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Specific binding of insulin-like growth factors 1 and 2 to the type 1 and type 2 receptors respectively

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1. Competitive binding and receptor cross-linking experiments have been used to examine the receptor–ligand interactions between three bovine insulin-like growth factors (IGF) and monolayer cultures of myoblasts and fibroblasts. 2. Labelled IGF-2 bound predominantly to the type 2 receptor with negligible label cross-linked to the type 1 receptor, notwithstanding the ability of IGF-2 to compete effectively for the binding of IGF-1 to the type 1 receptor. Approx. 100-fold higher concentrations of IGF-1 or the *N*-terminal truncated (des-Gly-Pro-Glu) IGF-1 (−3*N*:IGF-1) were required to produce competition equivalent to IGF-2. 3. All IGF peptides, but especially IGF-1, enhanced the binding of labelled IGF-2 to the type 2 receptor of lung fibroblasts. This unusual effect was probably a consequence of the displacement of labelled IGF-2 otherwise bound to a medium protein, a conclusion supported by the demonstration of a 38 kDa membrane protein cross-linked to labelled IGF-2. 4. Both IGF-1 and −3*N*:IGF-1 bound only to the type 1 IGF receptor in L6 myoblasts, rat vascular smooth-muscle cells and human lung fibroblasts. The peptides competed for labelled IGF-1 binding with potencies in the order −3*N*:IGF-1 > IGF-1 > IGF-2 ⩾ insulin. Since the IGF peptides were equipotent in skin fibroblasts, it was proposed that the apparently higher affinity of −3*N*:IGF-1 for receptors in the other cell types was instead a consequence of a low affinity of this peptide for the competing 38 kDa binding protein.

INTRODUCTION

Competitive binding and receptor–ligand cross-linking experiments have established the presence of two distinct receptors for IGF in vertebrate cells. In most studies IGF-1 has been shown to cross-link predominantly to the type 1 receptor which has a 130 kDa binding subunit (Kasuga *et al.*, 1981; Massagué & Czech, 1982; Beguinot *et al.*, 1985; Ballard *et al*, 1986). Both IGF-2 and insulin also bind to this subunit but with a lower affinity than found with IGF-1. IGF-2 can bind to a 250 kDa protein, the type 2 receptor (Kasuga *et al.*, 1981; Massagué & Czech, 1982). This latter receptor does not bind insulin, while IGF-1 competition for IGF-2 binding is variable apparently depending on cell type.

The demonstration that recombinant IGF-1 is a 10–100-fold less effective competitor for IGF-2 binding than observed with IGF-1 purified from serum (Ewton *et al.*, 1987; Rosenfeld *et al.*, 1987) implies that either natural IGF-1 preparations are less pure than previously recognized or an unexplained difference exists between recombinant and natural IGF-1. The discovery of a novel IGF-1 derivative (−3*N*:IGF-1) with glycine, proline and glutamic acid residues cleaved from the *N*-terminus (Francis *et al.*, 1986; Carlsson-Skwirut *et al.*, 1986) raises the possibility that the presence of this peptide in IGF-1 preparations may account for the anomalous binding results. In the present report we have tested this explanation with competitive binding and cross-linking experiments using pure IGF-1, IGF-2 and −3*N*:IGF-1.

MATERIALS AND METHODS

Peptides

Bovine IGF-1, IGF-2 and −3*N*:IGF-1 were purified from colostrum as described previously (Francis *et al.*, 1986, 1988). Each peptide was labelled with carrier-free [^{125}I]NaI (Amersham International, Bucks., U.K.) to a specific activity of approx. 80 Ci/g using the chloramine T method (Van Obberghen-Schilling & Pouyssegur, 1983) and separated from unreacted iodide and aggregates by chromatography on a Sephadex G-100 column equilibrated with 1 M-acetic acid containing 0.1% bovine serum albumin (RIA grade, Sigma, St. Louis, MO, U.S.A.). The insulin used was porcine Actrapid (Novo Industri A/S, Copenhagen, Denmark).

Cell cultures

Lung fibroblasts [HE(39)L] derived from a human fetus were purchased from Commonwealth Serum Laboratories, Melbourne, Australia. Rat L6 myoblasts were kindly provided by Dr. J. M. Gunn, Texas A & M University, College Station, TX, U.S.A., SD8 ArA rat vascular smooth-muscle cells by Dr. J. Funder, Prince Henry's Hospital, Melbourne, Australia, and skin fibroblasts (SF 1972) derived from a full-term human infant by Dr. W. Carey, Adelaide Children's Hospital, North Adelaide, Australia. The cells were grown as monolayers in 12-place multiwell dishes (Costar, Cambridge, MA, U.S.A.) in a medium consisting of Dulbecco-modified Eagle's Minimal Essential Medium supple-

Abbreviations used: IGF, insulin-like growth factor(s); IGF-1, insulin-like growth factor 1; IGF-2, insulin-like growth factor 2; −3*N*:IGF-1, *N*-terminal-truncated (des-Gly-Pro-Glu) IGF-1.

