rat homologue of IGF-II]. These IGF-binding sites have moderate affinity for MSA and IGF-I [$K_a = (2–5) \times 10^8$ M^{-1}] and 5–15-fold higher affinity for insulin (Jonas *et al.*, 1986). Their properties cannot be attributed solely to contamination with Type I or Type II IGF receptors (which are preferentially excluded from insulin–CDI-agarose) or to cross-reactivity with classical insulin receptors, because these three receptors each have characteristically different patterns of cross-reactivity with insulin, MSA and IGF-I. Moreover, Scatchard analyses of IGF-I and insulin binding revealed that the number of IGF-I-binding sites was only 6–18% of the total insulin-binding sites. Thus we ascribed the IGF-binding activity copurifying with human placental insulin receptors to a discrete sub-population of atypical insulin receptors with moderately high affinity for IGF-I and MSA. These receptors had the same subunit structure, as determined by covalent cross-linking to ^{125}I-MSA, as insulin and Type I IGF receptors, displayed intermediate reactivity toward polyclonal and monoclonal antibodies to insulin and Type I IGF receptors and contained sub-populations of receptors not recognized by polyclonal antibodies to the insulin receptor, B-2 and B-10 (Jonas *et al.*, 1986).

We now present evidence for two immunologically distinct populations of IGF-binding sites in human placental receptors purified by insulin-affinity chromatography. One population reacts with the monoclonal antibody αIR-3 and represents contaminating Type I IGF receptors; the other reacts only with antibodies to the insulin receptor. Examination of the latter in the absence of Type I IGF receptors reveals a unique sub-population of atypical insulin receptors, which differ from classical insulin receptors in their unusually high binding affinities for insulin, MSA and IGF-I.

This work was reported in part at the 69th Annual Meeting of the American Endocrine Society, Indianapolis (Jonas & Harrison, 1987).

EXPERIMENTAL

Materials

Porcine monocomponent insulin (Actrapid solution, 100 units/ml) was purchased from Novo (Copenhagen, Denmark). IGF-I was purchased from Amgen (Thousand Oaks, CA, U.S.A.). MSA (rat IGF-II) was purified from the conditioned medium of BRL-3A rat liver cells, as described by Marquardt *et al.* (1981). Cohn fraction IV of human plasma (Commonwealth Serum Laboratories, Melbourne, Australia), acidified and subjected to cation-exchange chromatography on SP Sephadex C-25 (Svoboda *et al.*, 1980), was used as a source of IGFs for estimation of non-specific binding; 1 μl (~ 0.05% pure) contained the equivalent of 80 ng of IGF-I and 0.8 ng of insulin (as determined by immuno-

Abbreviations used: IGF, insulin-like growth factor; MSA, multiplication-stimulating activity; PMSF, phenylmethanesulphonyl fluoride; CDI-agarose, 1,1′-carbonyl di-imidazole-activated agarose; PEG, poly(ethylene) glycol 6000; DTT, dithiothreitol.

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assay). Insulin, IGF-I and MSA were labelled with ^{125}I by the fractional chloramine-T procedure (Roth, 1975) to specific radioactivities of 110–160, 210–260 and 150–230 μCi/μg respectively. ^{125}I-insulin and ^{125}I-MSA were purified by chromatography on cellulose CF-II (Roth, 1975) and ^{125}I-IGF-I by hydrophobic-interaction chromatography (Baxter & Brown, 1982) on octyl-Sepharose CL-4B (Pharmacia).

Human immunoglobulin (Cohn fraction II) and *Staphylococcus aureus* [strain Cowan I, supplied as a formalin-fixed heat-killed 10% (w/v) suspension] were purchased from the Commonwealth Serum Laboratories; Triton X-100, bovine serum albumin (RIA grade), dithiothreitol (DTT), *N*-ethylmaleimide, *N*-acetyl-*O*-glucosamine, phenylmethanesulphonyl fluoride (PMSF), bacitracin and neuraminidase (type X from *Clostridium perfringens*) were from Sigma; CDI-activated agarose [Reacti-Gel (6x)] was from Pierce Chemical Co., Rockford, IL, U.S.A.; wheat-germ lectin–Sepharose 6MB was from Pharmacia; Trasylol (aprotinin) was from Bayer AG, Leverkusen, Germany; and poly(ethylene) glycol 6000 (PEG) was from BDH Chemicals, Kilsyth, Australia.

