Biochem. J. (1986) 233, 215–221 (Printed in Great Britain)

Comparative binding of bovine, human and rat insulin-like growth factors to membrane receptors and to antibodies against human insulin-like growth factor-1

Leanna C. READ,* F. John BALLARD,*§ Geoffrey L. FRANCIS,* Robert C. BAXTER,†
Christopher J. BAGLEY‡ and John C. WALLACE‡

*CSIRO (Australia), Division of Human Nutrition, Adelaide, South Australia 5000, †Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, N.S.W. 2050, and ‡Department of Biochemistry, University of Adelaide, Adelaide, South Australia 5000, Australia

1. The immunological properties of human, bovine and rat insulin-like growth factors (IGF) and insulin were compared in competitive binding studies with Tr10 and NPA polyclonal antisera raised in rabbits against human IGF-1. Bovine IGF-1 was 11–19% as effective as human IGF-1 in competing for binding with ^{125}I-labelled human IGF-1, whereas IGF-2 reacted poorly and insulin did not compete. 2. Similar competitive binding curves were obtained with the mouse monoclonal anti-(human IGF-1) antibody 3D1, except that bovine IGF-1 showed a severalfold greater affinity for the monoclonal antibody than for either polyclonal antiserum. 3. Membranes isolated from human placenta, sheep placenta and foetal-human liver were used as sources of cellular receptors. In human placental membranes, most of the binding of IGF-1 tracers could be attributed to a type-1 receptor, because insulin inhibited up to 65% of tracer binding. The other two tissues apparently contain only type-2 receptors, as evidenced by the very low potency of bovine or human IGF-1 in competing for binding with IGF-2 tracers and the absence of any competition by insulin. 4. In competition for binding with labelled bovine or human IGF-1 to human placental membranes, bovine IGF-1 had a similar potency to human IGF-1, whereas bovine IGF-1 was more potent in binding studies with tissues rich in type-2 receptors. 5. Rat IGF-2 was considerably less effective than human IGF-2 in competition for receptors on any of the membrane preparations.

INTRODUCTION

The insulin-like growth factors IGF-1 and IGF-2 are growth-hormone-dependent proteins first isolated from human serum (Rinderknecht & Humbel, 1978*a*,*b*). Similar proteins have since been detected in various body fluids and tissues of numerous species (Baxter *et al.*, 1982*a*, 1984; Haselbacher & Humber, 1982; Laubli *et al.*, 1982; Wilson & Hintz, 1982; D'Ercole *et al.*, 1984; Eigenmann *et al.*, 1984) and in medium conditioned by cultured cells (Atkinson *et al.*, 1980; D'Ercole *et al.*, 1980; Moses *et al.*, 1980; Adams *et al.*, 1983*a*, *b*). Nevertheless, few of these IGFs have been isolated in a pure state. Complete purification from serum has been achieved for human IGF-1, human IGF-2 and rat IGF-1 (Rinderknecht & Humbel, 1978*a*,*b*; Rubin *et al.*, 1982), and multiplication-stimulating activity, a family of IGF-2-like peptides, has been isolated from the culture medium of buffalo-rat liver cells (Moses *et al.*, 1980). Marked structural homology is apparent between rat and human IGF-1, with no differences identified in the first 29 *N*-terminal residues (Rubin *et al.*, 1982). Sequence analysis has also revealed that a low-M_r component of multiplication-stimulating activity shares 93% structural homology with human IGF-2 (Marquardt *et al.*, 1981). Consequently, this protein has now been designated 'rat IGF-2'.

Due to the paucity of pure IGF preparations, there is little information available concerning the comparative immunological and biological properties of IGFs from different species. The preceding paper (Francis *et al.*, 1986) describes the purification to near homogeneity of an IGF from bovine colostrum. This growth factor has been designated 'bovine IGF-1' because sequence determination indicated complete homology with human IGF-1 in the first 30 amino acids. In the present paper we have compared the binding of bovine IGF-1, human IGF-1, human IGF-2 and rat IGF-2 to cell receptors and to antibodies raised against human IGF-1. Three anti-(human IGF-1) antibodies were used for these comparisons, including two polyclonal antisera and one monoclonal antibody. For receptor-binding studies, several different tissues were included as sources of IGF receptors, chosen on the basis of their known affinities for IGF-1 and IGF-2. IGFs are known to bind with high affinity to two types of cell-surface receptors: type 1, which generally binds IGF-1 preferentially over IGF-2 and shows a low affinity for insulin, and type 2, which is selective for IGF-2 and has no affinity for insulin (Kasuga *et al.*, 1981; Massague & Czech, 1982; Czech *et al.*, 1983). The relative proportions of the two receptors vary considerably between tissues. For this reason, competitive binding studies were carried out with membranes isolated from sheep placenta or foetal-human liver, both tissues

Abbreviations used: IGF, insulin-like growth factor; rIGF-2, rat IGF-2; hIGF-1, human IGF-1; hIGF-2, human IGF-2; bIGF-1, bovine IGF-1.
§ To whom correspondence and reprint requests should be addressed.

containing predominantly type-2 receptors (Baxter, 1984, 1985) and human placental membranes, known to be rich in type-1 receptors (Daughaday *et al.*, 1981).

