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STRUCTURAL DIFFERENCES BETWEEN INSULIN AND SOMATOMEDIN-C/ INSULIN-LIKE GROWTH FACTOR-1 RECEPTORS REVEALED BY AUTOANTIBODIES TO THE INSULIN RECEPTOR

Helen A. Jonas*, Robert C. Baxter** and Len C. Harrison*

*Endocrine Laboratory and University of Melbourne Department of Medicine, Royal Melbourne Hospital, 3050, Victoria, Australia, and ** Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, 2050, New South Wales, Australia

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Five sera containing autoantibodies to the insulin receptor were used to compare the immunological characteristics of the insulin and the somatomedin-C/insulin-like growth factor-1 receptors of Triton-solubilized human placental membranes. Complete immunoprecipitation of [^{125}I] insulin-labelled receptors was achieved using all five antisera. Three antisera precipitated 90, 65 and 40% of [^{125}I] insulin-like growth factor-1-labelled receptors while the other two caused less than 7% immunoprecipitation. These results are consistent with the view that insulin and insulin-like growth factor-1 receptors are separate molecules which although structurally similar, possess a signficant degree of immunologic non-identity.

Somatomedin-C and insulin-like growth factor-1 are growth hormone dependent polypeptides, with cartilage-stimulating, mitogenic, and insulin-like metabolic activities (1). Because somatomedin-C and IGF-1[a] have similar N-termini (2) and are immunologically indistinguishable (3) the term "somatomedin-C/IGF-1" has been proposed (3). Although binding sites for IGF-1 and insulin exist in many tissues the peptides compete for occupancy of both receptors. In general the metabolic and mitogenic activities of each hormone (4) and their capacity to down-regulate each receptor (5) are in proportion to their ability to occupy that receptor. Both receptors appear to have similar disulphide-linked subunits as judged by affinity labelling and analysis in polyacrylamide gels (6 - 9). The presence of autoantibodies that inhibit both insulin and somatomedin-C binding (10) also suggests that insulin and IGF-1 receptors are structurally related.

a. IGF-1: insulin-like growth factor-1.

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In this study we have compared the immunological characteristics of solubilized insulin receptors and IGF-1 receptors from human placenta using several human auto-antisera to the insulin receptor. Autoantibodies to the insulin receptor, first described in a group of patients with severe insulin resistance and acanthosis nigricans (Type B syndrome) (11, 12), recognise determinants on or close to the insulin binding site (13), exert insulin-like effects in vitro (14) and immunoprecipitate solubilized insulin receptors (15).

MATERIALS AND METHODS

Materials: Porcine monocomponent insulin was purchased from Novo Research Institute. Somatomedin-C/IGF-1 was prepared as previously described (16). For use in competitive binding studies, this material was further purified by elution from a column of Octyl Sepharose CL-4B (Pharmacia) using a 0-25% gradient of acetonitrile in 0.1 M NH_4HCO_3 pH 7.8. IGF-1 (lot 16SP II) was kindly donated by Dr. R. Humbel, Zurich. For non-specific binding measurements a less pure preparation of somatomedin-C (5% pure, made by the method of Svoboda et al (2), up to the isoelectric focusing step) was used. Triton X-100 was purchased from Ajax Chemicals, Sydney; polyethylene glycol 6000 from Merck Institute; human gamma globulins (Fraction II, "Pentex") from Miles Laboratories, Inc., U.S.A.; and sheep anti-human IgG from Silenus Laboratories, Melbourne. Porcine insulin, used to estimate non-specific binding; human gamma globulin (Cohn fraction II); used as carrier protein in PEG[b] precipitation assays; and Staphylococcus aureus (strain Cowan I), supplied as a formalin-fixed, heat-killed, 10% (w/v) suspension, were obtained from the Commonwealth Serum Laboratories. Melbourne.

[^{125}I]-labelled insulin was prepared to a specific activity of 100 - 150 μCi/ug (17); [^{125}I]-labelled somatomedin-C/IGF-1 was prepared to a specific activity of approximately 100 μCi/ug and purified by hydrophobic interaction chromatography (16).

Serum containing antibodies to the insulin receptor (and in some cases to insulin) was obtained from patients with severe insulin resistance and acanthosis nigricans (Type B syndrome), heated at 56°C for 30 min and stored at -20°C. They were designated B-2, B-5, B-7 and B-8, as previously described (12). Another patient, designated B-10, was also studied.

