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Recombinant human insulin-like growth factor I induces its own specific carrier protein in hypophysectomized and diabetic rats

(growth hormone/insulin/streptozotocin diabetes)

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ABSTRACT **The physiology of the specific serum binding proteins which constitute the main storage pool for insulin-like growth factors (IGFs) in mammals is still incompletely understood. We have, therefore, investigated the regulation of these proteins in (*i*) hypophysectomized (hypox) rats infused with recombinant human growth hormone (rhGH) or recombinant human IGF I (rhIGF I) and (*ii*) streptozotocin-diabetic rats infused with insulin or rhIGF I. The main carrier protein, a GH-dependent complex of apparent molecular mass 200 kDa, contains N-glycosylated IGF-binding subunits (42, 45, and 49 kDa) that differ in their glycosyl but not in their protein moiety. These subunits are lacking in hypox and diabetic rats. They are induced by GH and insulin, respectively, and appear in the 200-kDa complex. Infusion of rhIGF I induces the subunits in both states; however, only in diabetic, not in hypox, rats do they form the 200-kDa complex. Glycosylated carrier protein subunits do not appear before 8 hr of rhIGF I infusion. During that period, hypox rats may become severely hypoglycemic. After 16 hr, glycosylated subunits are clearly induced, and blood sugar values are normal. We conclude: (*i*) The N-glycosylated subunits of the 200-kDa complex reflect the IGF I status. (*ii*) IGF I may mediate the induction of these subunits by GH. (*iii*) Significant association to the 200-kDa complex occurs only in the presence of GH. It is likely that GH, but not IGF I, induces a component, which itself does not bind IGF, but associates with the glycosylated IGF-binding subunits. (*iv*) The glycosylated subunits protect against IGF-induced hypoglycemia and may be involved in tissue-specific targeting of IGFs.**

In mammalian blood, insulin-like growth factors (IGFs) circulate in tight association with specific high-affinity carrier proteins (1–3). Although they constitute the main reservoir of IGFs in the organism, the significance of this storage pool is still under debate. Experimental evidence suggests three possible functions of IGF carrier proteins: (*i*) protection of the organism against acute insulin-like effects of the large quantities of IGFs in blood by decreased availability to tissue receptors (4–8), (*ii*) prolongation of the half-life of IGFs in the circulation (6, 9), and (*iii*) potentiation of the growth-promoting effects of IGFs (10).

Native serum from normal rats contains at least two IGF carrier protein complexes: upon neutral gel filtration on Sephadex G-200 one of them elutes with an apparent molecular mass of 150–200 kDa, the other with 40–50 kDa [in this paper termed 200- and 40-kDa complexes, respectively, according to our Sephadex G-200 elution profiles (see Fig. 1)]. The 200-kDa complex carries most of the endogenous rat IGF (11) and has been shown to be growth hormone (GH)-dependent (12–15): hypophysectomized (hypox) and diabetic rats, both of which are GH deficient and have low IGF I serum levels, lack the 200-kDa complex. It reappears after GH or insulin treatment, respectively, together with the rise of endogenous IGF. On the basis of these findings and in the context of the somatomedin hypothesis (16), the question arises whether or not these effects of GH or insulin are mediated by IGF I—i.e., whether IGF I treatment of hypox or diabetic rats induces IGF carrier protein.

MATERIALS AND METHODS

Animals. Hypophysectomy was carried out in 5- to 6-week-old male Tif RAI rats (courtesy of K. Müller and M. Cortesi, CIBA-Geigy, Basel). Only those rats whose body weight did not increase more than 2 g per week were used for the experiments. Two weeks after hypophysectomy, Alzet mini osmotic pumps (model 2001 or 2002; Alza) were implanted subcutaneously at the abdomen. Recombinant human (rh) GH [Nordisk, Gentofte, Denmark; 200 milliunits (mU) (= 67 μg)/day], dissolved in 0.9% NaCl, or rhIGF I (generous gift of J. Nüesch, CIBA-Geigy, Basel, and W. Rutter, Chiron; 200–300 μg/day), dissolved in 0.9% NaCl/0.1 M acetic acid, was infused for 8 hr to 8 days. Hypox control rats received 0.9% NaCl/0.1 M acetic acid.