MATERIALS AND METHODS

Materials

Carrier-free $Na^{125}I$ was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.), 1-tetrachloro-3a,6a-diphenylglycoluril (Iodogen) from Pierce Chemical Co. (Rockford, IL, U.S.A.) and chloramine-T from May and Baker, Dagenham, Essex, U.K. Bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, MO, U.S.A.) was extracted by the method of Chen (1967) to remove growth factors. Sephadex G-25 and G-50 (fine grade) resins were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), poly(ethylene glycol) 6000 from Sigma, goat anti-rabbit γ-globulin from Bio-Rad (Montreal, Canada) and sheep anti-mouse γ-globulin from Silenus (Dandenong, Vic., Australia).

Growth factors

bIGF-1 was purified to near homogeneity from colostrum. Pool-3 material from the final h.p.l.c. step of the purification described in the preceding paper (Francis *et al.*, 1985) was used as unlabelled bIGF-1 in binding studies. This material was estimated to be 60–70% pure and did not contain the *N*-terminal tripeptide Gly-Pro-Glu. Bovine IGF-1 for radioiodination was obtained from the fourth h.p.l.c. step of the purification of a different colostrum sample, but had a biological specific activity equivalent to that of the unlabelled peptide. The elution volume of the bIGF preparation used for iodination indicated that it was equivalent to pool-1 material, containing the *N*-terminal tripeptide, as described in the preceding paper (Francis *et al.*, 1986). hIGF-1 was isolated from serum as described by Baxter & Brown (1982), followed by reversed-phase h.p.l.c. on a phenyl-μ-Bondapak column (Waters Associates, Milford, MA, U.S.A.) with a linear 20–70% (v.v) acetonitrile gradient in 0.1% trifluoroacetic acid. The final product was equipotent on a weight basis by radioimmunoassay with hIGF-1 (lot 16 SPII) kindly donated by Dr. R. Humbel (University of Zurich, Zurich, Switzerland). hIGF-2 was purified from Cohn paste of human plasma as described by Baxter (1984) and showed equivalent potency by weight to hIGF-2 (lot 9SEIV) from Dr. R. Humbel in a sheep-placental-radioreceptor assay. Rat IGF-2 (rIGF-2), purified from the culture medium of BRL-3A rat liver cells by methods similar to those described by Moses *et al.* (1980) and Marquardt *et al.* (1981), was very generously provided by Dr. J. Florini (Syracuse University, Syracuse, NY, U.S.A.). Insulin was porcine Actrapid (Novo Industri A/S, Copenhagen, Denmark).

Antibodies

Polyclonal rabbit antibodies to hIGF-1 included an antiserum, designated 'NPA', donated by the National Hormone and Pituitary Program (Bethesda, MD, U.S.A.) and antiserum Tr10, similar to antiserum Tr4 described by Baxter *et al.* (1982*b*). The mouse monoclonal antibody 3D1 was prepared in the Immunology Unit, Department of Medicine, University of Sydney, N.S.W., Australia, as described by Baxter *et al.* (1982*a*). hIGF-1 purified by the method of Baxter & Brown (1982) was used as antigen for production of this antibody.

Iodination of growth factors

bIGF-1 and rIGF-2 were iodinated by using the Iodogen method (Fraker & Speck, 1978) to a specific radioactivity of 20 Ci/g. The reaction were carried out in a solution containing 0.05 M-KH_2PO_4 and 0.15 M-NaCl at pH 7.6. Urea (7 M) was included for iodination of bIGF-1 to overcome solubility problems at neutral pH (Francis *et al.*, 1986). Iodinated bIGF-1 and rIGF-2 were subsequently separated from unincorporated ^{125}I by filtration through a Sephadex G-25 (fine grade) column (1 cm × 30 cm) equilibrated with 0.01 M-KH_2PO_4/0.15 M-NaCl, pH 7.4, containing 0.2% bovine serum albumin. Chloramine-T was used for labelling of hIGF-1 and hIGF-2 (Baxter & Brown, 1982) to specific radioactivities of 120 and 200 μCi/μg respectively. Tracers were then purified by passage through a column (1 cm × 30 cm) of Sephadex G-50 (fine grade) in 0.1 M-NH_4HCO_3, pH 7.8, containing 0.1% bovine serum albumin. Iodinated hIGF-1 was further purified by hydrophobic-interaction chromatograpy (Baxter & Brown, 1982).

Quantification of growth factors

Freeze-dried preparations of hIGF-1, hIGF-2 and rIGF-2 were weighed on a micro-balance in amounts not less than 40 μg. Insufficient bIGF-1 was available for quantification by weight, so the A_{280} was taken as a measure of protein, with porcine insulin as the standard. Although we have indicated that pool-3 material used as unlabelled bIGF-1 in this and the following paper (Ballard *et al.*, 1986) was only 60–70% pure, no correction for purity differences has been made in calculations of the potency of bIGF-1. Actrapid insulin was supplied by the manufacturers in a solution containing sodium acetate as a buffering agent at pH 7 and 0.1% hydroxybenzoate, with the insulin concentration given as 100 units/ml. This concentration was assumed to be equivalent to 4 mg/ml.

