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Expression of the Insulin-Like Growth Factor-II/ Mannose-6-Phosphate Receptor in Multiple Human Tissues during Fetal Life and Early Infancy*

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ABSTRACT

The insulin like growth factor-II/mannose-6-phosphate (IGF-II/M6P) receptor has been detected in many cells and tissues. In the rat, there is a dramatic developmental regulation of IGF-II/M6P receptor expression, the receptor being high in fetal and neonatal tissues and declining thereafter. We have systematically studied the expression of the human IGF-II/M6P receptor protein in tissues from 10 human fetuses and infants (age 23 weeks gestation to 24 months postnatal). We have asked 1) whether there is differential expression among different organs, and 2) whether or not the human IGF-II/M6P receptor is developmentally regulated from 23 weeks gestation to 24 months postnatal. Protein was extracted from human tissues using a buffer containing 2% sodium dodecyl sulfate and 2% Triton X-100. Aliquots of the protein extracts were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting using an anti-IGF-II/M6P receptor antiserum (no. 66416) and ^{125}I-protein A or an immunoperoxidase stain. IGF-II/M6P receptor immunoreactivity was detected in all tissues studied with the highest amount of receptor being expressed in heart, thymus, and kidney and the lowest receptor content being measured in brain and muscle. The receptor content in ovary, testis, lung, and spleen was intermediate. The apparent molecular weight of the IGF-II/M6P receptor (220,000 kilos without reduction of disulfide bonds) varied among the different tissues: in brain the receptor was of lower molecular weight than in other organs. Immunoquantitation experiments employing ^{125}I-protein A and protein extracts from human kidney at different ages revealed a small, albeit not significant, difference of the receptor content between fetal and postnatal tissues: as in other species, larger amounts of receptor seemed to be present in fetal than in postnatal organs. In addition, no significant difference of the receptor content between human fetal liver and early postnatal liver was measured employing ^{125}I-protein A-immunoquantitation in three fetal and five postnatal liver tissue samples. The distribution of IGF-binding protein (IGEBP) species, another abundant and major class of IGF binding principles, was also measured in human fetal and early postnatal lung, liver, kidney, muscle, and brain using Western ligand blotting with ^{125}I-IGF-II: as with IGF-II/M6P receptor immunoreactivity there was differential expression of the different classes of IGFBPs in the various organs. In conclusion, 1) the IGF-II/M6P receptor is present in multiple human tissues, 2) the human IGF-II/M6P receptor is variably expressed in different organs, 3) there is a much lesser degree of developmental regulation of IGF-II/M6P receptor expression in the human than has been reported for the rat or sheep, 4) the differential pattern of distribution of IGF-II/M6P receptor and IGFBPs in the various organs throughout fetal and early postnatal human life points to an important and tissue specific role of the IGF binding principles in development and growth. (*J Clin Endocrinol Metab* **75:** 424–431, 1992)

THE insulin-like growth factor-II (IGF-II) is a polypeptide which belongs to the insulin family of polypeptide hormones that also includes IGF-I and relaxin (1–3). Although the aminoacid sequence and molecular structure of IGF-II have long been characterized (4, 5), its biological action and physiological role *in vivo* are still elusive: 1) it has been suggested that IGF-II plays a role in the growth and development of the central nervous system and of certain tumors (6–9). 2) Alternatively, IGF-II might be involved in fetal growth and development, since both serum IGF-II levels and tissue IGF-II messenger RNA content are high prenatally and decline after birth. Although this is true in mice, rats, and sheep (10–14), in the human, IGF-II levels are lower during prenatal life and do not change dramatically after birth (15–18). 3) Recently, IGF-II has been implicated in modulating the cellular uptake of lysosomal enzymes via the IGF-II/mannose-6-phosphate (M6P) receptor (19–22). It is hypothesized that a growth factor, IGF-II, might thus be a regulator of the major degradative pathway of the cell. IGF-II binds to two high affinity receptors which are present in many cells and tissues. The IGF-I receptor prefers IGF-I over IGF-II and also binds insulin. This receptor resembles the insulin receptor and contains tyrosine kinase activity (1, 2, 23, 24). It is thought that the IGF-I receptor mediates the metabolic and growth promoting action of the IGFs (25–27). The IGF-II receptor binds IGF-II with high affinity, recognizes IGF-I only weakly, and does not bind insulin at all. This receptor does not have intrinsic tyrosine kinase activity (24, 28). Interestingly, the IGF-II receptor has been shown by complementary DNA cloning to be identical to the cation-independent M6P receptor (29–31). These observations were confirmed by biochemical and immunological studies (32–36). The M6P receptor functions to target lysosomal enzymes