Antisera containing anti-receptor antibodies were from patients B-2 and B-10 with the type B syndrome of insulin resistance and acanthosis nigricans (Kahn *et al.*, 1981; Taylor *et al.*, 1982). Immunoglobulin G was prepared from serum B-10 by affinity chromatography on protein A–Sepharose CL-4B (Pharmacia). Monoclonal antibody to the Type I IGF receptor (αIR-3) (Kull *et al.*, 1983) was kindly donated by Dr. S. Jacobs (Research Triangle Park, Bethesda, U.S.A.). Monoclonal antibodies to the insulin receptor MC 51 (Roth *et al.*, 1982) and 47–9 (Soos *et al.*, 1986) were generous gifts from Dr. R. Roth (Stanford, CA, U.S.A.) and Dr. K. Siddle (Cambridge, U.K.) respectively. Rabbit antiserum to mouse immunoglobulins was purchased from Dakopatts (Glostrup, Denmark).

Human placental membranes were prepared and solubilized with Triton X-100 as previously reported (Harrison *et al.*, 1978), except that the placental tissue was homogenized in the presence of Trasylol (200 Kallekrein inhibitor units/ml, and PMSF (1 mM), and the washed microsomal membranes were solubilized in Triton X-100 (0.1 g/100 ml) containing Trasylol (1000 Kallikrein inhibitor units/ml), PMSF (2 mM) and bacitracin (500 units/ml).

Insulin–CDI-agarose was prepared as described (Newman & Harrison, 1985). Insulin receptors with full binding activity were purified to homogeneity from solubilized placental membranes by sequential affinity chromatography on wheat-germ lectin–agarose and insulin–CDI-agarose (Newman & Harrison, 1985) and stored in neutralized eluant buffer at −70 °C. MSA–agarose was prepared by covalently coupling 350 μg of MSA to 1 ml of CNBr-activated Sepharose 4B (Pharmacia) gel, using the procedures described by Oppenheimer & Czech (1983). Incorporation of MSA, assessed by inclusion of ^{125}I-MSA, was 66%. Wheat-germ lectin–agarose eluate (2 ml; 2 mg of protein) was swirled with the MSA–agarose for 16 h at 4 °C, and the non-adsorbed receptors were collected after transfer to a column. After washing the gel with 50 mM-Tris/HCl, pH 7.4, containing 1 M-NaCl and Triton X-100 (0.1 g/100 ml), receptors were eluted with 50 mM-sodium acetate, pH 5.0, containing 1 M-NaCl and 0.1 g of Triton X-100/100 ml, and then neutralized with equal volumes of 0.1 M-sodium phosphate, pH 7.5. The buffer compositions of the flow-through and eluate fractions were equalized before assay.

Binding assays

Solubilized placental membranes (160–170 μg of protein) or eluates from the insulin–CDI-agarose column (20–50 μl; 80–430 ng of protein) were incubated with 10000–15000 c.p.m. of ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I for 18–24 h at 4 °C in a total volume of 0.2 ml of 0.1 M-sodium phosphate buffer, pH 7.5, containing Triton X-100 (0.1 g/100 ml) (Triton/phosphate buffer). In competition-binding studies, when unlabelled insulin, MSA or IGF-I was included, incubation mixtures also contained 0.25 g of bovine serum albumin/100 ml. Receptor-bound hormone was precipitated by PEG (final concentration, 12.5 g/100 ml) in the presence of carrier human γ-globulin (final concentration, 0.05 g/100 ml), as previously described (Jonas *et al.*, 1982).

Total binding was expressed as a percentage of the total radioactivity added per assay tube. To determine specific binding of ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I to their receptors, the non-specific binding of radioactivity in the presence of unlabelled insulin (5 μg) or unlabelled IGF (2 μl of 0.05% pure preparation) was subtracted from total binding. Data from the competition-binding studies were analysed by the method of Scatchard (1949).

Immunoprecipitation assay. Receptor preparations were incubated for 1 h at 22 °C with the same concentrations of ^{125}I-labelled hormones, unlabelled hormones, BSA and Triton X-100 as described above, followed by serum B-2, B-10, IgG or control non-immune serum [0.05 ml; final dilutions of 1:1000 (serum) or 14 μg/ml (IgG) in Triton/phosphate buffer] for another 18–24 h at 4 °C. Antibody-bound receptors were precipitated by *S. aureus* [0.05 ml of 10% (w/v) suspension], as previously described (Jonas *et al.*, 1982), and total receptors (antibody-bound and non-antibody bound) by PEG.

To calculate specific binding of ^{125}I-labelled hormones to antibody-bound receptors, non-specific binding in the presence of control serum and *S. aureus* was subtracted from total binding (antibody and *S. aureus*). To determine the proportion of ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I binding precipitated by B-2 or B-10, specific immunoprecipitable-tracer binding was expressed as a percentage of the specific PEG-precipitable binding.