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mented with antibiotics and 5% fetal bovine serum (Ballard *et al.*, 1986).

IGF binding experiments

The procedure has been described previously (Ballard *et al.*, 1986). Briefly, confluent monolayers were washed twice over 90 min and incubated for 150 min at 15 °C in 0.5 ml of a solution containing 4×10^4 d.p.m. of radioligand, unlabelled peptides Hepes and binding buffer (0.1 M-Hepes/0.12 M-NaCl/5 mM-KCl/1.2 mM-$MgSO_4$/8 mM-glucose/25 mg of bovine serum albumin/ml, pH 7.6). At the completion of the binding period the monolayers were washed and the radioactivity determined (Ballard *et al.*, 1986). Binding was expressed as the percentage bound in the absence of competing peptide.

Cross-linking experiments

Binding of radioligand was carried out as described above except for the addition of 4×10^5 d.p.m. of radioligand per well. Subsequently the cells were washed three times in Hepes binding buffer without bovine serum albumin at 0 °C, after which 0.5 ml of 0.5 mM-disuccinimidyl suberate in the same buffer was added and left on the cells for 20 min at 15 °C. The cross-linking reaction was quenched with 1.5 ml of 0.1 M-Tris containing 1 mM-EDTA at pH 7.4 and the cells were left for a further 20 min. The medium was removed, the cells were solubilized in 200 μl of a dissociating solution composed of 2% SDS/10% (w/v) glycerol/100 mM-dithiothreitol/0.01% Bromophenol Blue/62.5 mM-Tris, pH 6.8, and the mixture was stored at −70 °C. Immediately before electrophoretic separation, the dissociated cell mixtures were heated at 95 °C for 5 min and portions (40 μl) were subjected to SDS/polyacrylamide-gel electrophoresis under reducing conditions on either 5% (w/v) acrylamide gels (Laemmli, 1970) or linear gradient gels containing between 5 and 25% (w/v) acrylamide. The M_r values for receptor–ligand complexes were deduced by comparison with a mixture of marker proteins (SDS-6H, Sigma) that was electrophoresed in an adjacent lane. Details of staining, destaining, drying and autoradiography have been given previously (Ballard *et al.*, 1986).

RESULTS

Binding experiments with ^{125}I-labelled IGF-1 and −3*N*:IGF-1

Monolayers of L6 myoblasts containing 200 μg of protein bound approx. 10% of the radioligand added in the absence of competing peptides. Essentially all binding of label was prevented by the addition of 200 ng of IGF-1, IGF-2 or −3*N*:IGF-1/ml, while 10 μg of insulin/ml reduced the binding of labelled IGF-1 to 38% (Fig. 1*a*). In this experiment 50% competition for tracer binding occurred with 5 ng of −3*N*:IGF-1/ml, 10 ng of IGF-1/ml, 20 ng of IGF-2/ml and 4 μg of insulin/ml. Very similar results were obtained with the vascular smooth-muscle line, SD8 ArA (results not shown).

Half-maximal competition for labelled IGF-1 binding to human lung fibroblasts (Fig. 1*b*) occurred at almost exactly the same concentrations of the IGF peptides as with L6 myoblasts. However, low concentrations of IGF-2 enhanced the binding of ^{125}I-labelled IGF-1 while the competition curve for −3*N*:IGF-1 was biphasic

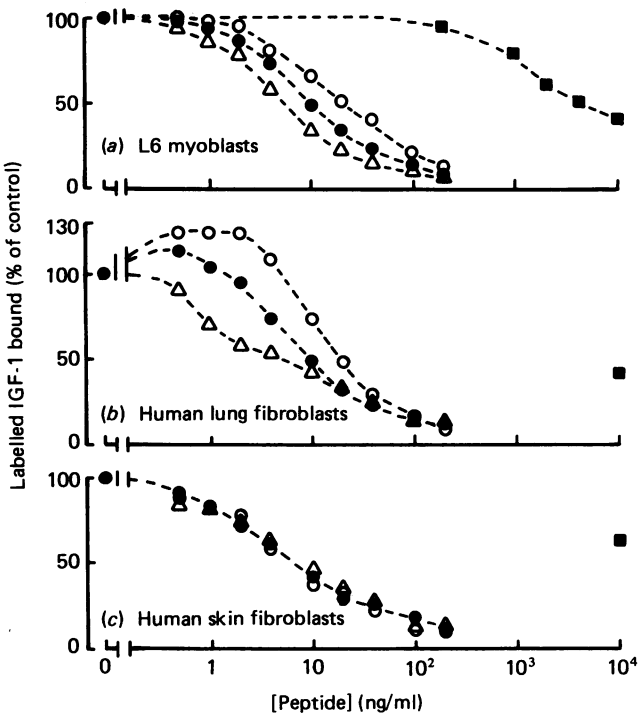

Fig. 1. Competition for the binding of ^{125}I-labelled IGF-1 in (*a*) L6 myoblasts, (*b*) human lung fibroblasts and (*c*) human skin fibroblasts