Rats were anesthetized with Innovar Vet (Pitman Moore, Washington Crossing, NJ), 0.2 ml/100 g of body weight, and bled by aortic puncture. Blood was kept on ice for 30 min and centrifuged for 15 min at 1000 × g and 4°C. Serum was stored in 1-ml samples at −20°C until used for determination of IGF I or for IGF carrier protein analysis.

Sera from untreated diabetic rats (Tif RAI) and from diabetic rats treated with insulin or rhIGF I were from the experiments of Scheiwiller *et al.* (17). Growth and metabolic indices in these animals have been reported (17, 18).

Sephadex Gel Filtration Experiments. *Radiochromatographic patterns of sera.* A 0.2-ml serum sample was diluted with 0.3 ml of Dulbecco's phosphate-buffered saline [pH 7.4, containing 60 μg of NaN_3 and 500 U of Trasylol (Bayer)] and incubated for 24 hr at 4°C with ^{125}I-labeled IGF II (3–4 × 10^5 cpm, 1.5–2 ng). The mixture was gel-filtered through a Sephadex G-200 column (2 × 70 cm) and the fractions were collected and their radioactivities were measured in a γ counter. To assess nonspecific binding, some of the incubations were carried out in the presence of 5 μg of unlabeled IGF (partially purified preparation, containing 2.5 μg each of immunoreactive IGF I and II per 300 μg of protein; kindly supplied by R. E. Humbel, Biochemisches Institut, Zurich). Radiochromatographic patterns of rat sera are qualitatively similar but less prominent when ^{125}I-IGF I is used instead of ^{125}I-IGF II. However, this procedure does not allow quantitation of the carrier protein because it measures only free or exchangeable IGF binding sites.

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Abbreviations: IGF, insulin-like growth factor; GH, growth hormone; rh-, recombinant human; hypox, hypophysectomized; U, unit.

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Fractionation of sera on Sephadex G-200. One-milliliter samples of pooled sera were run on a 2 × 70 cm column and eluted with Dulbecco's phosphate-buffered saline, pH 7.4, containing 0.02% NaN_3. Fractions corresponding to the peak regions of the radiochromatographic pattern of normal rat serum were pooled (apparent molecular mass ranges: pool I, 120–280 kDa; pool II, 60–120 kDa; pool III, 25–60 kDa), dialyzed against 0.1 M NH_4HCO_3, lyophilized, and dissolved in 1 ml (pool I and II) or 0.5 ml (pool III) of distilled water for "Western blots" and for determination of IGF I by radioimmunoassay (19).

Fractionation of Sera on Concanavalin A (Con A)-Sepharose. Con A-Sepharose (Pharmacia) was packed in 2-ml plastic syringes, forming columns with a bed volume of 1 ml. The columns were rinsed with 10 ml of starting buffer (0.05 M sodium phosphate buffer, pH 7.4, containing 0.2 M NaCl and 0.02% NaN_3). Serum or serum fractions (0.1 ml) diluted with 0.1 ml of starting buffer were eluted at 6 ml/hr with 4 ml of starting buffer (nonadsorbed fraction), followed by 4 ml of 0.25 M methyl α-D-mannopyranoside (Sigma) in starting buffer (adsorbed fraction). The two fractions were dialyzed against 0.1 M NH_4HCO_3, lyophilized, and taken up in 100 μl of distilled water for "Western blot" analysis.

Analysis of IGF Carrier Proteins by Ligand Blotting ("Western Blot"). The method described in detail by Hossenlopp *et al.* (20) was used with slight modifications. Five microliters of serum or 10 μl of the pooled serum fractions was added to 10 or 20 μl, respectively, of 0.1 M Tris·HCl buffer, pH 6.8, containing 4% SDS, 0.02 M NaEDTA, and 24% (vol/vol) glycerol and electrophoresed for 5 hr at 170 mV on SDS/15% polyacrylamide slab gels (15 × 15 × 0.15 cm) under nonreducing conditions (except the ^{14}C-labeled molecular weight markers: Rainbow Marker, Amersham). Electroblotting on nitrocellulose was performed in a Transblot cell (Bio-Rad) for 2 hr at 0.6–1.0 A under cooling with running tap water.