Immunodepletion of receptors. B-10 IgG or non-immune human serum diluted in Triton/phosphate buffer was preadsorbed on *S. aureus* by incubation [7–25 μg of B-10 IgG or 1 μl of serum/50 μl of a 10% (w/v) suspension of *S. aureus*] for 30 min at 4 °C, followed by washing with Triton/phosphate buffer. Monoclonal antibodies and non-immune mouse serum were preadsorbed on rabbit anti-mouse IgG and *S. aureus*. Rabbit anti-mouse IgG was incubated with *S aureus* (1 μl/20 μl of *S. aureus* suspension) for 30 min at 4 °C, washed, and then incubated with αIR-3 (8 μl/μl of ascites fluid), MC 51 (0.83 μl/μg of purified antibody), 47–9 (4 μl/μl of antibody preparation) or non-immune mouse serum (8 μl/μl of serum) for 4 h at 4 °C. The washed immunoadsorbents were mixed for 18 h at 4 °C

with the receptor preparations in Triton/phosphate buffer at dilutions equivalent to 140 μg of B-10 IgG/ml, 1:500 dilution of αIR-3 ascites fluid, 1:400 dilution of 47-9 and 50 μg of MC 51/ml. After centrifugation (2500 *g* for 10 min), the supernatants were assayed for specific ^{125}I-insulin binding and ^{125}I-IGF-I binding by PEG precipitation or B-10 immunoprecipitation.

RESULTS

Immunodepletion of affinity-purified receptors by antisera αIR-3 and B-10

In preliminary studies αIR-3 was shown to immunoprecipitate only a fraction of the ^{125}I-IGF-I binding activity of the receptors eluted from insulin–CDI-agarose. Therefore, we tested whether αIR-3 and B-10 would each recognize a different sub-population of IGF-I-binding sites in affinity-purified insulin receptors. We found that the characteristic competition-binding curves obtained by using ^{125}I-IGF-I and unlabelled insulin and IGF-I (Figs. 1*a* and 2*b*; Jonas *et al.*, 1986) were altered markedly following immunodepletion with B-10 or αIR-3 (Figs. 1*b* and 1*c*), and that quantitative removal of the IGF-I-binding sites could be achieved by sequential immunodepletion with B-10 and αIR-3 (Table 1).

The receptors remaining after immunodepletion with B-10 probably represented contamination with Type I IGF receptors, because they bound IGF-I [$K_a = (2–3) \times 10^9$ M^{-1}] with 10–30-fold the potency of insulin (Fig. 1*b*), and could be removed by further depletion with αIR-3 (Table 1) or rechromatography on insulin–CDI-agarose, which preferentially excludes Type I IGF receptors (Jonas *et al.*, 1986). The receptors remaining after depletion with αIR-3 (Fig. 1*c*) were the same as those obtained after rechromatography on insulin–CDI-agarose (Fig. 1*d*) in that they bound IGF-I [$K_a = (0.5–1.0) \times 10^9$ M^{-1}] with $\frac{1}{20}$th the potency of insulin and could be precipitated by B-10. Similar competition-binding curves could also be generated by B-10 precipitation of ^{125}I-IGF-I-labelled affinity-purified receptors (Fig. 2*c*). Thus the B-10 reactive IGF-binding sites co-purifying with insulin receptors during insulin-affinity chromatography represented a population of atypical insulin receptors with high and intermediate binding affinities for insulin and IGF-I, respectively.

Binding characteristics of the B-10-reactive atypical insulin receptors: comparison with B-10-reactive classical insulin receptors

Atypical insulin receptors eluted from the insulin–CDI-agarose column and precipitated with antiserum B-10 had different binding properties from classical insulin receptors that co-precipitated with B-10. The former, identified by ^{125}I-IGF-I binding (Fig. 2*c*) or ^{125}I-MSA binding (Fig. 3*c*), had moderately high affinity for IGF-I [$K_a = (0.2–1.0) \times 10^9$ M^{-1}] and MSA