The symbols used are: ●, IGF-1; ○, IGF-2; △, −3*N*:IGF-1; ■, insulin. In the absence of competing growth factors the percentage of label bound to the cells in this experiment is: (*a*), 11.4; (*b*), 9.4; (*c*), 3.1. Values are the means of three determinations at each concentration. In all cases the standard errors of the mean are smaller than the symbols.

showing more than the expected competition at low concentrations. A third pattern of competition was evident with the skin fibroblasts in which the curves for the three IGF peptides were indistinguishable and gave a half-maximal effect at 6 ng/ml. Additional binding experiments were highly reproducible except for the lung fibroblasts where the biphasic nature of the −3*N*:IGF-1 curve was not always clear.

Equivalent families of binding curves have been generated for all four cell lines with labelled −3*N*:IGF-1 replacing labelled IGF-1 (results not shown). The results obtained were remarkably similar in each case to those obtained for IGF-1.

In receptor cross-linking experiments with both labelled IGF-1 and labelled −3*N*:IGF-1, the amount of tracer peptide added to the wells was increased to 2.5 ng (4×10^5 d.p.m.) so that significant cross-linking could be detected. With L6 myoblasts, autoradiography of SDS/polyacrylamide gels showed a major band at approx. 130 kDa with much reduced labelling of larger peptides (Fig. 2*a*, lanes 1 and 9). The addition of 10 μg of insulin/ml during binding reduced the autoradiographic density of all visible bands (Fig. 2*a*, lane 2). Reduction of the major 130 kDa band as well as the minor bands to approx. the same extent as with insulin occurred when 40 ng of either IGF-1 or −3*N*:IGF-1/ml was added (Fig. 2*a*, lanes 3 and 7 respectively), while much greater

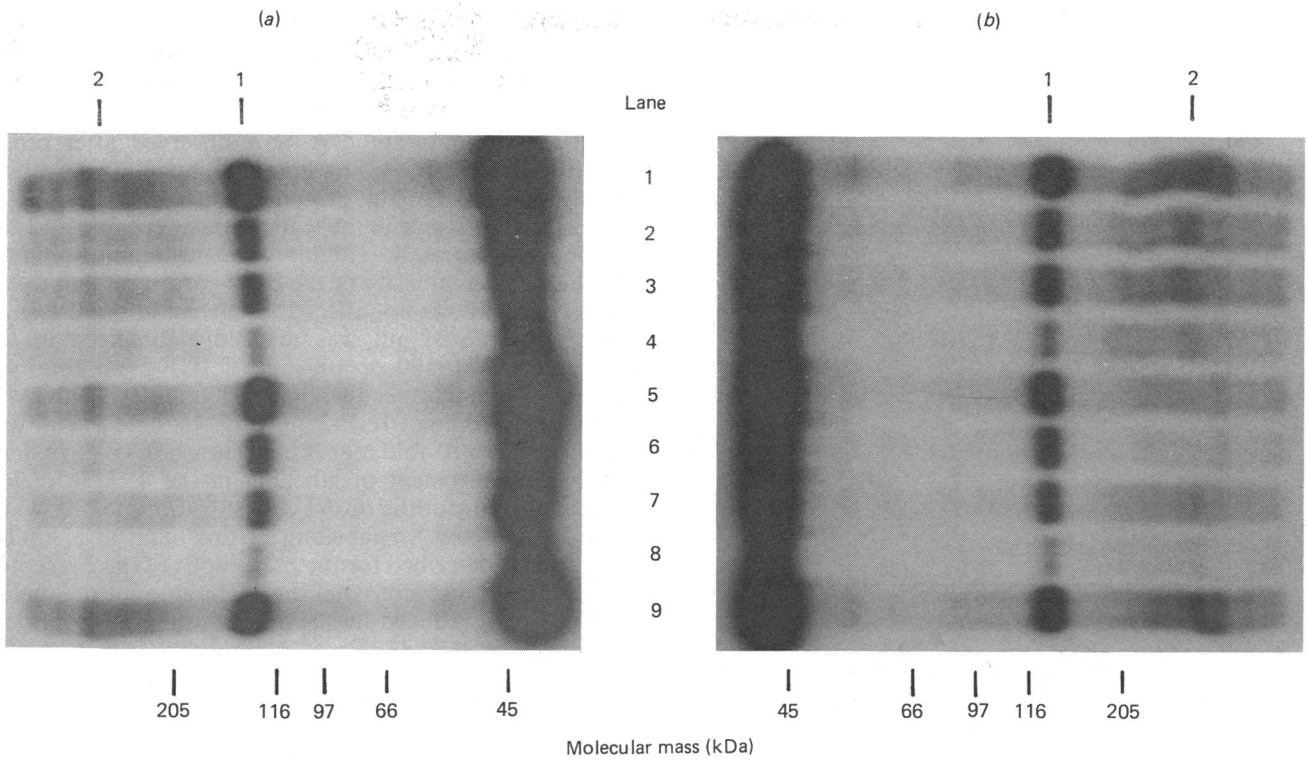

Fig. 2. Autoradiographs of dried gels after SDS/polyacrylamide-gel electrophoresis under reducing conditions from cross-linking experiments with (*a*) ^{125}I-labelled IGF-1 and (*b*) ^{125}I-labelled $-3N$:IGF-1 added to L6 myoblasts

