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Disulphide reduction alters the immunoreactivity and increases the affinity of insulin-like growth-factor-I receptors in human placenta

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We previously identified two forms of the insulin-like growth-factor-I (IGF-I) receptor in human placenta: a lower-affinity form reactive with an autoantiserum (B-2) to the insulin receptor and a higher-affinity non-immunoreactive form [Jonas & Harrison (1985) J. Biol. Chem. **260**, 2288–2294]. Evidence is now presented that the lower-affinity immunoreactive forms are convertible into higher-affinity non-immunoreactive forms via reduction of receptor disulphide bonds. Treatment of placental membranes with increasing concentrations of dithiothreitol (DTT): (1) converted native M_r-290000 heterotetrameric IGF-I receptors into M_r-180000 dimers (determined by chemical cross-linking of ^{125}I-IGF-I with disuccinimidyl suberate); (2) increased ^{125}I-IGF-I binding, owing to an increase in receptor affinity; and (3) abolished the reactivity of Triton-solubilized IGF-I receptors with antiserum B-2 and transformed the curvilinear plot of IGF-I binding to a linear form. In isolated complexes between receptor and B-2 antibody, DTT increased ^{125}I-IGF-I binding and released a single class of higher affinity IGF-I receptors of M_r 180000. Thus DTT-treated IGF-I receptors have similar properties to the higher-affinity non-immunoreactive forms of the native receptor, except that reduced dimeric forms are not detected by cross-linking of ^{125}I-IGF-I to native membranes. Cleavage of the inter-dimeric disulphide bonds is therefore not a prerequisite for higher-affinity binding or loss of immunoreactivity. These observations suggest that the thiol redox state of the IGF-I receptor *in vivo* is an important determinant of receptor conformation and therefore of the biological responses to IGF-I.

INTRODUCTION

Receptors for insulin-like growth factor-I (IGF-I) and insulin are functionally and structurally related. Each binds the other's hormone, albeit with low affinity (Zapf *et al.*, 1978; Massague & Czech, 1982*a*), reacts with antibodies to the insulin receptor (Jonas *et al.*, 1982; Kull *et al.*, 1982) and, in some cells and tissues, mediates similar metabolic and mitogenic functions (Rechler *et al.*, 1983). IGF-I receptors, like insulin receptors, are oligomers of disulphide-linked subunits of M_r 130000–135000 (α) and M_r 90000–95000 (β) (Bhaumick *et al.*, 1981; Kull *et al.*, 1983), which in plasma-membrane preparations appear as three major oligomeric forms with M_r values of 330000–360000, 310000–320000 and 240000–290000 (Chernausek *et al.*, 1981; Massague & Czech, 1982*a*). Intermediate-sized species (M_r 160000–210000), found to a limited extent in native membranes (Massague & Czech, 1982*a*), can be generated by partial reduction of these three major receptor forms (Chernausek *et al.*, 1981; Massague & Czech 1982*a*,*b*). It has been proposed that the three major oligomeric forms of native insulin and IGF-I receptors represent $(\alpha\beta)_2$, $(\alpha\beta)(\alpha\beta_1)$ and $(\alpha\beta_1)_2$ heterotetramers (Massague & Czech, 1982*a*), the β_1 subunit resulting from specific limited proteolytic cleavage of the β subunit (Massague *et al.*, 1981), and the intermediate forms generated by partial reduction consist of $\alpha\beta$ or $\alpha\beta_1$ dimers (Massague & Czech, 1982*a*). Alternatively, since the distribution of insulin-receptor oligomers in adipocytes is altered by disulphide or thiol reagents, the three receptor species could be explained by interconvertible redox forms (Yip & Moule, 1983).

Structural changes occur in insulin receptors after their exposure to the reducing agent dithiothreitol (DDT), as evidenced by altered elution volumes on Sepharose 6B (Maturo *et al.*, 1983), a smaller radiation-inactivation target size (Harmon *et al.*, 1983) and the appearance of lower-M_r forms (Massague & Czech, 1982*b*; Aiyer, 1983). DTT treatment is also accompanied by alterations in receptor binding of insulin (Jacobs & Cuatrecasas, 1980; Schweitzer *et al.*, 1980; Massague & Czech, 1982*b*; Harmon *et al.*, 1983) that are dose-related and apparently characteristic for different tissues. The influence of DTT on IGF-I receptor binding and structure has not been documented.