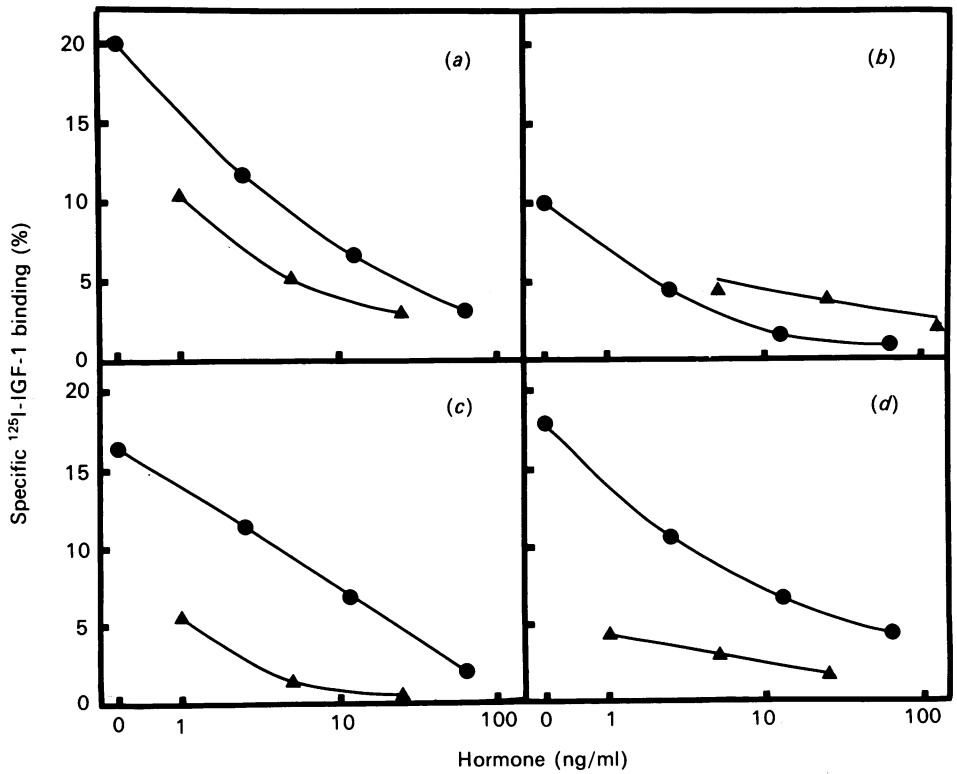

Fig. 1. Competition between ^{125}I-IGF-I and unlabelled hormones for binding to affinity-purified receptors after immunodepletion by antibodies αIR-3 and B-10

Affinity-purified receptors (2.7 μg/ml) immunodepleted with non-immune rabbit serum (*a*), B-10 (*b*) or αIR-3 (*c*), as described in the Experimental section, were assayed (0.1 ml aliquots) for ^{125}I-IGF-I binding in the presence of increasing concentrations of unlabelled insulin (▲) or IGF-I (●). Affinity-purified receptors (5.5 ml) were re-chromatographed on a second column of insulin–CDI-agarose (4 ml) and 0.1 ml aliquots of the eluate (1.8 μg/ml) assayed for ^{125}I-IGF-I binding (*d*). The competition-binding curves obtained in (*a*) could be regenerated by combining the supernatants remaining after immunodepletion with each antiserum.

Table 1. Sequential immunodepletion of the ^{125}I-IGF-I-binding activity in affinity-purified insulin receptors by antibodies αIR-3 and B-10

Affinity-purified receptors (0.8 μg/ml) were incubated with monoclonal antibody αIR-3, polyclonal B-10 IgG, or non-immune mouse serum (NIMS) adsorbed on *S. aureus* under the conditions described in the Experimental section. The supernatants were subjected to a second immunodepletion step using non-immune human serum (NIHS), non-immune mouse serum (NIMS), αIR-3 or B-10, and the final supernatants (0.1 ml aliquots) assayed for ^{125}I-IGF-I binding. The data are expressed as means ± S.D.

Antibodies used for immunodepletion	Specific ^{125}I-IGF-I binding (%)
NIMS, then NIHS	10.6±0.4
B-10, then NIMS	4.7±0.6
B-10, then αIR-3	0.2±0.2
αIR-3, then NIHS	6.1±0.3
αIR-3, then B-10	1.2±0.3

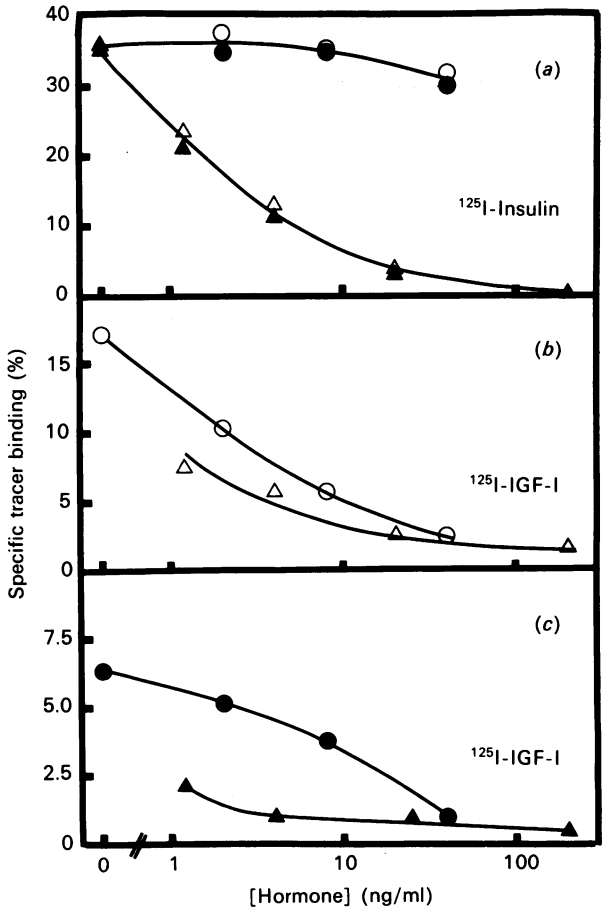

Fig. 2. Competition between ^{125}I-insulin or ^{125}I-IGF-I and unlabelled hormones for binding to affinity-purified receptors immunoprecipitated by B-10 IgG