The concentrations of non-radioactive ligands were: lanes 1 and 9, no addition; lane 2, 10 μg of insulin/ml; lane 3, 40 ng of IGF-1/ml; lane 4, 200 ng of IGF-1/ml; lane 5, 40 ng of IGF-2/ml; lane 6, 200 ng of IGF-2/ml; lane 7, 40 ng of $-3N$:IGF-1/ml; lane 8, 200 ng of $-3N$:IGF-1/ml. The positions of molecular mass markers in adjacent lanes are indicated. The type 1 and type 2 IGF receptor bands are marked.

competition was evident when 200 ng of either peptide/ml was present (Fig. 2*a*, lanes 4 and 8). Although 40 ng of IGF-2/ml had little effect on the band intensities (Fig. 2*a*, lane 5), increasing the concentration to 200 μg/ml produced essentially the same degree of competition as that observed with 40 ng of either IGF-1 peptide/ml (Fig. 2*a*, compare lane 6 with lane 3 or 7). It is pertinent that the density of autoradiographic bands in the 250 kDa region of the type 2 receptor was both diminished by insulin and not selectively diminished by IGF-2 to below the competition obtained with IGF-1.

Cross-linking to give a faint band at approx. 50 kDa can be seen following addition of either ^{125}I-labelled IGF-1 or ^{125}I-labelled $-3N$:IGF-1 to L6 myoblasts (Figs. 2*a* and 2*b*). The intensity of this autoradiographic band is diminished by all peptides. In general the labelling patterns with IGF-1 and $-3N$:IGF-1 in L6 myoblasts (Fig. 2) were very similar except that a band in the region expected for the type 2 receptor was visible with the latter tracer (Fig. 2*b*, lanes 1 and 9). The intensity of this band was selectively diminished by a low concentration of IGF-2 (Fig. 2*b*, lane 5) as compared with a similar concentration of IGF-1 or $-3N$:IGF-1 (Fig. 2*b*, lanes 3 and 7) or by the high insulin concentration (Fig. 2*b*, lane 2).

The patterns of labelled bands following cross-linking of labelled IGF-1 or $-3N$:IGF-1 to SD8 ArA smooth-muscle cells or human lung fibroblasts were very similar to those obtained with L6 myoblasts (results not shown) while insufficient labelling occurred with the skin fibroblast line to permit interpretation of the autoradiographs.

Binding experiments with ^{125}I-labelled IGF-2

Addition of unlabelled IGF-2 to L6 myoblasts in the presence of tracer amounts of labelled IGF-2 resulted in a 20% inhibition of binding at 6 ng/ml and a 50% inhibition at 40 ng/ml (Fig. 3). Much higher concentrations of IGF-1 were required to compete for the binding of label; for example 20% inhibition occurred with 500 ng of $-3N$:IGF-1/ml or 1.8 μg of IGF-1/ml (Fig. 3*a*). Very similar results were obtained using the SD8 ArA muscle cell line (results not shown) in which 20% competition for binding of labelled IGF-2 was demonstrated with 16 ng of IGF-2, 500 ng of $-3N$:IGF-1 and 2 μg of IGF-1/ml.

The competitive binding curves with both fibroblast lines were unusual because low concentrations of IGF-1 or $-3N$:IGF-1 and, in the case of lung fibroblasts, IGF-2, enhanced the amount of IGF-2 label bound (Figs. 3*b* and 3*c*). Repeat experiments confirmed this extra binding, although the magnitude of the response was variable. Whereas this effect precluded any quantitative assessment of the amounts of IGF-1 or $-3N$:IGF-1 required to inhibit IGF-2 binding to the fibroblasts, it seems that approx. 100-fold higher concentrations of the two IGF-1 peptides than IGF-2 would be needed to produce an equivalent degree of inhibition. It should be noted that