We previously identified two species of IGF-I receptors in human placenta, delineated on the basis of their immunoreactivity with an autoantiserum (B-2) to the insulin receptor. The B-2-immunoreactive IGF-I receptor binds with lower affinity to IGF-I ($K_a = 1.4 \times 10^9$ M^{-1}) than the B-2-non-reactive IGF-I receptor ($K_a = 4.8 \times 10^9$ M^{-1}) and exhibits a greater cross-reactivity with insulin, the relative IGF-I/insulin binding potencies

Abbreviations used: IGF-I, insulin-like growth factor I; DTT, dithiothreitol; K_a, association constant; PEG, polyethylene glycol 6000; NEM, *N*-ethylmaleimide.

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being 120 and 1100 respectively (Jonas & Harrison, 1985). The present paper describes the effect of disulphide reduction with DTT on the binding and immunoreactive properties of placental IGF-I receptors.

EXPERIMENTAL

Materials

Pig monocomponent insulin was purchased from Novo Research Institute. IGF-I (preparation I/4) was kindly given by Dr. R. Humbel, Zurich, Switzerland. For non-specific binding measurements, a 1% pure, insulin-free, preparation of somatomedin-C/IGF-I (Baxter & Brown, 1982) was kindly given by Dr. R. Baxter, Sydney, Australia. Pig insulin, used to estimate non-specific binding, human γ-globulin (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, Melbourne, Australia. Sheep anti-human IgG was purchased from Silenus Laboratories Pty. Ltd., Melbourne, Australia. DTT, NEM and *N*-acetyl-D-glucosamine were from Sigma, disuccinimidyl suberate was from Pierce Chemical Co., agarose-bound wheat-germ agglutinin was from Vector Laboratories, Burlingame, CA, U.S.A., bovine albumin ('Pentex') from Miles Laboratories, Naperville, IL, U.S.A., cellulose powder CFII was from Whatman, and polyethylene glycol 6000 (PEG) was from the Merck Institute, Darmstadt, Germany. All reagents for SDS/polyacrylamide-gel electrophoresis were from Bio-Rad Laboratories. ^{125}I-insulin was prepared to a specific radioactivity of 100–150 μCi/μg (Roth, 1975); ^{125}I-somatomedin-C/IGF-I, kindly given by Dr. R. Baxter, was prepared to a specific radioactivity of approx. 100 μCi/μg and purified by hydrophobic-interaction chromatography (Baxter & Brown, 1982). For some experiments IGF-I (preparation I/4) was labelled with ^{125}I to a specific radioactivity of approx. 80 μCi/μg and purified by chromatography on cellulose (Roth, 1975). Serum containing anti-receptor autoantibodies was from patient B-2 with the Type B syndrome of insulin resistance and acanthosis nigricans (Kahn & Harrison 1981).

Receptor preparations

Human placental membranes were prepared and solubilized in Triton X-100 as previously reported (Harrison *et al.*, 1978). For some experiments, solubilized placental membranes (5–10 mg of protein/ml) were applied to wheat-germ-agglutinin–agarose columns (1 ml/0.7 ml column), the columns were extensively washed, and bound glycoproteins were desorbed with 0.3 M-*N*-acetyl-D-glucosamine (1.2 mml/column) containing 0.1% Triton X-100, 150 mM-NaCl and 50 mM-Hepes, pH 7.6. To isolate B-2-reactive receptors, eluates from the above columns (0.9 mg of protein/ml) were incubated with antiserum B-2 or control non-immune serum (final dilution 1:100) for 16 h at 4 °C, followed by sheep anti-human IgG (20 μl/μl of serum B-2) for another 24 h at 4 °C. The immune complexes were centrifuged, washed twice and resuspended to the original volume of wheat-germ-agglutinin column eluate with 0.1% Triton/0.1 M-phosphate buffer, pH 7.5.

Binding assays

Particulate placental membranes (100–170 μg of protein) were incubated with tracer concentrations of ^{125}I-IGF-I (50–70 pg) or ^{125}I-insulin (50–60 pg) in the presence or absence of unlabelled IGF-I (1% pure; 100 μg/ml) or unlabelled insulin (20 μg/ml) in 0.2 ml of 0.1 M-sodium phosphate buffer, pH 7.5, containing bovine albumin (0.25%, w/v). In competition binding studies unlabelled IGF-I (preparation I/4) was used. After 18–36 h at 4 °C, membranes were precipitated by centrifugation at 3000 *g* for 20 min and bound radioactivity was measured in a gamma counter.