Affinity-purified receptors were incubated with ^{125}I-insulin (*a*) or ^{125}I-IGF-I (*b*, *c*) in the presence of increasing concentrations of unlabelled insulin (△,▲) or IGF-I (○,●), then precipitated by PEG (△,○) or B-10 IgG and *S. aureus* (▲,●). Assay tubes containing 80 ng (*a*) or 160 ng (*b*, *c*) of receptor protein.

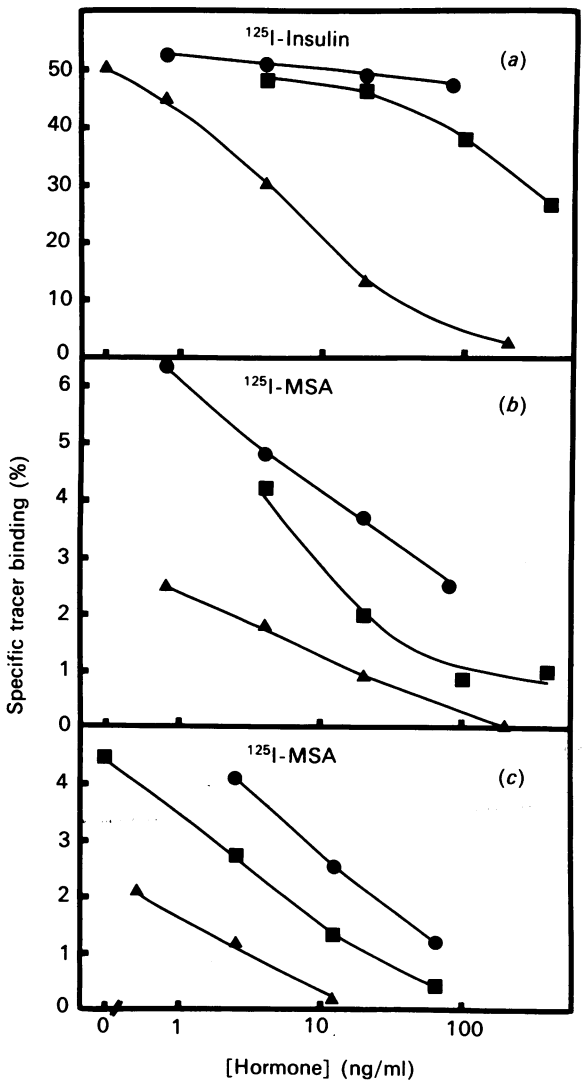

Fig. 3. Competition between ^{125}I-insulin or ^{125}I-MSA and unlabelled hormones for binding to Triton-solubilized placental membranes or affinity-purified receptors immunoprecipitated by B-10 IgG

Triton-solubilized placental membranes (160 μg; *a*, *b*) and affinity-purified receptors (155 ng; *c*) were incubated with ^{125}I-insulin (*a*) or ^{125}I-MSA (*b*, *c*) in the presence of increasing concentrations of unlabelled insulin (▲), MSA (■) or IGF-I (●), then immunoprecipitated by B-10 IgG and *S. aureus*.

[K_a = (1.5–2.0) × 10^9 M^{-1}], bound insulin, MSA and IGF-I with relative potencies of 40:5:1 respectively compared with 150:4:1 for the ^{125}I-insulin-bound receptors (Fig. 3; Jonas *et al.*, 1986), and occupied 20–25% of the total immunoprecipitated insulin receptors. Furthermore, the atypical insulin receptors could not be detected (by B-10 precipitation of ^{125}I-IGF-I or ^{125}I-MSA-binding) in the flow-through fractions of the insulin–CDI-agarose column, although high levels of B-10-precipitable ^{125}I-insulin binding (30–60%/100 μl) were present in these fractions. The latter finding is probably explained by the high affinity of the atypical receptors for insulin [K_a = (1.0–2.5) × 10^{10} M^{-1}] and the lower affinities of the insulin receptors excluded from the column [K_a =