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bearing the M6P recognition marker to lysosomes (37–39). In addition, a signaling role for the IGF-II/M6P receptor has been suggested by some authors (40–45). A truncated form of the IGF-II/M6P receptor lacking the cytoplasmic domain of the membrane receptor molecule has been identified in the circulation in many species (46–48). It has been hypothesized that the process of proteolytic cleavage that leads to the production of the serum form of the IGF-II/M6P receptor serves as a major degradative pathway for the receptor (49). Recently, the levels of IGF-II/M6P receptor protein in rat, sheep, and monkey serum and tissue have been measured (46, 47, 50, 51). In this study, we measured by immunoblotting the IGF-II/M6P receptor protein content in solubilized whole tissue extracts from human fetuses and infants ranging in age from 23 weeks before to 24 months after birth. Immunoblots were analyzed to determine if there is differential expression of the IGF-II/M6P receptor among different human tissues and whether or not there exists a developmental regulation of receptor protein expression in the human. Ligand blotting employing ^{125}I-IGF-II as the radioligand was used to compare the organ distribution of IGF-binding protein (IGFBP) species with that of the IGF-II/M6P receptor.

Subjects and Methods

Tissue collection

Tissues (kidney, brain, liver, lung, testis, ovary, spleen, thymus, muscle, heart) were collected from 10 aborted or stillborn fetuses and infants who had died suddenly (sudden infant death syndrome) or by accident. The time interval between death and removal of tissues at the Institutes of Forensic Medicine and Pathology, University of Munich, Munich, Germany, was between 4 and approximately 24 h. Tissues were removed aseptically, diced, and immediately frozen in liquid nitrogen. The study protocol had been approved by the Ethical Committee of the Children's Hospital (University of Munich, Munich, Germany).

Tissue extraction

Approximately 20 mg thawed tissue were placed in 1.5 mL plastic tubes and weighed; 0.4 mL 20 mmol Tris, 2% Triton X-100, pH 7.4, and 0.1 mL Laemmli buffer containing 2% sodium dodecyl sulfate (SDS) (49) was added, and the tissues homogenized for 3 min with a Polytron homogenizer (Polytron PT 10–35, Bachofer, Reutlingen, Germany). The samples were boiled for 5 min and then incubated overnight at 4 C. The protein homogenates were then boiled again for 1 min and centrifuged for 5 min at 10,000 × *g* in a Mikrorapid/K centrifuge (Hettich, Tuttlingen, Germany). No visible pellet was seen after extraction. Aliquots of the supernatants were stored at −20 C until further analysis. The protein content of the tissue extracts was determined using the BCA protein assay (Pierce Chemical Co., Rockford, IL, Cat. no. 23225). Dilutions of BSA were used as protein standards.