Solubilized placental membranes (50–110 μg of protein) or eluates from wheat-germ-agglutinin–agarose columns (45 μg of protein) were incubated with ^{125}I-IGF-I or ^{125}I-insulin in the presence or absence of excess unlabelled hormones in 0.2 ml of phosphate buffer containing 0.1% Triton. In competition binding studies using unlabelled IGF-I (preparation I/4), incubation mixtures also contained 0.25% bovine albumin. After 18–21 h at 4 °C, the mixtures were further incubated with antiserum B-2 or a control non-immune serum (0.05 ml of 1:200 dilution) or 0.05 ml of phosphate buffer containing Triton (0.02%). After 17–24 h at 4 °C, antibody-bound receptors were precipitated by *S. aureus* (0.05 ml of 10% suspension) and total receptors (antibody-bound and non-antibody-bound), by PEG (final concn. 12.5% w/v) in the presence of carrier human γ-globulin (final concn. 0.05%, w/v).

Total binding was expressed as a percentage of the total radioactivity added per assay tube. To determine specific binding of ^{125}I-insulin or ^{125}I-IGF-I, the non-specific binding of radioactivity in the presence of excess unlabelled hormone was subtracted from total binding. For antibody-bound receptors, non-specific binding in the presence of control serum was subtracted from control binding. Data from the competition binding studies were analysed by the method of Scatchard (1949) by using the EBDA/LIGAND program from the Biomedical Computing Technology Information Centre, Nashville, TN, U.S.A. In Fig. 2(*b*), the Scatchard plots were analysed for one or two classes of binding sites by using the separate LIGAND program (Munson & Rodbard, 1980).

Affinity labelling of DTT-treated receptors

Glycoproteins from the wheat-germ-agglutinin–agarose columns (0.9 mg/ml) were exposed to 10 mM-DTT (20 min at 22 °C), followed by 25 mM-NEM, then incubated at 4 °C for 24 h at an 8-fold dilution with ^{125}I-IGF-I (2.3×10^6 c.p.m./ml) in the presence or absence of excess unlabelled IGF-I in sodium phosphate buffer, pH 7.5, containing 1% bovine albumin and 0.05% Triton. Receptor–B-2 antibody complexes, resuspended to the original volume of the glycoprotein eluate, were similarly exposed to DTT and NEM, and the supernatant was incubated at a 5-fold dilution with ^{125}I-IGF-I.

Disuccinimidyl suberate, freshly dissolved in dimethyl sulphoxide (20 mM), was added to give a final concentration of 0.2 mM. After 15 min at 0 °C, 100 μl samples were boiled for 5 min with equal volumes of SDS/polyacrylamide-gel-electrophoresis sample buffer [4% (w/v) SDS and 0.02% (w/v) Bromophenol Blue in 0.125 M-Tris/HCl, pH 6.8] in the presence or absence of 50 mM-DTT. Electrophoresis was performed as described by Laemmli (1970) in 5.0%-polyacrylamide slab gels with an aacrylamide/bisacrylamide ratio of 100:1 (non-reduced samples) or in 7.5% gels with an acrylamide/bisacrylamide ratio of 36:1 (reduced samples). Gels were stained

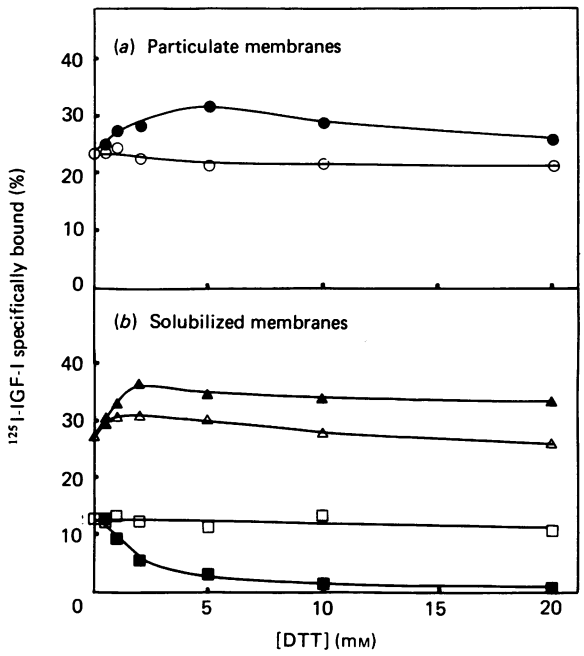

Fig. 1. Effects of DTT on the binding and immunoreactivity of human placental IGF-I receptors