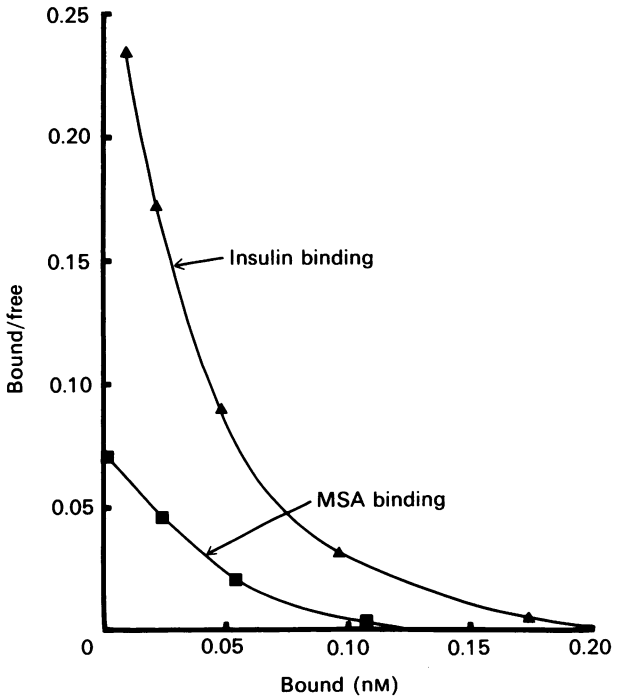

Fig. 4. Scatchard plots of insulin and MSA binding to Triton-solubilized placental membranes immunoprecipitated by B-10 IgG

Triton-solubilized unfractionated receptors (160 μg) were incubated with ^{125}I-insulin (▲) or ^{125}I-MSA (■) in the presence of increasing concentrations of unlabelled insulin or MSA, respectively, then immunoprecipitated by B-10 IgG and *S. aureus*. The scale of the Scatchard plot for insulin binding was reduced by a factor of 5; i.e. the original 'bound/free' and 'bound' values for insulin binding were 5-fold greater than indicated in the Figure.

$(0.4–1.3) \times 10^9$ M^{-1}] compared with those bound to the column [$(1.5–5.0) \times 10^9$ M^{-1}].

The B-10-reactive atypical insulin receptors were not an artefact of receptor purification, because they could be also detected by B-10 precipitation of ^{125}I-MSA-labelled receptors from Triton-solubilized unfractionated placental membranes (Fig. 3*b*). As with the affinity-purified receptors, the B-10-reactive ^{125}I-MSA-binding sites in solubilized membranes showed different patterns of cross-reactivity with insulin, MSA and IGF-I compared with the B-10 reactive ^{125}I-insulin-binding sites (Figs. 3*a* and 3*b*) and only occupied a minor proportion (10–20%) of the total insulin receptors in the immunoprecipitate (Fig. 4).

Although the atypical and classical insulin receptors bind MSA with different affinities, we were not able to separate them by MSA-affinity chromatography. When wheat-germ lectin–agarose-purified preparations were applied to an MSA–agarose column, the eluted receptors (7% of the total insulin receptor population) bound insulin with higher affinity than the receptors remaining in the flow-through fractions ($K_a = 4.5 \times 10^9$ M^{-1} and 0.8×10^9 M^{-1}, respectively). However, their relative binding potencies for insulin, MSA and IGF-I were the same.

Specific detection of the atypical insulin receptors in unfractionated placental extracts could not be achieved by ^{125}I-MSA binding alone, because MSA also interacts with Type I IGF receptors. Thus only 25–50% of the ^{125}I-MSA-labelled receptors in the Triton-solubilized unfractionated placental membranes could be immunoprecipitated by B-10. Furthermore, the relative potencies of unlabelled insulin, MSA and IGF-I for displacing ^{125}I-MSA from unfractionated placental receptors varied from each placental membrane preparation (12:8:1 and 3:6:1, for example), depending on the different proportions of atypical receptors and Type I IGF receptors in these preparations. Less than 5% of the total ^{125}I-IGF-I binding to solubilized membranes could be precipitated by B-10, because the Type I IGF receptors in the unfractionated extracts bound IGF-I with 5 times the affinity of MSA (Jonas *et al.*, 1986), while the atypical insulin receptors bound IGF-I with one-fifth the affinity of MSA (Fig. 3*b*).

Immunoprecipitation of the atypical insulin receptors with monoclonal antibodies to the insulin receptor

Monoclonal antibody MC 51, which cross-reacts minimally with Type I IGF receptors (< 1%, Roth *et al.*, 1983), immunodepleted the atypical and the classical insulin receptors of affinity-purified preparations to similar extents. The ^{125}I-insulin-binding activity remaining in the supernatant was reduced from 48% to 12%, and the ^{125}I-MSA binding activity, from 11% to 2%.