Triton-solubilized human placental membranes were prepared as previously reported (17).

Binding Assays: Solubilized placental membranes (110 - 140 μg protein determined by the Bio-Rad protein assay, using bovine gamma globulin as standard) were incubated with tracer concentrations of [^{125}I] insulin (10,000 cpm, 70 pg) or [^{125}I] IGF-1 (10,000 cpm; 105- 115 pg) and increasing concentrations of unlabelled insulin (0 - 5 μg/ml) or IGF-1 (0 - 3 μg/ml) in a total volume of 0.2 ml in 0.1 M sodium phosphate buffer, pH = 7.5, at a final Triton concentration of 0.1%. Steady state was reached by 2 h at 22°C or 16 h at 4°C data not shown). To precipitate receptor-bound hormone, incubation mixtures were cooled to 4°C and mixed with 0.1 ml cold (4°C) 0.1 M sodium phosphate containing 0.02% Triton and 0.24% carrier human gamma globulin, followed by 0.3 ml cold buffer containing 25% PEG. The mixture was shaken vigorously, allowed to stand for 10 min at 4°C, then centrifuged at 3000 x g for 20 min.

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b. PEG: polyethylene glycol 6000.

Unbound radioactivity in the supernatant was removed by aspiration, and the pellet, containing bound radioactive hormone, was counted in a gamma counter. Specific binding was determined by subtracting the radioactivity that remained bound in the presence of unlabelled insulin (20 μg /ml) or unlabelled IGF-1 (5% pure; 20 μg/ml). When PEG precipitation was performed in the presence of anti-receptor sera, other controls ([^{125}I] insulin or [^{125}I] IGF-1 in Triton-buffer without solubilized membranes) were also included to correct for the precipitation of [^{125}I] insulin bound to insulin antibodies or [^{125}I] IGF-1 bound to serum binding proteins.

Immunoprecipitation of Receptors: Solubilized placental membranes were incubated with tracer [^{125}I] insulin or [^{125}I] IGF-1 for 1 h at 22°C, using the concentrations of protein, tracer and Triton described above. Antisera (10 μl diluted in phosphate buffer to yield final assay dilutions of 1/40 to 1/800) were added and the incubation allowed to proceed at 4°C for 24 h. Precipitation of the labelled receptor-antibody complexes was achieved by the addition of 0.05 ml sheep anti-human IgG (titer = 1.5 mg/ml) and 50 μg 'carrier' human gamma globulins ("Pentex") for 16 hr at 4 C, or, by the addition of 0.05 ml Staph. A[c] (washed x 3 in 0.1 M phosphate buffer containing 0.02% Triton) for 30 min at 4°C. The immune complexes were precipitated by centrifugation at 3000 x g for 20 min, the supernatants aspirated, and the pellets counted in a gamma counter. Two controls were included in each experiment: 1) normal human gamma globulin for non-specific trapping of radioactivity; and 2) antisera incubated with [^{125}I] insulin in Triton-buffer without solubilized membranes, to correct for insulin antibodies.

RESULTS

The presence of binding sites for both insulin and IGF-1 in Triton-solubilized human placental membranes was confirmed by binding studies involving competition between tracer concentrations of [^{125}I] insulin or [^{125}I] IGF-1 and increasing amounts of the unlabelled hormones (Figure 1). Porcine insulin was approximately 400 times more potent than IGF-1 in competing with [^{125}I] insulin for its receptor while the potency of IGF-1 compared to insulin in competing with [^{125}I]-IGF-1 for its receptor was about 150. The Scatchard plot (18) of the insulin binding data was curvilinear and could be resolved into two components (K_1 = 3.3 x 10^9 M^{-1}, R_1 = 0.29 pmoles/mg; and K_1 = 3.0 x 10^9 M^{-1}, R_2 = 1.95 pmoles/mg)[d] by Rosenthal analysis (19). Scatchard analysis of IGF-1 binding was consistent with a single class of binding sites (K_1 = 1.2 x 10^9 M^{-1}, R_1 = 0.51 pmoles/mg).

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c. Staph. A: 10% (w/v) suspension of Staphylococcus aureus.

d. K_1, K_2: association constants for high and low affinity binding sites, respectively.