The air-dried nitrocellulose sheets were soaked for 30 min at 4°C in "saline" [0.15 M NaCl containing 0.01 M Tris·HCl, pH 7.4, NaN_3 at 0.5 g/liter, and 3% Nonidet P-40 (Sigma)], incubated overnight at 4°C in "saline"/1% human serum albumin (Fluka), and finally incubated for 10 min in "saline"/0.1% Tween 20 (Serva). Then each sheet was incubated at room temperature for 6 hr in a sealed plastic bag with 18 ml of "saline" containing 1% human serum albumin/0.1% Tween 20 and 4 × 10^6 cpm of ^{125}I-labeled IGF II. After two washings for 15 min in "saline"/0.1% Tween and then three washings for 15 min in "saline" the membranes were air-dried and exposed for 1–3 days at −70°C to an x-ray film (Kodak, X-Omat AR) in a Kodak X-Omatic cassette.

Digestion of Samples with N-Glycanase. Samples (20–30 μg of protein) were incubated for 30 min at room temperature in 19 μl of 0.2 M sodium phosphate buffer, pH 8.6, containing 16% glycerol and 0.79 mM EDTA with or without 1.5 U of N-Glycanase (Genzyme). Samples were electrophoresed and blotted as described above.

RESULTS

Composition of IGF Carrier Complexes in Normal Rat Serum. When normal rat serum preequilibrated with ^{125}I-IGF II is gel-filtered over Sephadex G-200 at neutral pH the specific IGF–carrier protein complexes elute as radioactive peaks at apparent molecular masses of 200 kDa (peak I), 90–100 kDa (peak II), and 40 kDa (peak III). Preincubation in the presence of excess unlabeled IGF results in the displacement of these peaks and an increase of nonbound tracer (Fig. 1 *Upper*). The typical "Western blot" pattern of normal rat serum (Fig. 1 *Lower Left*) is similar to that described by Hossenlopp *et al.* (21): We find a main triplet of specific IGF-binding bands at apparent molecular masses of 42, 45, and 49 kDa, a band around 32 kDa (sometimes with an additional band at 29 kDa), and one at 24 kDa. The most intensely labeled band is the 45-kDa band of the triplet. This pattern is identical with ^{125}I-IGF II or ^{125}I-IGF I, except that the 32-kDa band appears less intense with ^{125}I-IGF I. All these bands disappear when incubation with ^{125}I-IGF of the nitrocellulose is performed in the presence of excess unlabeled IGF (not shown).