More extensive titration studies with monoclonal antibody 47-9 showed that the dilutions required to immunodeplete classical and atypical insulin receptors from Triton-solubilized placental membranes were similar (Fig. 5). The immobilized antibody removed 95% of the B-10-precipitable ^{125}I-insulin-binding activity from the solubilized membranes and 81% of the B-10-precipitable ^{125}I-MSA-binding activity, but reacted poorly with Type I IGF receptors and depleted total ^{125}I-IGF-I binding by only 22%. Total ^{125}I-MSA binding was depleted by 55%, reflecting the ability of ^{125}I-MSA to bind to both the atypical insulin receptors and the Type I IGF receptors in unfractionated placental extracts.

Storage stability of the atypical insulin receptors

When affinity-purified receptors were stored at −20 °C for 13 days they lost 96% of their ^{125}I-insulin-binding activity and 40–50% of their ^{125}I-IGF-I-binding activity compared with the receptors stored at −70 °C. The loss of insulin-binding activity was due to a 25-fold reduction in the number of insulin-binding sites, while the decline in IGF-I binding activity was accompanied by the disappearance of B-10-reactive, but not αIR-3-reactive, receptors. In keeping with the latter results, the ^{125}I-IGF-I activity remaining after storage at −20 °C was readily displaced by IGF-I, but not insulin (relative binding potencies, 60:1), while the ^{125}I-IGF-I-binding activity of the receptors stored at −70 °C could be displaced equipotently by both hormones. These results suggested that the atypical and classical insulin receptors, unlike Type I IGF receptors, were unstable to storage at −20 °C.

Treatment of affinity-purified receptors with dithiothreitol and neuraminidase

We previously showed that insulin receptors, but not Type I IGF receptors, in solubilized placental membranes lose their ligand-binding activities after exposure to DTT (Jonas & Harrison, 1986). When affinity-purified recep-

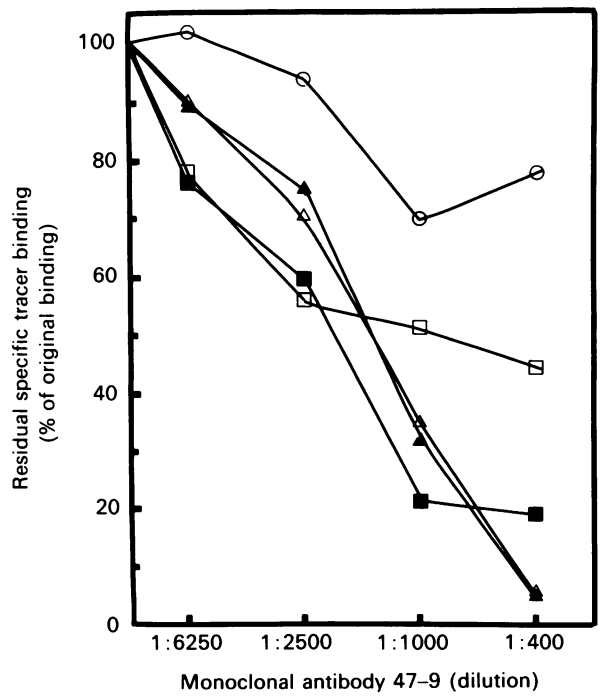

Fig. 5. Immunodepletion of the ^{125}I-IGF-I-, ^{125}I-MSA- and ^{125}I-insulin-binding activities of Triton-solubilized placental membranes by monoclonal antibody 47-9

Triton-solubilized unfractionated receptors (1.1 mg/ml) were incubated with monoclonal antibody 47-9 (final dilutions 1:400 to 1:6250) preadsorbed on rabbit anti-mouse IgG (1:100 dilution) and *S. aureus*, as described in the Experimental section, and the supernatants (0.15 ml aliquots) assayed for ^{125}I-IGF-I binding (○), ^{125}I-MSA binding (□) and ^{125}I-insulin binding (△) by PEG precipitation; and ^{125}I-MSA binding (■) and ^{125}I-insulin binding (▲) by immunoprecipitation with B-10 IgG and *S. aureus*. Specific tracer binding to the receptors remaining after immunodepletion with rabbit anti-mouse IgG and *S. aureus* alone was 20% (○), 11% (□), 37% (△), 6.2% (■) and 38% (▲). The residual specific tracer binding remaining after immunodepletion with 47-9 was expressed as a percentage of control binding.

tors were incubated with 5 mM-DTT, both ^{125}I-IGF-I and ^{125}I-insulin binding decreased by 60%. The proportions of bound ^{125}I-insulin and ^{125}I-IGF-I precipitated by B-10 also declined (from 86% to 56% and from 32% to 24%, respectively), suggesting that DTT had induced similar conformational changes in the classical and atypical insulin receptors.

Neuraminidase treatment of affinity-purified insulin receptors (2–10 μg/ml for 1 h at 37 °C) has been shown to enhance significantly their ^{125}I-insulin-binding activity (Fujita-Yamaguchi *et al.*, 1985). When we preincubated affinity-purified receptors with the same concentrations of neuraminidase for 1 h at 37 °C, ^{125}I-insulin and ^{125}I-MSA binding increased slightly (from 31% to 38% and from 12% to 14.5%, respectively, per 64 ng of receptor) but the relative potencies of unlabelled insulin, MSA and IGF-I for displacing each labelled hormone were unaltered.