The insulin stock was stored at 4 °C and diluted immediately before use. Other growth factors were dissolved in 0.01 M-HCl at 10–400 ng/μl and stored at −80°C for no more than 1 week before use in binding and biological assays. When needed, stocks were thawed once only and diluted appropriately in a solution containing 0.05 M-KH_2PO_4, 0.15 M-NaCl and 0.1% bovine serum albumin, pH 7.6. Each series of experiments described in this and the following paper (Ballard *et al.*, 1986) was done at the one time in triplicate at each concentration, with the same diluted samples of growth factors.

Isolation of membranes

Human placental membranes were isolated by the method of Williams & Turtle (1979), and sheep placental and foetal-human liver membranes were prepared as described for rat liver membranes by Baxter & Turtle (1978).

Binding to anti-(human IGF-1) antibodies

Radioimmunoassays were carried out at 2 °C for 16 h in 0.5 ml of a medium consisting of 0.03 M-KH_2PO_4 at pH 7.5, 0.2% protamine sulphate, 0.2% NaN_3 and 0.25% bovine serum albumin (phosphate binding medium). Polyclonal antisera Tr10 and NPA were incubated with

^{125}I-labelled hIGF-1 (10000 c.p.m., 0.05 ng), together with 20 μl of different concentrations of unlabelled peptides. Antisera were used at final dilutions of 1:50000 (Tr10) or 1:20000 (NPA), concentrations that bound 40–50% of added tracer. Monoclonal antibody 3D1 was incubated with either ^{125}I-labelled hIGF-1 (10000 c.p.m., 0.05 ng) or bIGF-1 tracer (13000 c.p.m., 0.5 ng) at a final dilution of 1:400000 (hIGF-1 tracer) or 1:250000 (bIGF-1 tracer), such that approx. 30% of tracer bound in the absence of competing peptides. For precipitation of polyclonal antibodies, 0.5 μl of normal rabbit serum and 25 μl of goat anti-rabbit γ-globulin were added to each tube, whereas 3D1 antibodies were precipitated with 2 μl of normal mouse serum together with 20 μl of sheep anti-mouse γ-globulin/tube. After 30 min at 2 °C, 1 ml of cold 6% (w/v) poly(ethylene glycol) 6000 in 0.15 M-NaCl was added and tubes were immediately centrifuged for 30 min at 4000 *g* before removal of supernatants by aspiration. Radioactivity bound to tubes in the absence of antibody was subtracted from the total to obtain the antibody-bound radioactivity. No correction was made for radioactivity bound in the presence of excess amounts of unlabelled growth factors.

Comparative binding to membrane receptors

Binding to human placental membranes was carried out in 600 μl tubes containing 40 μg of membrane protein per tube in a total volume of 200 μl of a solution containing 0.1 M-Hepes, 0.12 M-NaCl, 5 mM-KCl, 1.2 mM-$MgSO_4$, 1.3 mM-$CaCl_2$, 8 mM-glucose and 1% bovine serum albumin at pH 7.5. The reaction mixture included ^{125}I-labelled hIGF-1 (8000 c.p.m., 0.04 ng) or bIGF-1 (8000 c.p.m., 0.3 ng), together with various concentrations of unlabelled growth factors. After 16 h at 4 °C, tubes were centrifuged for 10 min at 10000 *g* before aspiration of the supernatants. Residual radioactivity in control tubes incubated without membranes was subtracted from the total to obtain membrane-bound radioactivity. No correction was made for radioactivity bound in the presence of excess unlabelled growth factors.

Human-foetal liver membranes (100 μg of protein/tube) or sheep placental membranes (40 μg of protein/tube) were incubated at 22 °C for 2 h in a total volume of 300 μl of phosphate binding medium, with hIGF-2 (10000 c.p.m., 0.03 ng) or rIGF-2 (14000 c.p.m., 0.5 ng) as the iodinated peptide. Other details were as described for human placental membranes.

RESULTS

Comparative binding of bIGF-1 and hIGF-1 to polyclonal anti-hIGF-1 antisera

The relative potencies of growth factors in competitive binding to antibodies or to cell receptors were assessed by the concentrations required to decrease binding of the radioligand by 50%. Half-maximal inhibition of ^{125}I-labelled hIGF-I binding to Tr10 antiserum was observed with hIGF-1 at 1.6 ng/ml, bIGF-1 at 14 ng/ml or hIGF-2 at 39 ng/ml (Fig. 1*a*). NPA antiserum was more sensitive than Tr10, with 50% inhibition of labelled hIGF-1 binding at considerably lower concentrations of all competing growth factors (Fig. 1*b*). In Table 1, the concentrations giving 50% inhibition of tracer binding have been used to calculate relative potencies, the most effective competing growth factor having been arbitrarily