Particulate placental membranes (1.7 mg/ml in phosphate buffer) or solubilized placental membranes (0.8 mg/ml in 0.2% Triton/phosphate buffer) were incubated with increasing concentrations of DTT (0.5–20 mM) for 20 min at 22 °C, and the reaction was stopped by the addition of 2.5-fold higher concentrations of NEM. For control incubations, DTT and NEM were pre-mixed before their addition to membranes. Samples (0.1 ml) were assayed for ^{125}I-IGF-I binding, as outlined in the Experimental section. ●, ■, ▲, Membranes treated with DTT; ○, □, △, control incubations. (*a*) ^{125}I-IGF-I binding to particulate membranes (○, ●); non-specific binding was 7.7%. (*b*) ^{125}I-IGF-I binding to solubilized membranes, precipitated by serum B-2 and *S. aureus* (□, ■) or PEG (△, ▲); non-specific binding was 3.6% and 9.2% respectively. PEG-precipitable binding was not assayed in the presence of serum B-2, since the serum dilution (1:1000) and incubation conditions used did not alter ^{125}I-IGF-I binding (Fig. 2*b*, and Jonas & Harrison, 1985).

in 0.1% (w/v) Coomassie Blue (R-250) in 50% (w/v) trichloroacetic acid and destained in 7% (v/v) acetic acid/20% (v/v) methanol. Autoradiography was performed at −70 °C by using Agfa Curix film and a Dupont Cronex Lightning-Plus enhancing screen.

RESULTS

Effects of DTT on the binding and immunoreactivity of human placental IGF-I receptors

Specific binding of ^{125}I-IGF-I increased after particulate placental membranes were exposed to increasing concentrations of DTT, followed by blocking with NEM, the maximium increase, from 25±2% to 342±2% (mean± S.D.) per 170 μg of protein occurring after incubation with 5 mM-DTT (Fig. 1*a*). A similar increase in ^{125}I-IGF-I binding, measured by PEG precipitation, was observed after Triton-solubilized placental membranes were exposed to the same conditions of disulphide reduction (Fig. 1*b*). In contrast, immunoprecipitable ^{125}I-IGF-I binding decreased from 13±1% to 5±1% (mean±S.D.) per 80 μg of protein after exposure of solubilized receptors to 2 mM-DTT, reaching almost undetectable values at 10 mM-DTT (Fig. 1*b*).

Insulin receptors in the same membrane preparations responded quite differently to DTT. ^{125}I-insulin binding to either particulate membranes (44.8±1.3% per 170 μg of protein) or to solubilized membranes (20.6±1.2% per 80 μg of protein) increased by 10–20% after exposure to 0.5 mM- and 1 mM-DTT, but at higher concentrations of DTT decreased by 20% and 60% respectively. Furthermore, the ability of serum B-2 to precipitate ^{125}I-insulin-binding sites was not altered by DTT (results not shown).

Scatchard analysis of IGF-I binding to DTT-treated membranes

To determine whether IGF-I binding sites on receptors recognized by B-2 antibodies were destroyed by DTT treatment, or converted into higher affinity sites on receptors no longer able to bind B-2 antibodies, estimates of receptor numbers and affinities were obtained by Scatchard (1949) analysis.

Treatment of particulate membranes with DTT increased the average binding affinity, but caused no change in the total number of IGF-I binding sites (Fig. 2*a*). With the solubilized membranes (not exposed to DTT) the Scatchard plot of IGF-I binding to PEG-precipitable receptors was curvilinear, in keeping with the presence of two classes of IGF-I receptors in solubilized placental membranes, i.e. B-2-immunoreactive lower-affinity receptors and B-2-non-immunoreactive higher-affinity receptors (Jonas & Harrison, 1985). Treatment of solubilized membranes with DTT caused no change in the total number of binding sites, but the Scatchard plots were steeper and straighter, consistent with a single class of higher-affinity binding sites and the concomitant loss of lower-affinity binding sites (Fig. 2*b*). When ^{125}I-IGF-I binding to solubilized membranes (not exposed to DTT) was precipitated by B-2 antibodies and *S. aureus*, a linear Scatchard plot was obtained, consistent with a single class of lower-affinity binding sites. Treatment with DTT abolished virtually all immunoprecipitable ^{125}I-IGF-I (Fig. 2*c*). We therefore reasoned that the DTT-induced decrease in the number of lower-affinity immunoreactive receptors (Fig. 2*c*) might be explained by their conversion into the higher-affinity non-immunoreactive receptors.