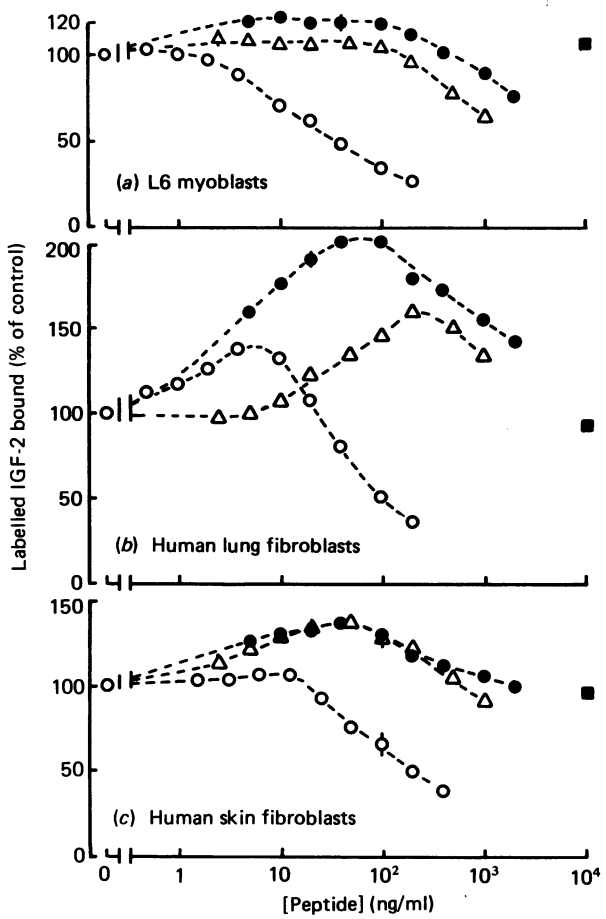

Fig. 3. Competition for the binding of ^{125}I-labelled IGF-2 in (*a*) L6 myoblasts, (*b*) human lung fibroblasts and (*c*) human skin fibroblasts

The symbols used are: ●, IGF-1; ○, IGF-2; △, −3*N*:IGF-1; ■, insulin. In the absence of competing growth factors the percentage of label bound to the cells in this experiment is: (*a*), 30.8; (*b*), 15.6; (*c*), 20.2. Values are the means of three determinations at each concentration. Standard errors of the mean are shown when they are sufficiently large in relation to the symbols.

insulin did not affect the binding of labelled IGF-2 in any of the four cell lines.

Cross-linking of labelled IGF-2 to L6 myoblasts followed by SDS-electrophoresis under reducing conditions and autoradiography identified a protein band of approx. 250 kDa (Fig. 4*a*, lanes 1 and 2). Insulin (10 μg/ml) did not reduce the intensity of this band (Fig. 4*a*, lane 3). The higher concentration of IGF-1 or −3*N*:IGF-1 tested (1 μg/ml) produced some competition for IGF-2 binding (Fig. 4*a*, lanes 5 and 9 respectively). On the other hand almost complete competition for binding occurred wiith 40 ng of IGF-2/ml (Fig. 4*a*, lane 6). Slight binding of label to a peptide with a molecular mass of 130 kDa expected for the type 1 receptor was detected in the control incubations. In these cross-linking experiments with labelled IGF-2 the gels used for separation contained 5–25% acrylamide gradients, a modification adopted to identify more clearly any small proteins that may be cross-linked to the radioligand. However, in these studies with either L6 myoblasts (Fig. 4*a*) or SD8 ArA muscle cells (results not shown), no marked labelling of small proteins was observed, although faint bands of approx. 80 and 90 kDa were detected.

The autoradiographic pattern observed after cross-linking labelled IGF-2 to HE(39)L human lung fibroblasts gave two main protein bands, one at approx. 250 kDa that is presumably the type 2 receptor and the second with a mobility similar to the ovalbumin standard (45 kDa). The intensity of the band corresponding to the larger protein was not affected by the addition of insulin (Fig. 4*b*, lane 3) but was markedly enhanced in the presence of 20, 100 or 1000 ng of IGF-1/ml (Fig. 4*b*, lanes 4, 5 and 6 respectively). Labelling of this band was reduced by 40 ng of IGF-2/ml (Fig. 4*b*, lane 8) and was abolished at a 10-fold higher concentration (Fig. 4*b*, lane 9). No enhancement of labelling in the 45 kDa region occurred at any of the IGF-1 concentrations tested, while IGF-2 competed for labelling of this band in a similar way to that described for the presumptive type 2 receptor (Fig. 4*b*). We also note that the slight amount of label at 130 kDa was reduced by the addition of IGF-2 but less obviously by IGF-1.

Specific binding of labelled IGF-2 to a protein of approx. 250 kDa was detected in a cross-linking experiment with human skin fibroblasts (results not shown). With these cells there was little indication of either an increased band intensity in the presence of IGF-1 or of substantial IGF-2 binding to smaller proteins.