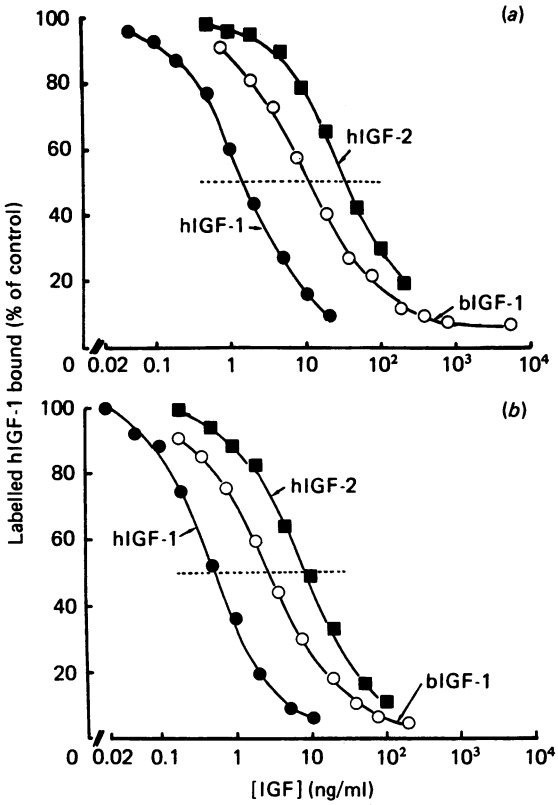

Fig. 1. Competition of different growth factors with ^{125}I-labelled hIGF-1 for binding to polyclonal anti-(human IGF-1) antiserum (*a*) Tr10 and (*b*) NPA

The symbols used are: ●, hIG-1; ○, bIGF-1; ■, hIGF-2. In the absence of competing growth factors (control), the amount of ^{125}I-labelled hIGF-1 bound to Tr10 and NPA antiserum represented 40% and 50% of added tracer. Values are triplicate measurements at each concentration.

assigned a potency of 100%. Both polyclonal antibodies showed less affinity (11–19%) for bIGF-1 than for hIGF-1 (100%) and little cross-reactivity (4–6%) with hIGF-2, whereas insulin, tested at concentrations up to 10 μg/ml, did not compete for tracer binding.

Radioimmunoassays using monoclonal antibody 3D1

Both hIGF-1 and bIGF-1 were used as tracers for binding to 3D1. Saturating concentrations of antibody bound 90–95% of ^{125}I-labelled hIGF-1, with 50% tracer binding at a final dilution of 1:160000. 3D1 antiserum also bound strongly to bIGF-1, with maximal binding equivalent to 80% of added tracer and 50% binding at an antibody dilution of 1:120000. Competitive binding curves were similar with the two radioligands. Half-maximal competition for hIGF-1 tracer occurred with hIGF-1 at 4.0 ng/ml, bIGF-1 at 7.6 ng/ml and hIGF-2 at 58 ng/ml (Fig. 2*a*), whereas slightly higher concentrations of each peptide (5.3 ng/ml for hIGF-1, 14.5 ng/ml for bIGF-1 and 75 ng/ml for hIGF-2) were required for 50% effects with bIGF-1 tracer (Fig. 2*b*). Relative to the potency of hIGF-1, bIGF-1 was 36% and 50% as effective in competing with labelled bIGF-1 and labelled hIGF-1 respectively, for binding to the antibody. There was little (7%) cross-reactivity with hIGF-2 in either assay and insulin did not compete. The monoclonal

Table 1. Relative potencies of bovine, human and rat IGFs in competitive binding to anti-(human IGF-1) antibodies and cell receptors

Potencies were calculated from data in Figs. 1–5 as the ratio (expressed as %) of growth-factor concentrations required for 50% inhibition of tracer binding, the most potent peptide having been assigned a potency of 100%. Insulin, tested at concentrations up to 10 μg/ml, had no activity in any of the assays, except human placenta. Abbreviation used: nd, not done.

Assay	Potency (% relative to the most active peptide)				
	hIGF-1	bIGF-1	hIGF-2	rIGF-2	Insulin
Polyclonal antiserum Tr10/hIGF-1 tracer	100	11	4	nd	< 0.02
Polyclonal antiserum NPA/hIGF-1 tracer	100	19	6	nd	< 0.006
Monoclonal antiserum 3D1/hIGF-1 tracer	100	50	7	nd	< 0.04
Monoclonal antiserum 3D1/bIGF-1 tracer	100	36	7	nd	< 0.05
Human placenta/ hIGF-1 tracer	100	31	26	8	0.2
Human placenta/ bIGF-1 tracer	100	50	29	18	0.3
Sheep placenta/ hIGF-2 tracer	2	3	100	nd	< 0.03
Sheep placenta/ rIGF-2 tracer	< 3	3	100	29	< 0.03
Fetal human liver/ hIGF-2 tracer	< 3	3	100	nd	< 0.03