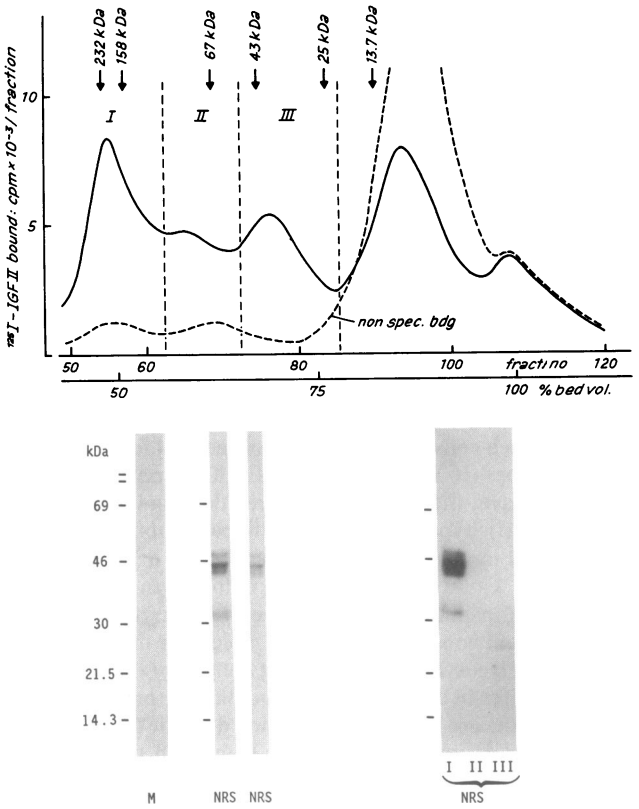

FIG. 1. (*Upper*) Radiochromatographic pattern of normal rat serum (NRS) in the presence (broken line) and absence (solid line) of 5 μg of unlabeled IGF. (*Lower*) "Western blot" analysis of whole serum (*Left*) and of pooled serum fractions I, II, and III obtained by neutral gel filtration on Sephadex G-200 (*Right*). Five microliters of serum or 10 μl of the corresponding pools processed as described in the text were used for "Western blot" analysis. Arrows with numbers in *Upper* represent molecular mass markers, as does lane M in *Lower*. The two NRS lanes represent the same "Western blot" after 56- (left lane) and 20-hr (right lane) exposure to the x-ray film. Triplet bands are more distinctly resolved after 20 hr; the 32-kDa band becomes more prominent after 56 hr.

To assign the bands of the Western blot to the native binding peaks of the radiochromatographic pattern normal rat serum was gel-filtered on Sephadex G-200, and fractions corresponding to the three specific IGF-binding regions (pools I, II, and III) were pooled and analyzed by "Western blot" (Fig. 1 *Lower Right*). Pool I, which contains the native 200-kDa carrier complex, gave a "Western blot" pattern similar to that of whole serum. Pools II and III gave only faint bands at 45 and 24 kDa, respectively. It thus appears that the native 200-kDa carrier complex consists of subunits represented by the 42- to 49-kDa triplet and the 32-kDa band. The native 40-kDa carrier protein (peak III, *Upper*) contains the 24-kDa band as the main constituent.

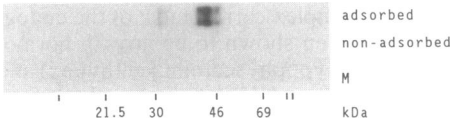

FIG. 2. "Western blot" analysis of the Con A nonadsorbed (10 μl) and adsorbed (10 μl) fraction of pool I from normal rat serum. M, molecular mass marker.

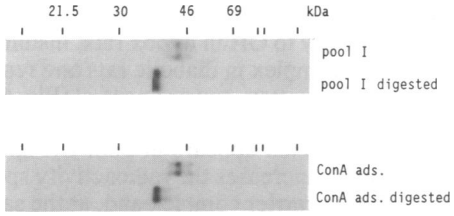

FIG. 3. "Western blots" of pool I (*Upper*) and of the Con A-adsorbed fraction of whole rat serum (*Lower*) before and after N-Glycanase digestion. Aliquots (10 μl) were lyophilized, incubated with or without N-Glycanase, and analyzed by "Western blotting."

Further evidence that the 32-kDa band is a component of or stems from the 200-kDa complex was obtained by Con A-Sepharose chromatography of pool I and subsequent "Western blot" analysis of the nonadsorbed and adsorbed fractions (Fig. 2). The fraction that is bound by Con A and eluted with methyl α-D-mannose contains the 42- to 49-kDa triplet and the 32-kDa band. This suggests that the native 200-kDa carrier complex (*i*) is glycosylated and (*ii*) dissociates into subunits of 32, 42, 45, and 49 kDa during SDS/PAGE.

When pool I or the Con A-adsorbed serum fraction is digested with N-Glycanase prior to "Western blot" analysis the triplet disappears, giving rise to a single band of lower molecular mass (37 kDa) (Fig. 3). These results indicate that the triplet may represent different degrees of N-glycosylation of one and the same protein.

Effects of GH and rhIGF I Treatment on IGF Carrier Proteins of Hypox Rats. The radiochromatographic pattern of hypox rat serum (Fig. 4 *Upper*) shows that the native 200-kDa carrier complex is missing, whereas the 40-kDa peak is significantly increased. GH induces the 200-kDa and, at the same time, reduces the 40-kDa peak without completely restoring the normal binding pattern. In agreement with these patterns, the triplet representative of the 200-kDa carrier complex is lacking in the "Western blots" of hypox rat serum and reappears under GH treatment (Fig. 4 *Lower Left*). Surprisingly, the triplet also appears in serum from IGF I-treated hypox rats, although the radiochromatographic pattern does not differ from that of saline-treated hypox controls. In contrast to GH treatment, the 49-kDa band of the triplet gives the strongest signal.