R_1, R_2: concentrations of high and low affinity binding sites, respectively.

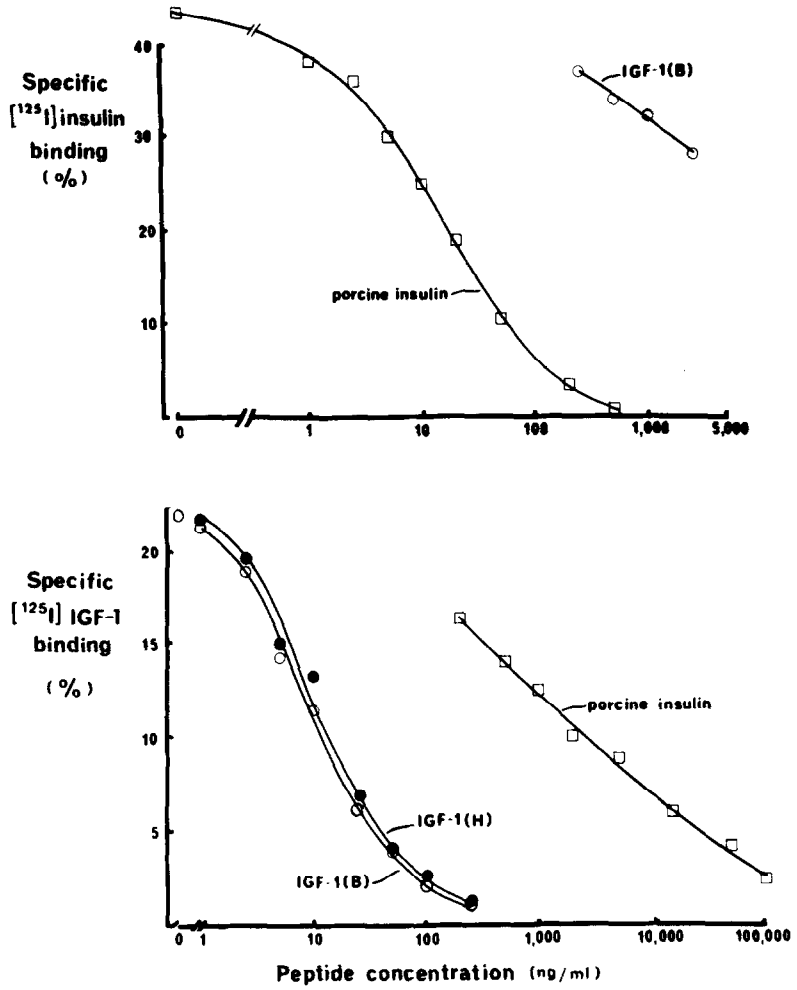

FIGURE 1: Competition between [^{125}I] insulin (upper panel) or [^{125}I] IGF-1 (lower panel) and increasing concentrations of unlabelled peptide for binding to solubilized placental membranes. IGF-1 (B) and IGF-1 (H) were prepared by Drs. Baxter and Humbel respectively. After incubation at 4°C for 18 h with 110 µg solubilized membranes, bound and free radioactivity were separated by PEG precipitation and specific binding was calculated as described under "Materials and Methods". Non-specific binding was: [^{125}I] insulin, 7.3% and [^{125}I] IGF-1, 6.0%.

When solubilized membranes, pre-labelled with [^{125}I] insulin, were incubated with increasing dilutions of anti-receptor sera, 30-55% of [^{125}I] insulin was immunoprecipitated. After labelling with [^{125}I]-IGF-1, 7-13% of the label was precipitated by sera B-2, B-5 and B-8, but less than 1%, by sera B-7 and B-10 (Figure 2A). The fraction of the total labelled receptors immunoprecipitated was determined by comparison with labelled receptors precipitated by PEG. PEG precipitation was performed after incubation with