Effect of DTT treatment on B-2-immunoreactive receptors isolated by immunoprecipitation

To test the hypothesis that the lower-affinity IGF-I receptors could be converted into higher-affinity forms by reduction, and to exclude the possibility that the effects of DTT were mediated indirectly via non-receptor proteins, receptors were partially purified by elution from wheat-germ-agglutinin–agarose and isolated by immunoadsorption to B-2 IgG bound to anti-human IgG, before being exposed to DTT as described in the legend to Fig. 3.

DTT treatment released ^{125}I-IGF-I-binding activity from the immune complexes, which, although precipitable by PEG, could not be reprecipitated by fresh additions of serum B-2 and *S. aureus* (Fig. 3). Scatchard analysis revealed a single class of IGF-I-binding sites with an

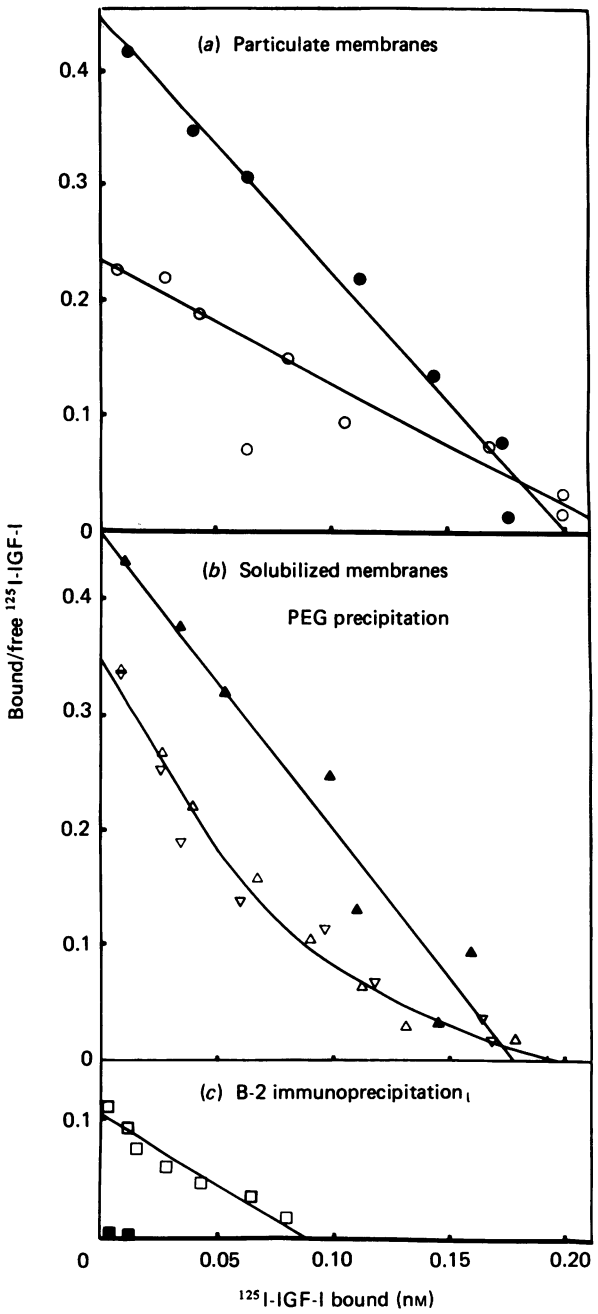

Fig. 2. Scatchard analysis of IGF-I binding to DTT-treated human placental membranes

Particulate placental membranes (5.2 mg/ml in phosphate buffer) were incubated with 10 mM-DTT for 20 min at 22 °C, followed by 25 mM-NEM, or incubated with 25 mM-NEM alone, washed twice with cold phosphate buffer and resuspended to their original volume. A portion was reserved for binding assay, and the remainder was solubilized in the same volume of 1.0% Triton/phosphate buffer for 1 h at 22 °C and centrifuged at 100000 *g* for 90 min at 4 °C to remove insoluble material. Particulate membranes (104 μg/tube) and solubilized membranes (50 μg/tube) were assayed for ^{125}I-IGF-I binding in the presence of increasing concentrations of unlabelled IGF-I, as described in the Experimental section, and the binding data were subjected to Scatchard analysis. ●, ▲, ■, Membranes treated with DTT; ○, ▽, △, □, membranes treated with NEM alone. (*a*) ^{125}I-IGF-I binding to particulate membranes (○, ●); non-specific binding was 3.9%. (*b*) and (*c*) ^{125}I-IGF-I binding to solubilized membranes precipitated by PEG in the presence (▽) or absence (△, ▲) of serum B-2 or by serum B-2 (1:1000 dilution) and *S. aureus* (□, ■); non-specific binding was 7.3%, 6.7% and 3.6% respectively.