When "Western blot" analysis is carried out separately in pools I, II, and III (Fig. 4 *Lower Right*) the following differences between the three treatment groups appear: the triplet is not found in any of the three serum pools from untreated hypox rats. Only pool III contains significant IGF-binding bands, a single band at 24 kDa and a double band at 33/34 kDa. After GH treatment, the 42- and 45-kDa bands of the triplet together with

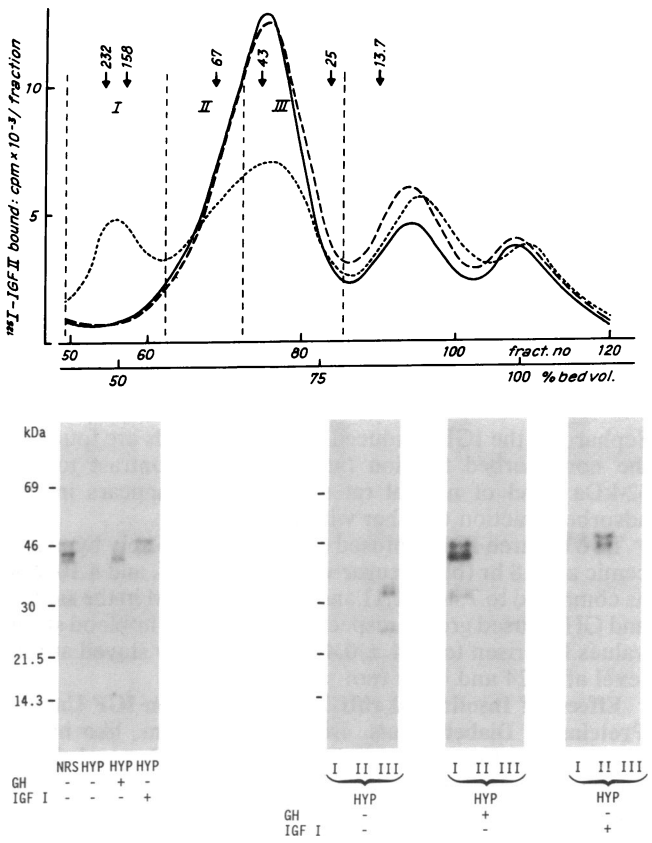

FIG. 4. (*Upper*) Radiochromatographic Sephadex G-200 patterns of sera from hypox rats treated with saline (——), rhGH (200 mU/day for 8 days; ---), or rhIGF I (200 μg/day for 6 days; ———). (*Lower*) "Western blots" of corresponding whole sera (*Left*) and of pools I–III from these sera (*Right*). Methodological details are given in the text. NRS, normal rat serum; HYP, hypox. Sera were pooled from three to five rats per treatment group. Arrows with numbers in *Upper* represent molecular mass markers.

the 32-kDa band appear in pool I. After IGF I infusion, however, the triplet appears nearly exclusively in pool II. Correspondingly, the major portion of the GH-induced endogenous IGF I is found in pool I, whereas the major portion of the infused rhIGF I is detected in pool II (Table 1).

The time course of the induction of the triplet by IGF I and GH is shown in Fig. 5: The triplet is clearly induced after 16 hr of IGF I or GH infusion. After 8 hr, rhIGF I induces a strong double band at 33/34 kDa which is not observed after 8 hr of GH infusion. When chromatographed on Con A-

Table 1. Distribution of immunoreactive IGF I among pools I, II, and III of sera from untreated and treated rats

Treatment group	Pool I (120–280 kDa)		Pool II (60–120 kDa)		Pool III (25–60 kDa)	
	ng equiv/ml	%	ng equiv/ml	%	ng equiv/ml	%
Normal untreated	96	56	38	22	37	22
Hypox saline-treated	6	11	13	24	35	65
Hypox + rhGH (200 mU/day)	50	44	37	32	28	24
Hypox + rhIGF I (200 μg/day)	75	24	202	65	34	11
Diabetic saline-treated	16	36	22	49	7	15
Diabetic + insulin (2.5 U/day)	121	61	52	27	24	12
Diabetic + rhIGF I (300 μg/day)	105	36	136	47	51	17