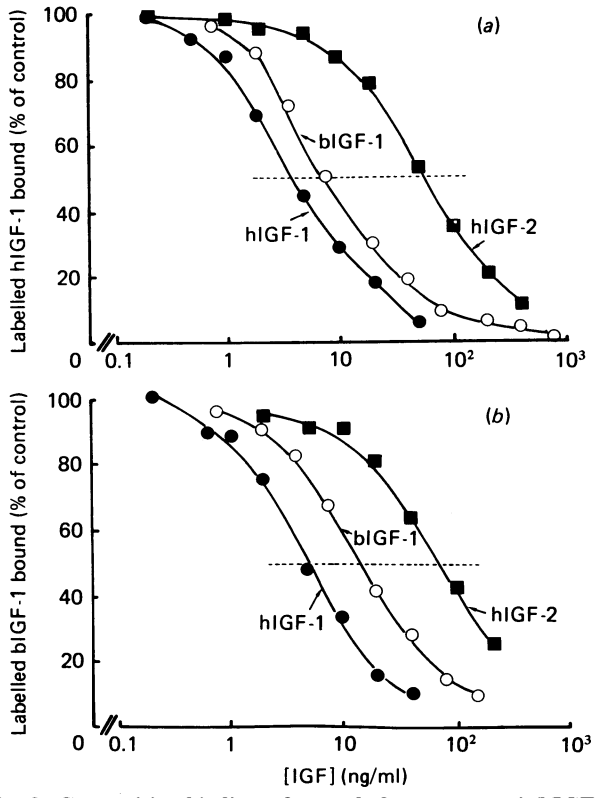

Fig. 2. Competitive binding of growth factors to anti-(hIGF-1) monoclonal antibody 3D1 in the presence of (*a*) ^{125}I-labelled hIGF-1 and (*b*) ^{125}I-labelled bIGF-1

The symbols used are: ●, hIGF-1; ○, bIGF-1; ■, hIGF-2. In the absence of competing growth factors (control), 33% of added hIGF-1 tracer and 28% of bIGF-1 tracer bound to monoclonal antibody 3D1. Values are triplicate measurements at each concentration.

antibody therefore behaved similarly to the polyclonal antisera, but showed greater affinity for bIGF-1.

Binding to human placental membranes

Both hIGF-1 and bIGF-1 were used as radioligands in competitive binding studies with human placental membranes (Fig. 3*a*). With hIGF-1 as tracer, the order of potency of competing peptides, with concentrations (ng/ml) giving half-maximal effects shown in parentheses, was: hIGF-1 (12) > bIGF-1 (39) > hIGF-2 (46) > rIGF-2 (260) > insulin (5600). Higher concentrations of competing growth factors were required for half-maximal effects when bIGF-1 was used as tracer (33 ng/ml for hIGF-1, 66 ng/ml for bIGF-1, 115 ng/ml for hIGF-2, 180 ng/ml for rIGF-2 or 10000 ng/ml for insulin). Nevertheless, potencies expressed relative to that of hIGF-1 were nearly identical in the two assays, bIGF-1 competing 31–50% as well as hIGF-1, whereas IGF-2 preparations, particularly rIGF-2, were less potent again (Table 1) and insulin competed poorly, with only 0.2–0.3% of the potency of hIGF-1. It was also observed that maximal concentrations of insulin inhibited no more than 65% of the binding of either IGF-1 tracer, whereas other competing peptides decreased tracer binding by at least 85% (Fig. 3).

Sheep placental membranes

Sheep placental membranes were highly specific for IGF-2 peptides, binding 12–19% of added IGF-2 tracers, but only 1–2% of labelled hIGF-1 or bIGF-1 under identical conditions (results not shown). Selectivity towards IGF-2 was also apparent in competitive binding studies using either hIGF-2 or rIGF-2 as the radioligand (Fig. 4). The decreasing order of potencies of competing growth factors with half-maximal concentrations (ng/ml) in parentheses was: hIGF-2 tracer: hIGF-2

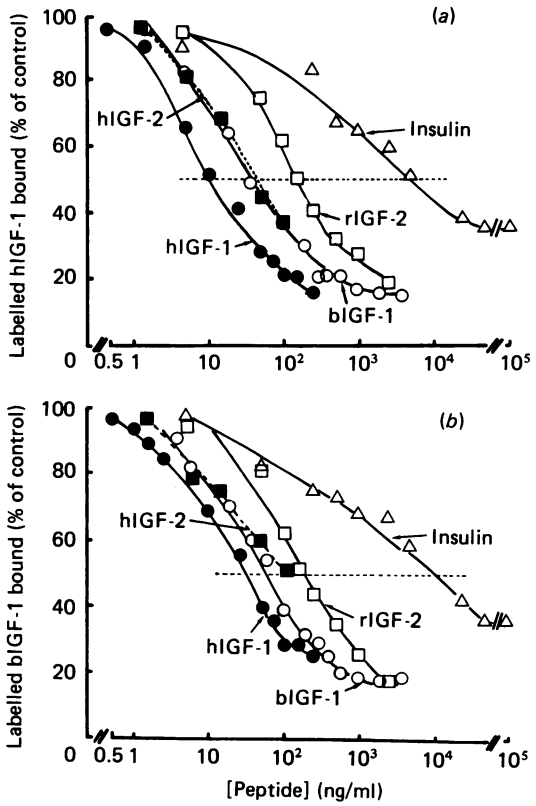

Fig. 3. Competition of growth factors with (*a*) ^{125}I-labelled hIGF-1 or (*b*) ^{125}I-labelled bIGF-1 for binding to human placental membranes

The symbols used are: ●, hIGF-1; ○, bIGF-1; ■, hIGF-2; □, rIGF-2; △, insulin. In the absence of competing growth factors (control), 27% of hIGF-1 tracer and 14% of bIGF-1 tracer bound to membranes. Values are triplicate measurements at each concentration.