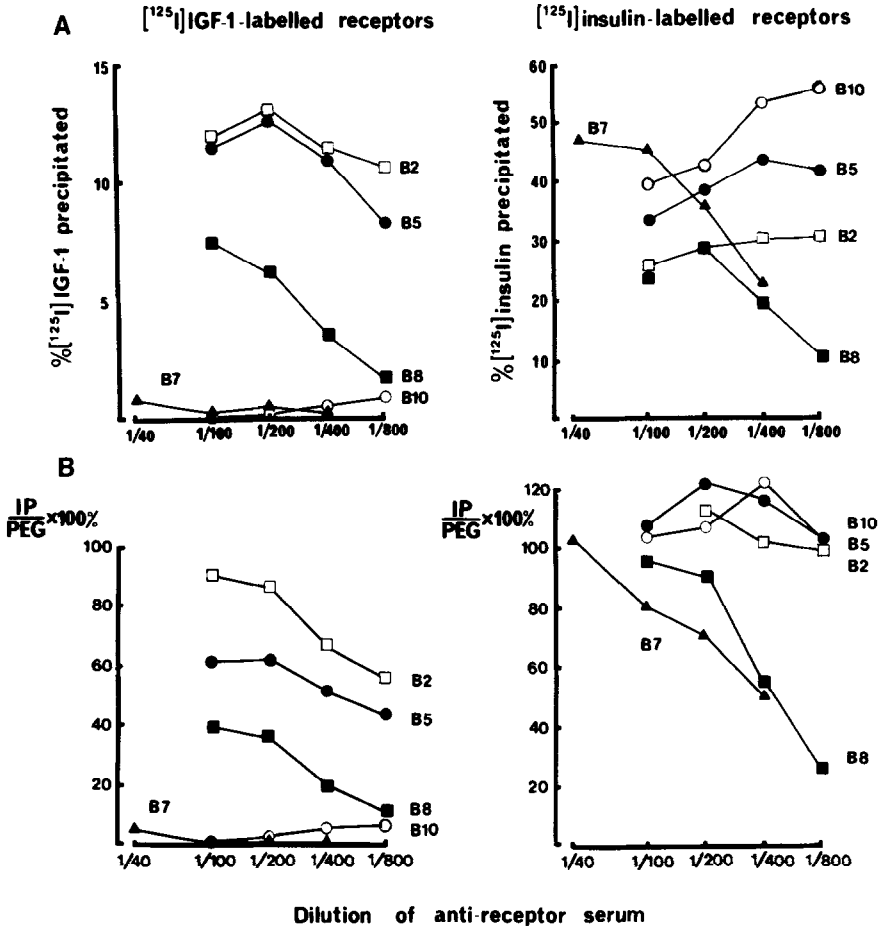

FIGURE 2: Precipitation of [^{125}I] IGF-1 or [^{125}I] insulin-labelled receptors by anti-receptor sera. Antisera (B-2, -5, -7, -8 and -10) were incubated with pre-labelled solubilized membranes (132 μg protein) at 4°C for 24 h and the labelled receptor-antibody complexes precipitated with an excess of Staph. A, as described under "Materials and Methods". At the end of the antibody incubation period, labelled receptors were also precipitated with PEG, as outlined under "Materials and Methods". Figure 2A represents [^{125}I] IGF-1 or [^{125}I] insulin specifically immunoprecipitated. These values were corrected for non-specific trapping of radioactivity ([^{125}I] IGF-1, 7.2 - 9.1%; [^{125}I] insulin, 2.8 - 3.7%) and, for each dilution of serum, binding of [^{125}I] insulin to insulin antibodies. At final serum dilutions of 1/100, for example, anti-insulin binding was: B-2, 1.0%; B-5, 10.0%; B-7, 0%; B-8, 1.9% and B-10, 12.0%. The same results were obtained when anti-human IgG was used to precipitate labelled receptor-antibody complexes. In Figure 2B, the immunoprecipitation data ("IP") are expressed as a percent of the labelled hormone-receptor complexes precipitated by PEG ("PEG"). The PEG precipitates were corrected for non-specific binding ([^{125}I] IGF-1, 6.0%; [^{125}I] insulin, 7.5%) and, for each dilution of serum, binding of [^{125}I] insulin or [^{125}I] IGF-1 to endogenous insulin antibodies or PEG-precipitable binding proteins. At final serum dilutions of 1/100, for example, anti-insulin binding was: B-2, 1.1%; B-5, 7.5%; B-7, 0%; B-8, 1.5% and B-10, 10.5%; and IGF-1 binding: B-2, 3.4%; B-5, 2.0%; B-7, 1.2%; B-8, 1.0% and B-10, 2.2%.

each antiserum, to allow for any displacement of pre-bound label by antibodies. When the immunoprecipitation data was expressed as a percent of