Rats were treated as described in legends of Figs. 4 and 6. Pools I–III of the sera were obtained after Sephadex G-200 gel filtration. Immunoreactive IGF I was determined (19) using rabbit antiserum against human IGF I (gift from the late K. Reber, Hoffmann–La Roche, Basel) at a final dilution of 1:20,000 and pure native human IGF I (kindly supplied by R. E. Humbel) as a standard. Aliquots (0.25 ml) were acid treated and run on Sep-Pak C_{18} cartridges (Waters) according to the protocol supplied by Immunonuclear (Stillwater, MN). After reconstitution with 1 ml of phosphate-buffered saline/0.2% human serum albumin, pH 7.4, all samples were assayed at three different dilutions. Values of endogenous rat IGF I are expressed in human ng equivalents/ml of serum. It is important to note that 1 human ng equivalent of endogenous rat IGF I equals about 4 ng in our RIA (J.Z., unpublished data).

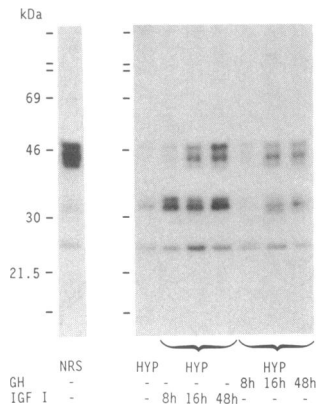

FIG. 5. Time course of the induction of IGF carrier protein in hypox rats as analyzed by "Western blotting." Hypox (HYP) rats were infused for 8, 16, and 48 hr with rhIGF I (300 μg/day) or rhGH (200 mU/day).

Sepharose, the IGF I-induced 33/34-kDa bands are found in the nonadsorbed fraction (not shown) in contrast to the 32-kDa band of normal rat serum, which appears in the adsorbed fraction together with the triplet.

Two of three IGF I-infused rats became severely hypoglycemic after 8 hr (blood sugar values 1.28, 2.05, and 4.10 mM as compared to 7.44 ± 1.11 and 7.44 ± 1.39 mM in the saline- and GH-infused group, respectively). After 16 hr blood sugar values had risen to 4.94 ± 0.44 mM, and they stayed at this level after 24 and 48 hr (not shown).

Effects of Insulin and rhIGF I Treatment on IGF Carrier Proteins of Diabetic Rats. Diabetic rat serum, like hypox serum, lacks the native 200-kDa carrier complex and shows an increase of the small molecular weight complex. The latter, however, does not elute at an apparent molecular mass of 40 kDa as in normal or hypox rats, but at 55 kDa (Fig. 6

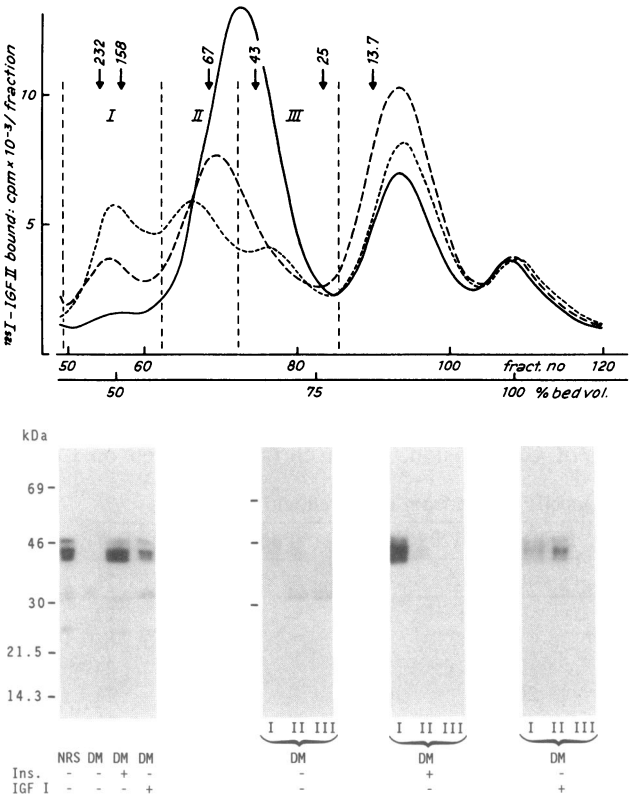

FIG. 6. (*Upper*) Radiochromatographic patterns of sera from diabetic rats infused for 6 days with saline (——), insulin at 2.5 U/day (---), or rhIGF I at 300 μg/day (———). (*Lower*) "Western blots" of the corresponding whole sera (*Left*) and of pools I–III from these sera (*Right*). NRS, normal rat serum; DM, diabetes mellitus. Sera were pooled from three to five rats per treatment group. Arrows with numbers in *Upper* represent molecular mass markers.