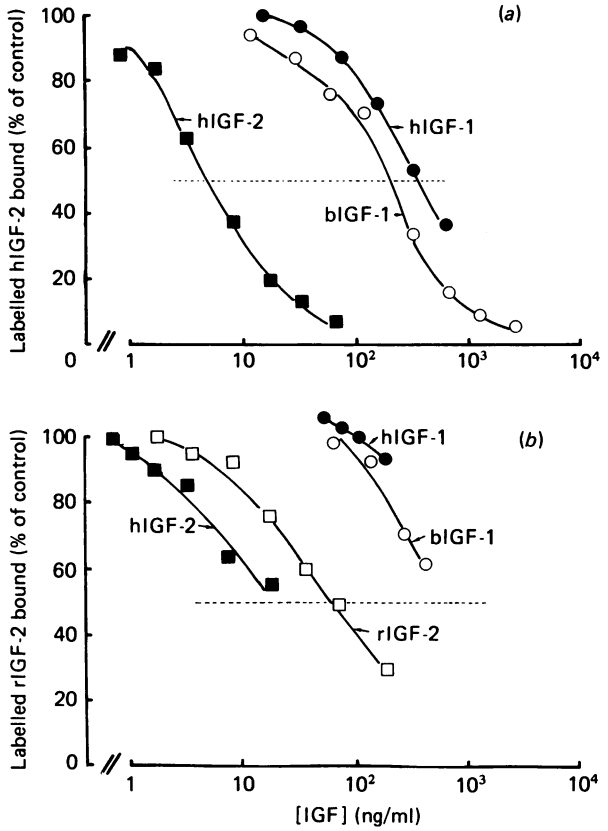

Fig. 4. Competition of growth factors with (*a*) ^{125}I-labelled hIGF-2 or (*b*) ^{125}I-labelled rIGF-2 for binding to sheep placental membranes

The symbols used are: ●, hIGF-1; ○, bIGF-1; ■, hIGF-2; □, rIGF-2. In the absence of competing growth factors (control), 19% of hIGF-2 tracer and 12% of rIGF-2 tracer bound to membranes.

(5.2) > bIGF-1 (215) > hIGF-1 (360); rIGF-2 tracer: hIGF-2 (20) > rIGF-2 (70) > bIGF-1 (~ 800) > hIGF-1 (> 800).

IGF-2 tracers appeared to bind entirely to type-2 receptors in sheep placental membranes, since insulin tested in concentrations up to 17 μg/ml showed no competing activity.

Competitive binding to foetal-human liver membranes

Foetal-human liver membranes showed similar IGF-binding specificity to that of sheep placental membranes, i.e. less than 2% of hIGF-1 tracer was bound (results not shown) and insulin was totally ineffective in competing for binding with labelled hIGF-2. Only hIGF-2 was used as tracer in these competitive binding studies. Competition curves were similar to those for sheep placental membranes, although higher concentrations of unlabelled growth factors were required for 50% effects (Fig. 5), as shown by the following order of sensitivities with half-maximal concentrations (ng/ml) given in parentheses: hIGF-2 (26) > bIGF-1 (800) > hIGF-1 (> 800). Table 1 shows that the relative potencies of competing peptides were similar in foetal-liver and sheep placental membranes.

DISCUSSION

bIGF-1 showed considerable affinity for antibodies raised against hIGF-1; unlabelled bIGF-1 competed for binding of hIGF-1 tracer to 3D1 antiserum 50% as well as did unlabelled hIGF-1. If allowance is made for bIGF-1 being only 60–70% pure, it appears likely that extensive homology must exist between bIGF-1 and hIGF-1. Nevertheless, other evidence suggests that some structural differences exist between hIGF-1 and bIGF-1. Thus polyclonal antisera to hIGF-1, presumably containing antibodies to multiple sites of the molecule, are less reactive to bIGF-1 than is the monoclonal antibody, whereas differences in the solubility properties of bIGF-1 and hIGF-1 provide evidence of structural diversity [the preceding paper (Francis *et al.*, 1986)].