affinity [$K_a = 2.0 (\pm 0.3) \times 10^9$ M^{-1} (mean ± S.E.M.)] comparable with that of the total IGF-I receptor population after partial purification on wheat-germ-agglutinin–agarose columns and exposure to DTT [$K_a = 1.6(\pm 0.1) \times 10^9$ M^{-1}]. Treatment of the immune complexes with pre-mixed DTT and NEM did not release a significant amount of ^{125}I-IGF-I binding activity (Fig. 3).

The amount of specific ^{125}I-IGF-I binding in DTT-treated immune complexes (7.8 ± 1.3%; mean ± S.D.) did not differ from that in the non-reduced complexes (7.0 ± 1.3%), despite the release of ^{125}I-IGF-I binding activity after DTT treatment. This suggests that IFG-I-binding sites were more susceptible to disulphide reduction (and subsequent conversion into higher-affinity states) than were the antibody-binding sites. In fact, Scatchard analysis showed that the number of receptors remaining in the DTT-treated immune complexes was significantly less than in control complexes [$1.0(\pm 0.2) \times 10^{-10}$ M versus $1.5(\pm 0.2) \times 10^{-10}$ M (mean ± S.E.M.)], but that their affinity was significantly higher [$K = 6.3(\pm 0.2) \times 10$ M versus $4.5(\pm 0.6) \times 10^8$ M^{-1}]. Thus it appeared that the conversion of lower-affinity B-2-reactive IGF-I receptors into higher-affinity B-2-non-reactive IGF-I receptors involved intermediate immunoreactive forms with increased binding affinity for IGF-I.

Affinity cross-linking of ^{125}I-IGF-I to receptors before and after treatment with DTT

To confirm that the IGF-I receptors had undergone disulphide reduction after exposure to DTT, solubilized placental membranes purified by elution from wheat-germ-agglutinin–agarose were covalently coupled to ^{125}I-IGF-I by using disuccinimidyl suberate, before and after incubation with 10 mM-DTT. In solubilized membranes not exposed to DTT, the specifically cross-linked ^{125}I-IGF-I was resolved as M_r-290000 complexes, whereas after exposure to DTT or with receptors released from DTT-treated immunoprecipitates it was resolved as M_r-180000 complexes (Fig. 4). Electrophoresis of all the samples under reducing conditions revealed specific bands in the M-140000 region (Fig. 4), the size of the binding (α) subunit of the receptor. The same results were obtained for particulate membranes exposed to DTT and affinity-cross-linked to ^{125}I-IGF-I (results not shown).

LIGAND analysis of data in Fig. 2(*b*)

One- or two-site model?		D.F.	Mean square	Residuals (+)	Residuals (−)	No. of runs
△, ▽	One	14	116.3	7	9	3 ($P < 0.01$)
	Two	12	48.1	6	10	11 ($P > 0.05$)
▲	One	5	79.0	2	5	5 ($P > 0.05$)
	Two	Program unable to fit a two-site model				

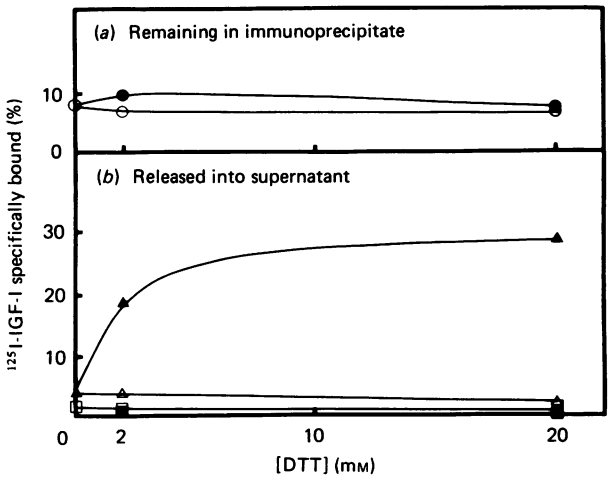

Fig. 3. Effect of DTT treatment on B-2-immunoreactive receptors isolated by immunoprecipitation