Upper). In the "Western blot" the triplet is lacking (Fig. 6 *Lower Left*). In analogy to GH in hypox rats, insulin induces the 200-kDa carrier complex in diabetic rats and restores the radiochromatographic pattern toward normal (Fig. 6 *Upper*). As expected, insulin also induces the Western blot triplet (Fig. 6 *Lower Left*) which appears in pool I (Fig. 6 *Lower Right*). IGF I infusion increases the radioactivity specifically bound to the 200-kDa carrier complex and, at the same time, reduces and shifts (65 kDa) the small molecular weight binding peak. "Western blot" analysis shows that IGF I induces the triplet (Fig. 6 *Lower Left*). Consistent with the reappearance of the 200-kDa complex in the radiochromatographic pattern, and in contrast to IGF I-infused hypox rats, the triplet appears in both pools I and II with similar intensity (Fig. 6 *Lower Right*).

DISCUSSION

N-Glycosylated Forms of One and the Same Protein Participate in the Formation of the 200-kDa IGF Carrier Protein. The major portion of the IGF-binding activity in normal rat serum elutes in the gamma globulin region upon neutral Sephadex gel filtration, elutes in the albumin region after acid treatment, and is decreased in hypox rats and restored by GH treatment (12–15). This activity resides in a glycosylated protein complex, as also shown by Baxter and Martin (22). On "Western blot" analysis it yields four subunits with apparent molecular masses of 32, 42, 45, and 49 kDa. Furthermore, N-Glycanase digestion converts the 42- to 49-kDa triplet into a single band with an apparent molecular mass of 37 kDa, suggesting that the triplet represents the same protein with different degrees of N-glycosylation. This is also consistent with our amino acid sequence data of the purified 42- and 45-kDa subunits, which give the same amino acid composition and a unique sequence of the first 31 amino acids (23). Similarly, Baxter and Martin (22), who purified IGF carrier protein from adult rat serum, found two components with apparent molecular masses of 50 and 56 kDa (nonreduced) and a unique sequence of the first 15 N-terminal amino acids, which are identical to those of our 42/45-kDa subunits. Interestingly, the purified 32-kDa protein that we find as a "constituent" of the native 200-kDa complex reveals the same 31 N-terminal amino acids as our 42/45-kDa proteins (23). It is also digestible with N-Glycanase, yielding a band at 28 kDa (23). Possibly, it results from C-terminal proteolytic cleavage of one of the 42- to 49-kDa subunits.

The "Western Blot" Triplet: Correlation with the IGF I Status. The lack of the 42- to 49-kDa triplet in the "Western blots" of hypox and diabetic rat sera coincides with the lack of the native large carrier complex in the gel filtration experiments and reflects a low IGF I state. This is also true for fasted rats, who have low IGF I levels and whose serum shows a reduction of the 200-kDa complex and of the triplet signal (J.Z., unpublished data). On the other hand, the restoration of the IGF I in the serum, which leads to resumption of growth in hypox and diabetic (17, 24, 25) as well as in refed rats, is associated with the reappearance of the glycosylated 42- to 49-kDa subunits. In this context, it is interesting to note that the major portion of the endogenously induced IGF I or of the infused exogenous rhIGF I is associated with the serum fraction that exhibits the strongest triplet signal (Figs. 1, 4, and 6, and Table 1). A correlation between the "Western blot" triplet and the IGF I status is also observed in rat osteoblastic cells *in vitro* (26).