The only other IGF-1 purified to homogeneity is derived from rat serum (Rubin *et al.*, 1982). Rat IGF-1 was found to be 30–40% as potent as hIGF-1 in competing with hIGF-1 tracer for binding to the polyclonal antiserum raised in rabbits (Daughaday *et al.*, 1982), yet showed negligible affinity for mouse monoclonal antibody 3D1 (Baxter *et al.*, 1982*a*), perhaps an expected result if high rat–mouse homology led to the mouse antiserum being raised against determinants differing

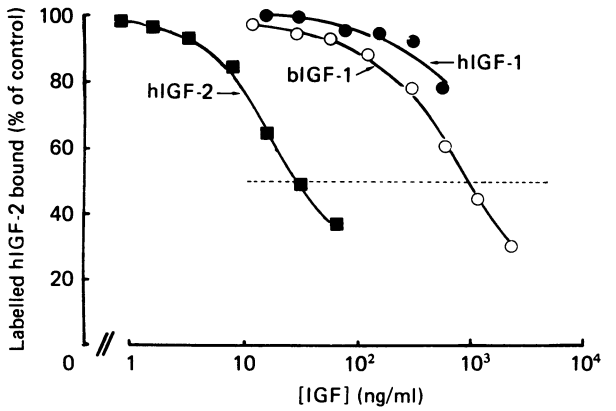

Fig. 5. Competitive binding of growth factors to human-foetal liver plasma membranes in the presence of ^{125}I-labelled hIGF-2

The symbols used are: ●, hIGF-1; ○, bIGF-1; ■, hIGF-2. Binding of ^{125}I-labelled hIGF-2 in the absence of competing growth factors (control) represented 13% of added tracer. Values are triplicate measurements at each concentration.

between human and mouse. Further evidence for this interpretation of the difference in antibody reactivities between rat IGF-1 and the human/bovine forms comes from the finding that all three IGFs share apparently complete homology over the first 29 residues from the *N*-terminus (Rinderknecht & Humbel, 1978*a*; Rubin *et al.*, 1982; Francis *et al.*, 1986).

The affinities of antibodies 3D1, NPA and Tr10 for hIGF-2 and insulin shown in Table 1 are similar to those reported previously for antisera raised against hIGF-1, i.e. less than 7% cross-reactivity with hIGF-2 and negligible binding to insulin (Van Wyk *et al.*, 1980; Zapf *et al.*, 1981; Baxter *et al.*, 1982*a*,*b*; Hintz *et al.*, 1982; Laubli *et al.*, 1982; Russell *et al.*, 1984).

Although human placental membranes contain both type-1 and type-2 IGF receptors, cross-linking studies have indicated that hIGF-1 binds preferentially to the type-1 receptor (Daughaday *et al.*, 1981; Massague & Czech, 1982). Our results are consistent with both hIGF-1 and bIGF-1 binding mainly to a type-1 receptor, because insulin inhibited up to 65% of the binding of either radioligand. The affinity of human placental membranes for hIGF-1 and bIGF-1 are similar if allowance was made for bIGF-1 being only 60–70% pure.

The receptor types in foetal-human liver or sheep placental membranes have not, to our knowledge, been characterized by affinity cross-linking studies. However, competitive binding properties suggest that these membranes contain almost entirely type-2 receptors (Baxter, 1984, 1985). Thus we observed that IGF-1 tracers bound very poorly to either membrane preparation, and very high concentrations of unlabelled IGF-1 were required to compete for binding with IGF-2 tracer; insulin was not active, even at concentrations exceeding 10 μg/ml. Bovine IGF-1 was slightly more potent than hIGF-1 in competing with either rIGF-2 or hIGF-2 tracer for binding to type-2 receptors. This may be another example of differences in the potency of bovine and human IGF-1, or, alternatively, bIGF-1 may contain 2–3% of IGF-2.

We observed that rIGF-2 was considerably less potent than hIGF-2 in competitive binding measurements with human or sheep placental membranes. Others have found similar results when using rat- or chick-embryo fibroblasts, human fibroblasts, BRL-3A cells or rat liver membranes (Rechler *et al.*, 1980; Van Wyk *et al.*, 1980; Adams *et al.*, 1983*b*). It is unlikely that these observations can be explained simply by impurities in rIGF-2 preparations, considering that, in one of the above studies (Rechler *et al.*, 1980), it was found that rIGF-2 was less effective in binding to cell membrane receptors yet equipotent to hIGF-2 in competing for binding with labelled rIGF-2 to rat serum IGF-binding proteins. It appears, therefore, that the same class of IGF from different species may not be equipotent biologically and, further, the order of potency of these IGFs may vary depending on the target tissue.

In conclusion, we have shown firstly that bIGF-1 cross-reacts strongly with 3D1 monoclonal antibody to hIGF-1 and, to a lesser extent, with NPA and Tr10 polyclonal antisera to hIGF-1. These reactivities contrast with the reported affinities of rIGF-1, which cross-reacts very poorly with monoclonal 3D1 antiserum but shows considerable affinity for NPA antiserum. Secondly, our results provide evidence that human, bovine and rat IGFs show considerable differences in binding to cell receptors. The extent to which these differences reflect the biological potencies of IGF preparations requires comparative receptor binding and functional studies in the same tissues.

We thank Dr. James Florini for generously providing us with rat IGF-2, and the National Hormone and Pituitary Program, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, for supplying IGF-1 antiserum. The expert technical assistance of Ms. Faye Upton and Ms. Paula Gravestock is acknowledged. Support for this work was provided in part by the National Health and Medical Research Council of Australia, the Reserve Bank Rural Credits Development Fund and the J. S. Davies Bequest to the University of Adelaide.