DISCUSSION

Specificity of binding to type 1 and type 2 receptors

In the present investigation we have compared the binding of three highly purified IGF peptides to the receptors on two rat muscle cell lines, human skin fibroblasts and human lung fibroblasts in an attempt to clarify some of the uncertainties and apparent anomalies of IGF binding. Our results show that IGF-1, −3*N*:IGF-1 and IGF-2 all compete at low concentrations for the binding of labelled IGF-1 to the different cell monolayers. When addition of labelled IGF-1 was followed by cross-linking, separation of proteins by SDS/polyacrylamide-gel electrophoresis and autoradiography, it was evident that essentially all the cross-linked radioactivity was associated with the 130 kDa subunit of the type 1 receptor. The small amount of radioactivity migrating in the 250 kDa region probably represents cross-linking to type 1 subunit dimers rather than binding to the type 2 receptor, because this radioactivity is reduced by insulin and not selectively by IGF-2. It seems likely that earlier studies which demonstrated cross-linking of IGF-1 to the type 2 receptor (Massagué & Czech, 1982; De Vroede *et al.*, 1984; Beguinot *et al.*, 1985; Ballard *et al.*, 1986) were a consequence of IGF-2 impurities in the labelled IGF-1. Thus Ewton *et al.* (1987) have shown that recombinant IGF-1 bound only to the type 1 receptor of L6 myoblasts while IGF-1 purified from human serum bound to both receptors.

For each of the cell lines examined in the present investigation, labelled IGF-2 bound predominantly to the type 2 receptor and non-radioactive IGF-2 competed for binding at concentrations about two orders of magnitude lower than observed with IGF-1. This large difference in the effectiveness of competitive binding has been observed with both synthetic and recombinant

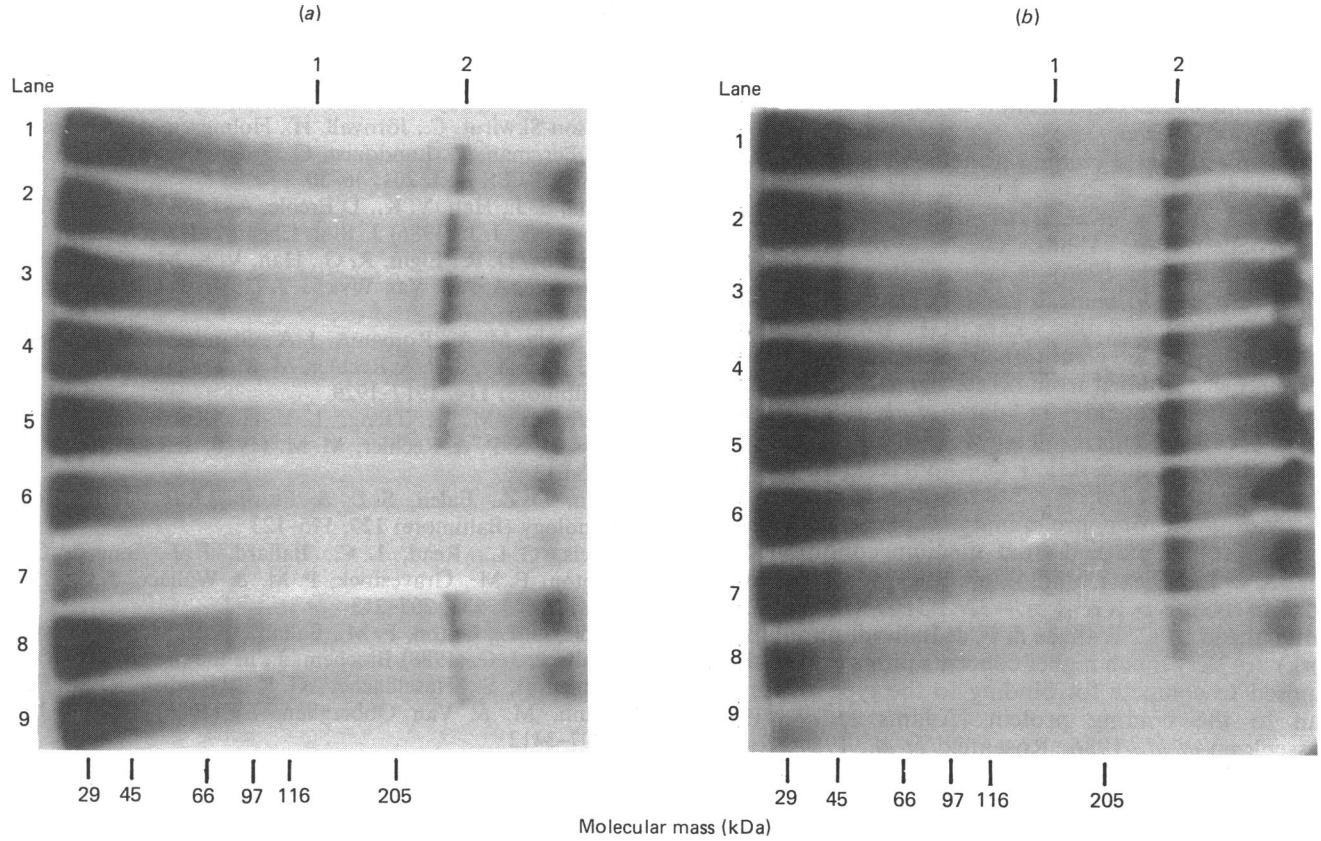

Fig. 4. Autoradiographs of dried gels after SDS/polyacrylamide-gel electrophoresis under reducing conditions from cross-linking experiments with ^{125}I-labelled IGF-2 added to (*a*) L6 myoblasts and (*b*) human lung fibroblasts