Suspensions of receptor–B-2-antibody complexes (see the Experimental section) were incubated with 0, 2 mM- or 20 mM-DTT for 20 min at 22 °C, followed by 2.5-fold higher concentrations of NEM, and then centrifuged at 3000 *g* for 20 min. For control incubations, DTT and NEM were premixed before their addition to the immune complexes. To determine whether DTT treatment had released receptors from the immune complexes, samples of the supernatants (equivalent to 40 μl of the original supension) were incubated with tracer amounts of ^{125}I-IGF-I or ^{125}I-insulin (final volume 0.2 ml) in the presence or absence of excess unlabelled hormone, and the bound tracer was precipitated with PEG or by using fresh additions of serum B-2 and *S. aureus* as described in the Experimental section. The DTT-treated immune complexes were washed, resuspended in 0.1% Triton/phosphate buffer and samples equivalent to 40 μl of original suspension were swirled for 16 h at 4 °C with tracer amounts of ^{125}I-IGF-I or ^{125}I-insulin (final volume 0.2 ml) in the presence or absence of excess unlabelled hormone. Specific hormone binding was determined after centrifugation and counting of radioactivity of the pellets. ●, ▲, ■, B-2 immune complexes treated with DTT; ○, △, □, control incubations. (*a*) Specific ^{125}I-IGF-I binding remaining in B-2 immune complexes (○, ●); non-specific binding was 1.5%. ^{125}I-insulin binding remaining after 20 mM-DTT or treatment with premixed DTT and NEM was 24% and 15% respectively. ^{125}I-IGF-I binding and ^{125}I-insulin binding remaining the 'control immune complexes' were < 1%. (*b*) ^{125}I-IGF-I binding released from the B-2 immune complexes precipitated by PEG (△, ▲) or by serum B-2 (1:1000 dilution) and *S. aureus* (□, ■); non-specific binding was 11.3% and 3.8% respectively). ^{125}I-insulin binding from the same complexes, precipitated by PEG or serum B-2 and *S. aureus* was 1.4% and 0.4% respectively, regardless of exposure to DTT. ^{125}I-IGF-I binding and ^{125}I-insulin binding released from the 'control immune complexes' were < 1%, regardless of the mode of assay or exposure to DTT.

DISCUSSION

The increase in ^{125}I-IGF-I binding after DTT treatment of placental membranes can be accounted for by the conversion of lower-affinity into higher-affinity IGF-I receptors. Disulphide reduction also causes a loss of the B-2 antibody-binding sites on placental IGF-I receptors. These sites appear to be separate from the hormone-binding sites and somewhat less susceptible to DTT. We therefore propose that the two types of human placental IGF-I receptor previously reported by us (Jonas & Harrison, 1985), a B-2-immunoreactive form with lower binding affinity for IGF-I and a B-2-non-immunoreactive

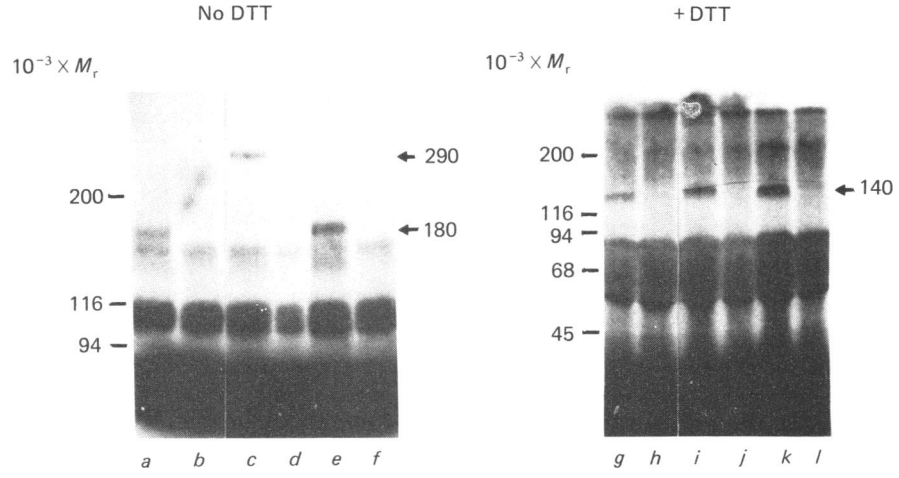

Fig. 4. Autoradiogram showing ^{125}I-IGF-I cross-linked to DTT-treated receptors