REFERENCES

Adams, S. O., Nissley, S. P., Headwerger, S. & Rechler, M. M. (1983*a*) Nature (London) **302**, 150–153

Adams, S. O., Nissley, S. P., Kasuga, M., Foley, T. P. & Rechler, M. M. (1983*b*) Endocrinology (Baltimore) **112**, 971–978

Atkinson, P. R., Weidman, E. R., Bhaumick, B. & Bala, R. M. (1980) Endocrinology (Baltimore) **106**, 2006–2012

Ballard, F. J., Read, L. C., Francis, G. L., Bagley, C. J. & Wallace, J. C. (1986) Biochem. J. **233**, 223–230

Baxter, R. C. (1984) Proc. Endocrine Soc. Aust. **27**, 3

Baxter, R. C. (1985) Adv. Clin. Chem. in the press

Baxter, R. C. & Brown, A. S. (1982) Clin. Chem. **28**, 485–487

Baxter, R. C. & Turtle, J. R. (1978) Biochem. Biophys. Res. Commun. **84**, 350–357

Baxter, R. C., Axiak, S. & Raison, R. L. (1982*a*) J. Clin. Endocrinol. Metab. **54**, 474–476

Baxter, R. C., Brown, A. S. & Turtle, J. R. (1982*b*) Clin. Chem. **28**, 488–495

Baxter, R. C., Zaltsman, A. & Turtle, J. R. (1984) J. Clin. Endocrinol. Metab. **58**, 955–959

Chen, R. F. (1967) J. Biol. Chem. **242**, 173–181

Czech, M. P., Oppenheimer, C. L. & Massague, J. (1983) Fed. Proc. Fed. Am. Soc. Exp. Biol. **42**, 2598–2601

Daughaday, W. H., Mariz, I. K. & Trivedi, B. (1981) J. Clin. Endocrinol. Metab. **53**, 282–288

Daughaday, W. H., Parker, K. A., Borwosky, S., Trivedi, B. & Kapadia, M. (1982) Endocrinology (Baltimore) **110**, 575–581
D'Ercole, A. J., Applewhite, G. T. & Underwood, L. E. (1980) Dev. Biol. **75**, 315–318
D'Ercole, A. J., Stiles, A. D. & Underwood, L. E. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 935–939
Eigenmann, J. E., Patterson, D. F., Zapf, J. & Froesch, E. R. (1984) Acta Endocrinol. (Copenhagen) **105**, 294–301
Fraker, P. J. & Speck, J. C. (Jr.) (1978) Biochem. Biophys. Res. Commun. **80**, 849–857
Francis, G. L., Read, L. C., Ballard, F. J., Bagley, C. J., Upton, F. M., Gravestock, P. M. & Wallace, J. C. (1986) Biochem. J. **233**, 207–213
Haselbacher, G. & Humber, R. (1982) Endocrinology (Baltimore) **110**, 1822–1824
Hintz, R. L., Liu, F. & Seegan, G. (1982) J. Clin. Endocrinol. Metab. **55**, 927–930
Kasuga, M., Van Obberghen, E., Nissley, S. P. & Rechler, M. M. (1981) J. Biol. Chem. **256**, 5305–5308
Laubli, U. K., Baier, W., Binz, H., Celio, M. R. & Humbel, R. E. (1982) FEBS Lett. **149**, 109–112
Marquardt, H., Todaro, G. J., Henderson, L. E. & Oroszlan, S. (1981) J. Biol. Chem. **256**, 6859–6865
Massague, J. & Czech, M. P. (1982) J. Biol. Chem. **257**, 5038–5045
Moses, A. C., Nissley, S. P., Short, P. A., Rechler, M. M. & Podskalny, J. M. (1980) Eur. J. Biochem. **103**, 387–400
Rechler, M. M., Zapf, J., Nissley, S. P., Froesch, E. R., Moses, A. C., Podskalny, J. M., Schilling, E. E. & Humbel, R. E. (1980) Endocrinology (Baltimore) **107**, 1451–1459
Rinderknecht, E. & Humbel, R. E. (1978*a*) J. Biol. Chem. **253**, 2769–2776
Rinderknecht, E. & Humbel, E. (1978*b*) FEBS Lett. **89**, 283–286
Rubin, J. S., Mariz, I. K., Jacobs, J. W., Daughaday, W. H. & Bradshaw, R. A. (1982) Endocrinology (Baltimore) **110**, 734–740
Russell, W. E., Van Wyk, J. J. & Pledger, W. J. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 2389–2392
Van Wyk, J. J., Svoboda, M. E. & Underwood, L. E. (1980) J. Clin. Endocrinol. Metab. **50**, 206–208
Williams, P. F. & Turtle, J. R. (1979) Biochim. Biophys. Acta **579**, 367–374
Wilson, D. M. & Hintz, R. L. (1982) J. Endocrinol. **95**, 59–64
Zapf, J., Walter, H. & Froesch, E. R. (1981) J. Clin. Invest. **68**, 1321–1330

Received 19 April 1985/9 July 1985; accepted 10 September 1985