The concentrations of non-radioactive ligands in (*a*) were: lanes 1 and 2, no addition; lane 3, 10 μg of insulin/ml; lane 4, 100 ng of IGF-1/ml; lane 5, 1 μg of IGF-1/ml; lane 6, 40 ng of IGF-2/ml; lane 7, 400 ng of IGF-2/ml; lane 8, 100 ng of $-3N$:IGF-1/ml; lane 9, 1 μg of $-3N$:IGF-1/ml. The concentrations of non-radioactive ligands in (*b*) were: lanes 1 and 2, no addition; lane 3, 10 μg of insulin/ml; lane 4, 20 ng of IGF-1/ml; lane 5, 100 ng of IGF-1/ml; lane 6, 1 μg of IGF-1/ml; lane 7, 10 ng of IGF-2/ml; lane 8, 40 ng of IGF-2/ml; lane 9, 400 ng of IGF-2/ml. The positions of molecular mass markers in adjacent lanes are indicated. The type 1 and type 2 IGF receptor bands are marked.

IGF-1 (Rosenfeld *et al.*, 1987; Ewton *et al.*, 1987) as well as in experiments with purified type 2 receptor (Scott & Baxter, 1987). It is not always possible to determine whether other results showing more IGF-1 competition for IGF-2 binding (Rechler *et al.*, 1980; Thorsson *et al.*, 1985; Krett *et al.*, 1986; Casella *et al.*, 1986) can be explained by IGF-2 impurities in the IGF-1 preparations or whether the binding is associated with the type 1 receptor. Certainly IGF-2 competes effectively for IGF-1 binding to the type 1 receptor (Fig. 1). This result is apparently inconsistent with the situation when only tracer amounts of IGF-2 are present, as cross-linking experiments such as those in Fig. 4 demonstrate minimal binding to the type 1 receptor. Perhaps the low amounts of IGF-2 added as tracer selectively bind to the type 2 receptor and much larger amounts are needed for binding or competition for the type 1 receptor.

Binding differences between cell types

The competitive binding curves clearly differ between the three cell lines (Figs. 1 and 3). We believe that these differences are best explained by the production of another binding protein by the lung fibroblasts in addition to the type 1 and type 2 receptors. Interpretation of our results in the context of an extra binding protein requires that: (*a*) it is present in the medium as well as on the cell surface; (*b*) it binds the growth factors with an order of affinities IGF-2 > IGF-1 > $-3N$:IGF-1; and (*c*) it binds IGF-1 with higher affinity than IGF-1 binds to the type 2 receptor.

Provided these criteria can be satisfied, the observed binding patterns with labelled IGF-2 (Fig. 3*b*, Fig. 4*b*) are predictable because IGF-1 would displace IGF-2 tracer from the binding protein in the medium, thus permitting increased cell surface labelling. Moreover, since IGF-1 has very low affinity for the type 2 receptor (Fig. 3) it will not complete with the labelled IGF-2 for binding to the cells. A similar explanation holds for the enhancement of labelled IGF-2 binding by $-3N$:IGF-1, since the higher concentrations of $-3N$:IGF-1 needed to produce the effect (Fig. 3*b*) would be a consequence of the lesser affinity of this growth factor for the binding protein. The second major binding difference with lung fibroblasts, an enhanced competition by $-3N$:IGF-1 in the IGF-1 receptor assay (Fig. 1*b*), would also result from a relatively low affinity of $-3N$:IGF-1 for the putative binding protein. Moreover, the somewhat variable positioning of the $-3N$:IGF-1 competition

curves between experiments with lung fibroblasts can be explained by different amounts of binding protein being produced.

A number of experimental findings support the interpretation given above. (*a*) Several studies have demonstrated that a binding protein is released into the medium as well as being present on the surface of fibroblasts (Adams *et al.*, 1984; Clemmons *et al.*, 1986; De Vroede *et al.*, 1986). The size of this protein (Clemmons *et al.*, 1986) is consistent with the band shown in Fig. 4(*b*) being a complex between a 38 kDa binding protein and the 7 kDa IGF. (*b*) The binding protein in fibroblast conditioned medium as well as one of similar size isolated from cerebrospinal fluid exhibit a selective affinity for IGF-2 (Adams *et al.*, 1984; Hossenlopp *et al.*, 1986). This result agrees with the much greater ability of IGF-2 than IGF-1 or $-3N$:IGF-1 to compete for binding of labelled IGF-2 to the 38 kDa protein (Fig. 4*b*). Moreover, our preliminary experiments with a binding protein purified from medium conditioned by MDBK cells indicate binding affinities in the predicted order of IGF-2 > IGF-1 > $-3N$:IGF-1 (L. Szabo, D. Mottershead, J. C. Wallace & F. J. Ballard, unpublished work). (*c*) Very much higher concentrations of IGF-1 are required to compete for binding to the type 2 receptor than to the binding protein (Adams *et al.*, 1984; Hossenlopp *et al.*, 1986; Rosenfeld *et al.*, 1987; Ewton *et al.*, 1987).