TABLE 1: Effect of anti-receptor sera on PEG precipitation of [^{125}I] IGF-1 incubated with or without solubilized human placental membranes

Triton buffer ±solubilized membranes	Precipitation of [^{125}I] IGF-1 (% of total)					
	normal globulins	Anti-receptor serum B-2	B-5	B-7	B-8	B-10
+	17.0	15.9	19.6	17.2	21.2	17.3
-	1.2	3.4	2.0	1.2	1.0	2.2

[^{125}I] IGF-1 (10,000 cpm) was incubated with solubilized membranes in 0.1% (v/v) Triton-buffer or with Triton-buffer only. Anti-receptor sera (final dilution 1/100) or normal human gamma globulins (0.1 mg/ml), were added for a further period, as outlined in the legend to Figure 2. Radioactivity was then precipitated with PEG, and the precipitates corrected for non-specific binding.

receptor bound-[^{125}I] insulin or -[^{125}I] IGF-1 precipitated by PEG (that is, as a percentage of the total labelled receptors), all the five anti-receptor sera were found to completely precipitate [^{125}I] insulin-labelled receptors. Serum B-2, B-5 and B-8 precipitated, respectively, 90, 65 and 40% of the [^{125}I] IGF-1-labelled receptors, but minimal precipitation (less than 7%) was observed with sera B-7 and B-10 (Figure 2B).

The failure of antisera B-7 and B-10 to precipitate [^{125}I] IGF-1-receptor complexes could not be attributed to an interaction of [^{125}I] IGF-1 with serum binding proteins (4) because only low levels of radioactivity were precipitated by PEG in the absence of solubilized receptors (Table 1). Furthermore, there was no diminution in the amount of [^{125}I] IGF-1 bound to the receptor after incubation with the antisera (Table 1), suggesting that: 1) non-PEG precipitable binding proteins were not competing with the receptor for [^{125}I) IGF-1, and 2) endogenous IGF-1 was not displacing [^{125}I] IGF-1 from its receptor.

DISCUSSION

We have shown that three antisera containing autoantibodies to the insulin receptor (B-2, B-5 and B-8) also immunoprecipitate solubilized human placental IGF-1 receptors, whereas two other antisera (B-7 and B-10) show no appreciable cross-reaction with IGF-1 receptors. Because both receptors bind

insulin and IGF-1, and are composed of similarly sized, disulphide-linked subunits (6-9), it is not unexpected that antisera to insulin receptors also recognize IGF-1 receptors. Because the antisera are polyclonal (12) separate antibodies to insulin and IGF-1 receptors may be present in antisera B-2, B-5 and B-8. This possibility could be tested by adsorption of sera against tissues lacking IGF-1 receptors. However, the fact that two antisera showed little or no crossreactivity with IGF-1 receptors confirms that insulin and IGF-1 receptors are discrete molecules, and allows us to conclude that they also contain regions of structural dissimilarity.

Previously, the interaction of only antiserum B-2 with human placental receptors was characterized in detail (15, 20). Antiserum B-2 was apparently specific for the insulin receptor and did not precipitate receptors for another growth factor, multiplication stimulating activity (15). This was in keeping with subsequent reports (6, 9) that receptors for MSA[e] are structurally different from those for insulin and IGF-1. Precipitation of solubilized ^{125}I-labelled placental membranes by antiserum B-2 followed by electrophoresis and autoradiography revealed reduced bands of 126,000, 90,000 and 42,000 (20). The same bands were eluted from a B-2 antibody affinity column but somatomedin-C binding activity was not present in the eluate (20), possibly due to the denaturing conditions required for elution (which abolished most of the insulin binding activity) or, less likely, to impaired affinity of bound antibodies for somatomedin-C (IGF-1) receptors. These bands were assumed to be the subunits of the insulin receptor, but on the present evidence might represent subunits of both insulin and IGF-1 receptors. Recent reports of phosphorylation of the insulin receptor, precipitated using B-2 antiserum (21), may need to be interpreted accordingly. However, two antisera (B-7 and B-10) have been shown to have restricted specificities for the insulin receptor and this provides a unique opportunity to further delineate insulin and IGF-1 receptors, whose structures are otherwise closely related.

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e. MSA: multiplication stimulating activity.

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