^{125}I-IGF-I was cross-linked with disuccinimidyl suberate to receptors released after treatment of immune complexes with 10 mM-DTT and then 25 mM-NEM (lanes *a*, *b*, *g* and *h*) and to receptors in the original wheat-germ-agglutinin–agarose eluate after treatment premixed DTT and NEM (lanes *c*, *d*, *i* and *j*) or DTT followed by NEM (lanes *e*, *f*, *k* and *l*). Cross-linking was performed in the presence (lanes *b*, *d*, *f*, *h*, *j*, *l*) or absence (lanes *a*, *c*, *e*, *g*, *i*, *k*) of excess unlabelled IGF-I. The samples were examined without further reduction in 5% polyacrylamide gels (lanes *a–f*) or under reducing conditions (50 mM-DTT) in 7.5%-polyacrylamide gels (lanes *g–l*). The procedures for affinity labelling, SDS/polyacrylamide-gel electrophoresis and autoradiography are described in the Experimental section. The non-specific bands corresponded in Coomassie-Blue-stained proteins in the gels, chiefly bovine albumin and contaminating proteins in the albumin preparation.

form with higher binding affinity for IGF-I, may represent convertible redox forms.

High concentrations of DTT (10–20 mM) induced higher-affinity binding of ^{125}I-IGF-I and complete conversion of the heterotetrameric IGF-I receptors into dimers. However, cleavage of the disulphide bonds maintaining the oligomeric configurations was not necessarily a prerequisite for higher-affinity hormone binding, since there was no ^{125}I-IGF-I cross-linking to lower-M_r forms in solubilized non-DTT-treated placental membranes, which contain both lower- and higher-affinity hormone-binding sites (Jonas & Harrison, 1985). Thus IGF-I receptors in human placental membranes might exist as a spectrum of redox forms not necessarily distinguished by molecular size. If IGF-I receptor sub-types underwent thiol redox transitions *in vivo* they could be catalysed by an enzyme, a possible candidate being protein disulphide-isomerase (EC 5.3.4.1) which reacts with a wide range of protein substrates to catalyse net formation, isomerization and reduction of protein disulphides, depending on the overall thiol/disulphide redox potential (Freedman, 1984). Our failure to observe dimeric IGF-I receptors in native placental membranes might be explained by the relative inaccessibility or resistance of the intersubunit receptor disulphide bonds to such an enzyme.

Our results also highlight some of the differences between the structurally similar receptors for IGF-I and insulin. Although ligand binding to both receptors was stimulated by low concentrations of DTT, higher concentrations decreased the binding of ^{125}I-insulin to its receptors. This biphasic response to DTT has also been noted by other investigators for insulin binding to human placental (Jacobs *et al.*, 1980) and rat liver (Schweitzer *et al.*, 1980; Harmon *et al.*, 1983) membranes. The binding sites for the polyclonal B-2 antibodies were also different, because disulphide reduction of human placental IGF-I receptors, but not insulin receptors, abolished their capacity to be precipitated by antiserum B-2.

The regulation of receptor conformation by the thiol redox state might play a role in modifying the biological functions of IGF-I receptors. If IGF-I receptors, like insulin receptors (Clark & Harrison, 1982, 1983), were to undergo disulphide exchange with their ligand, this reaction would be dependent on their thiol redox status. The other covalent receptor modification of potential biological importance is receptor autophosphorylation. IGF-I receptors, like insulin receptors (Kasuga *et al.*, 1982; Avruch *et al.*, 1982), undergo ligand-induced tyrosine phosphorylation on their β-subunits (Jacobs *et al.*, 1983; Rubin *et al.*, 1983). The tyrosine kinase activity of the insulin receptor is dependent on its degree of autophosphorylation (Rosen *et al.*, 1983; Yu & Czech, 1984) and is in fact stimulated *in vitro* by low concentrations of DTT (Shia *et al.*, 1983; Petruzelli *et al.*, 1984; Fujita-Yamaguchi & Kathuria, 1985) and *in vivo* by factors likely to modify redox status, such as alterations in metabolism (Blackshear *et al.*, 1984) and the location of receptors in the cell (Lerea & Livingston, 1984). It is therefore conceivable that the conformation and affinity of insulin and IGF-I receptors modulated by redox mechanisms is paralleled by changes in the phosphorylation states of the receptors. We have speculated that the two forms of IGF-I receptor in human placenta could mediate the metabolic and growth-promoting functions of IGF-I (Jonas & Harrison, 1985). Redox regulation of their conversion *in vivo* might represent a mechanism for co-ordinating biological activity in response to changing functional requirements of the cell.

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