In addition to explaining the differences in binding between lung fibroblasts and skin fibroblasts, the production of a small amount of binding protein with the properties listed above could also account for the enhanced competition of $-3N$:IGF-1 in the myoblast IGF-1 binding study (Fig. 1*a*). Other explanations are possible for the different binding results between the cell types. They include ligand-induced recruitment of receptors, ligand-induced changes in binding properties or the existence of sub-types of IGF receptors (King *et al.*, 1982; Massagué *et al.*, 1982; Oppenheimer *et al.*, 1983; Gammeltoft *et al.*, 1985; Morgan & Roth, 1986; Casella *et al.*, 1986). However, the lack of evidence at the present time in support of these possibilities with respect to the fibroblast and myoblast cell lines must favour the results being tentatively explained by the differential production of a binding protein.

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REFERENCES

Adams, S. O., Kapadia, M., Mills, B. & Daughaday, W. H. (1984) Endocrinology (Baltimore) **115**, 520–526

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

Beguinot, F., Kahn, C. R., Moses, A. C. & Smith, R. J. (1985) J. Biol. Chem. **260**, 15892–15898

Carlsson-Skwirut, C., Jörnvall, H., Holmgren, A., Andersson, C., Bergman, T., Lundquist, G., Sjögren, B. & Sarar, V. R. (1986) FEBS Lett. **201**, 46–50

Casella, S. J., Han, V. K., D'Ercole, A. J., Svoboda, M. E. & Van Wyk, J. J. (1986) J. Biol. Chem. **261**, 9268–9273

Clemmons, D. R., Elgin, R. G., Han, V. K. M., Casella, S. J., D'Ercole, A. J. & Van Wyk, J. J. (1986) J. Clin. Invest. **77**, 1548–1556

De Vroede, M. A., Romanus, J. A., Standaert, M. L., Pollet, R. J., Nissley, S. P. & Rechler, M. M. (1984) Endocrinology (Baltimore) **114**, 1917–1929

De Vroede, M. A., Tseng, L. Y.-H., Katsoyannis, P. G., Nissley, S. P. & Rechler, M. M. (1986) J. Clin. Invest. **77**, 602–613

Ewton, D. Z., Falen, S. L. & Florini, J. R. (1987) Endocrinology (Baltimore) **120**, 115–123

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

Francis, G. L., Upton, F. M., Ballard, F. J., McNeil, K. A. & Wallace, J. C. (1988) Biochem. J., in the press

Gammeltoft, S., Haselbacher, G. K., Humbel, R. E., Fehlmann, M. & Van Obberghen, E. (1985) EMBO J. **4**, 3407–3412

Hossenlopp, P., Seurin, D., Segovia-Quinson, B. & Binoux, M. (1986) FEBS Lett. **208**, 439–444

Kasuga, M., Van Obberghen, E., Nissley, S. P. & Rechler, M. M. (1981) J. Biol. Chem. **256**, 5305–5308

King, G. L., Rechler, M. M. & Kahn, C. R. (1982) J. Biol. Chem. **257**, 10001–10006

Krett, N. L., Heaton, J. H. & Gelehrter, T. D. (1986) Biochem. Biophys. Res. Commun. **134**, 120–127

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

Massagué, J. & Czech, M. P. (1982) J. Biol. Chem. **257**, 5038–5045

Massagué, J., Blinderman, L. A. & Czech, M. P. (1982) J. Biol. Chem. **257**, 13958–13963

Morgan, D. O. & Roth, R. A. (1986) Biochem. Biophys. Res. Commun. **138**, 1341–1347

Oppenheimer, C. L., Pessin, J. E., Massagué, J., Gitomer, W. & Czech, M. P. (1983) J. Biol. Chem. **258**, 4824–4830

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

Rosenfeld, R. G., Conover, C. A., Hodges, D., Lee, P. D. K., Misra, P., Hintz, R. L. & Li, C. H. (1987) Biochem. Biophys. Res. Commun. **143**, 199–205

Scott, C. D. & Baxter, R. C. (1987) Endocrinology (Baltimore) **120**, 1–9

Thorsson, A. V., Hintz, R. L., Enberg, G. & Hall, K. (1985) J. Clin. Endocrinol. Metab. **60**, 387–391

Van Obberghen-Schilling, H. & Pouyssegur, J. (1983) Exp. Cell Res. **147**, 369–378

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