In view of the nearly identical radiochromatographic patterns of sera from untreated and IGF I-infused hypox rats and the conspicuous difference between the patterns of IGF I- and GH-treated hypox rats, it was unexpected that IGF I, like GH, induced the "Western blot" triplet. This apparent discrepancy may result from the complex binding reaction of tracer in whole serum. The amount of tracer that binds to the

native carrier proteins depends not only on their concentrations but also on their degree of saturation with IGF and on the exchangeability of bound ligand. To explain why the predominant "Western blot" triplet in pool II of the IGF I-infused hypox rats is not reflected by a corresponding binding peak in the radiochromatographic pattern one has to assume that the IGF I-induced subunits in pool II are saturated with IGF I that is not readily exchangeable. This assumption is supported by the finding that the majority of the circulating infused IGF I is bound to carrier protein in pool II. Thus, binding of tracer to the newly induced carrier protein would be inhibited. On the other hand, the GH-induced 200-kDa complex in pool I appears to be only partly saturated with endogenous IGF I. Therefore, tracer can bind and give rise to a new radioactive peak.

In treated and untreated diabetic rats the "Western blot" patterns are more consistent with the radiochromatographic serum patterns. In contrast to IGF I infusion in hypox rats, exogenous IGF I, like insulin, leads to the induction of the 200-kDa carrier complex. This is reflected by the distinct reappearance of the 42- to 49-kDa triplet in pool I. The triplet also appears in pool II with similar intensity. These findings are consistent with the nearly equal distribution of the circulating infused IGF I between pools I and II (Table 1).

GH-Dependent Formation of the 200-kDa Complex and IGF Targeting. The finding that the IGF I-induced carrier protein subunits are able to form the 200-kDa complex in diabetic, but not in hypox, rats suggests that GH may be necessary to accomplish formation of this complex. Although GH secretion has been reported to be largely suppressed in diabetic rats (27), residual secretion might still be sufficient to account for a substantial formation of the large carrier complex under IGF I infusion. Another possibility, however, is that treatment with IGF I, like insulin (27), partly restores GH secretion in diabetic rats. Although it has been reported that intraventricular somatomedin C/IGF I infusion suppresses GH secretion in normal rats (28), the contrary might be true in the diabetic situation due to an insulin-like effect of IGF I.

The following findings suggest that IGF I mediates the effects of GH on the synthesis of IGF carrier protein subunits: (*i*) IGF I infusion clearly induces the subunits of the native 200-kDa carrier complex in the absence of GH. (*ii*) Serum IGF I levels attained during rhIGF I infusion are in the same range as endogenous rat IGF I levels induced by GH (Table 1). Beyond that and equally important, GH itself appears to accomplish what IGF I initiates. What might be responsible for the formation of the native 200-kDa carrier complex from the IGF I-induced glycosylated subunits? It has been reported (29) that an acid-labile protein which itself does not bind IGF participates in the formation of the 200-kDa carrier complex. Recent evidence for the existence of such an acid-labile component has been provided by Baxter (30). It is possible that the synthesis of this acid-labile component is critically dependent on GH but is not induced by IGF I. This difference may be physiologically relevant. We have recently observed that IGF I infusion into hypox rats for 6–18 days has a considerably greater effect on the weights of the spleen, the thymus, and the kidneys than has GH, whereas IGF I, in contrast to GH, does not increase muscle weight (25). These differential effects of IGF I and GH might well be explained by the different molecular weight species of carrier complexes which may serve the function of tissue-specific targeting of IGF I, dependent on the GH secretory activity of the pituitary. Although such an explanation would primarily be compatible with an endocrine route of action of IGF I, data from cell culture experiments also point to a possible role of carrier proteins in the paracrine function of IGF I (10).

Finally, the induction of the glycosylated subunits of the 200-kDa carrier complex may be an important means by which the organism protects itself against the hypoglycemic effects of IGF I (1, 6) and directs the potential of IGF I toward tissues concerned with growth. Although blood sugar values after 6 or 18 days of IGF I infusion in hypox rats are not different from those of saline-infused hypox controls (14, 25), we have observed that a number of hypox rats infused with doses of rhIGF I 300 μg/day or higher have died during the first hours of the infusion. This has never happened under saline or GH treatment. Indeed, blood sugar values during the infusion experiment described in Fig. 5 provide a plausible explanation for this observation: After 8 hr of rhIGF I infusion severe hypoglycemia occurred in two of three animals. With the appearance of the "Western blot" triplet after 16 hr blood sugar levels were almost normal throughout the rest of the infusion period.

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