DISCUSSION

We have shown that the IGF-binding species which co-purify with human placental insulin receptors during affinity chromatography on insulin coupled to CDI-agarose and have the same subunit structure as insulin receptors and Type I IGF receptors (Jonas *et al.*, 1986) consist of two immunologically distinct populations. The population reactive with monoclonal antibody αIR-3 represents Type I IGF receptors; the population reactive with polyclonal (B-10) or monoclonal antibodies to the insulin receptor represents atypical insulin receptors. Examination of the latter in the absence of Type I IGF receptors confirms their separate identity from classical insulin receptors: they have moderately high affinity for MSA ($K_a = \times 10^9$ M^{-1} compared with 5×10^7 M^{-1}), different relative potencies for insulin, MSA and IGF-I (40:5:1 compared with 150:4:1) and they adsorb more strongly to insulin–CDI-agarose. They occupy only 10–20% of the total insulin receptor population. Although the atypical and classical insulin receptors are distinct, their immunological properties are very similar, as are their binding properties in response to DTT, storage at −20 °C and neuraminidase digestion.

Our findings, that atypical insulin receptors account for 25–50% of the ^{125}I-MSA binding to Triton-solubilized placental membranes, may explain the unusual IGF-II binding properties reported by other investigators for human placental membranes. Thus, ^{125}I-IGF-II bound to human placental membranes could be displaced by different extents by unlabelled insulin, IGF-I and IGF-II: 33% displacement by 20 ng of insulin/ml and 70 ng of IGF-II/ml (Daughaday *et al.*, 1981); approximately equipotent displacement by insulin, IGF-I and IGF-II (Hintz *et al.*, 1984), 50% displacement by 15 ng of IGF-II/ml and 35% displacement by 0.5 μg of IGF-I/ml or 5 μg of insulin/ml (Barenton *et al.*, 1987). We propose that these variable binding patterns might reflect different proportions of atypical insulin receptors, Type I IGF receptors and (possibly) Type II IGF receptors in different placental preparations.

Tollefsen *et al.* (1987) have confirmed that human placental receptors purified by elution from an insulin-affinity column contain IGF-I-binding activity [$K_a = (0.3–1.0) \times 10^9$ M^{-1}], which is competitively displaced by low concentrations of insulin. Casella *et al.* (1986) have also reported high-affinity binding of IGF-II to M_r 130000 subunits in receptor preparations purified from human placenta by affinity chromatography on Sepharose-linked αIR-3. However, it is unlikely that the latter receptors corresponded to atypical insulin receptors because they contained binding sites for αIR-3 and had low affinity for insulin.

The atypical insulin receptor is unrelated to previously reported variant forms of the Type I IGF receptor (Jonas & Harrison, 1985; Morgan & Roth, 1986; Burant *et al.*, 1987) because the latter display higher affinities for IGF-I than insulin. The atypical receptor is unlikely to be a chimaeric form of the insulin and the Type I IGF receptor, because it cannot be immunoprecipitated by αIR-3, which recognizes both the M_r 180000 precursor and the tetrameric form of the Type I IGF receptor (Jacobs *et al.*, 1983).

Misra *et al.* (1986) have shown that ^{125}I-IGF-II binding to intact IM-9 cells (to M_r 135000 subunits) can be displaced by unlabelled insulin, IGF-II and IGF-I in that order of potency, and inhibited by a panel of polyclonal and monoclonal antibodies to the insulin receptor, but not by αIR-3. We have also demonstrated similar effects of anti-receptor antibodies on ^{125}I-MSA

binding to IM-9 cells, and shown that the relative potencies of insulin, MSA and IGF-I for displacing ^{125}I-MSA from intact IM-9 cells (52:6:1) or from IM-9 receptors purified by affinity chromatography on insulin–CDI-agarose (34:4:1) are similar to those measured for the atypical insulin receptors from human placenta (H. A. Jonas & A. J. Cox, unpublished work).

The cellular origin of the atypical insulin receptors in placenta is not yet known. It is likely that these receptors may co-exist with classical insulin receptors (and Type I IGF receptors) in the same cells, because all three receptors are present in IM-9 lymphocytes (Misra *et al.*, 1986; H. A. Jonas & A. J. Cox, unpublished work) and homogeneous populations of bovine endothelial cells (Cox *et al.*, 1987). Because they bind significant amounts of MSA/IGF-II in unfractionated placental membranes and because IGF-II is synthesized and secreted by human placental explants (Fant *et al.*, 1986), the atypical insulin receptors may have a specific role in placental growth and/or function.

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