Immunoblotting

Aliquots of the tissue extracts amounting to 0.2 mg protein per organ examined were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) under nonreducing conditions using the discontinuous buffer system of Laemmli (52). Prestained molecular weight markers were used as follows: lysozyme, 14,300; β-lactoglobulin, 18,400; carbonic anhydrase, 29,000; ovalbumin, 43,000; BSA, 68,000; phosphorylase B, 97,400; myosin, 200,000 (BRL, Eggenstein, Germany, prestained protein molecular weight standards, Cat. no. 6041LA). The electrophoresed samples were transferred onto nitrocellulose (Schleicher & Schuell, Dassel, Germany, Cat. no. 401180) by electroelution (500 mA; 80V), and stained using 0.1% Fast Green (Merck, Darmstadt, Germany, Cat. no. 4022) in 1% acetic acid. After destaining in 0.01 molar NaOH, immunoblotting was performed using a specific antiserum against the human IGF-II/M6P receptor (no. 66416, a kind gift from Dr. W. Sly, St. Louis, MO) following an earlier described protocol (50). The antiserum was used at a final dilution of 1:2000. An immunoperoxidase staining kit (Vectastain ABC kit, Vector Laboratories, Inc., Burlingame, CA) was used to detect specific protein bands on the nitrocellulose filters employing horse radish peroxidase and an avidin-biotin enhancing step. After drying overnight, the nitrocellulose sheets were photographed.

Immunoquantitation

In order to estimate the variation between samples (organs from different fetuses or infants) and to analyze the variation of the measuring technique (immunoblotting), the relative IGF-II/M6P receptor content of protein extracts from two organs with high/intermediate receptor content (liver and kidney) was quantitated. Immunoblotting was performed using antiserum 66416 and ^{125}I-protein A according to Sklar *et al.* (50). After drying overnight, the nitrocellulose sheets were autoradiographed using Kodak X-AR film (X-Omat AR, no. 150, 1451, Kodak, Rochester, NY) and enhancing screens (Du Pont, New England Nuclear, Boston, MA) at −70 C. The specific receptor bands detected on x-ray films were cut out from the nitrocellulose filters and were counted in a γ-counter (Berthold, LB 2103, Munich, Germany). The results of three independent experiments (three independent extractions from the same organ of three human fetuses (gestational age 23, 35, and 41 weeks) and of four (kidney) and, respectively, five (liver) infants (3, 4, 13, 18, and 24 months postnatal) were analyzed using Student's *t* test. Values for fetal tissue receptor content were compared with those of postnatal tissue receptor content.

Western ligand blotting

Aliquots (200 μg) of the tissue extracts were subjected to SDS-PAGE (12.5% acrylamide-bis) under nonreducing conditions as described by Laemmli (52). Proteins were then transferred onto nitrocellulose filters by electroelution as described for the immunoblotting procedure. Ligand blotting was then carried out essentially as described by Hossenlopp *et al.* (53). In brief, the nitrocellulose filters were blocked in buffer containing 1% BSA, and subsequently incubated with ^{125}I-IGF-II (Amersham, Braunschweig, Germany) diluted in 0.15 mol NaCl, 10 mmol Tris-HCl, 1% BSA, 0.1% Tween-20, pH 7.4 (20 mL/filter/bag) in the presence or absence of 2 μg/mL unlabeled human recombinant IGF-II (a kind gift from Dr. A. Skottner, Stockholm, Sweden) for 12 h at 4 C temperature. The nitrocellulose sheets were then washed extensively in a 0.15 mol NaCl, 10 mmol Tris-HCl, 0.1% Tween-20, pH 7.4 buffer, and finally in a 0.15 mol NaCl, 10 mmol Tris-HCl, pH 7.4 buffer, and dried overnight. Autoradiographs were obtained by exposing the filters to Kodak-X-Omat films (Eastman Kodak) using enhancer screens (DuPont, Newton, CT) at −70 C.

Results

Specificity of immunodetection of IGF-II/M6P receptors in human tissues

When protein extracts from human lung tissues were immunoblotted using anti-IGF-II/M6P receptor antiserum 66416, a single band at approximately 220 kilos mol wt corresponding to the approximate molecular weight of the IGF-II/M6P receptor upon SDS-PAGE was detected on the nitrocellulose sheets and no other band was stained (Fig. 1). When control serum from normal rabbit was used instead of the antiserum no protein band was detected (data not shown). The specific receptor band was found in all lung tissues examined irrespective of the age of the donor: it was

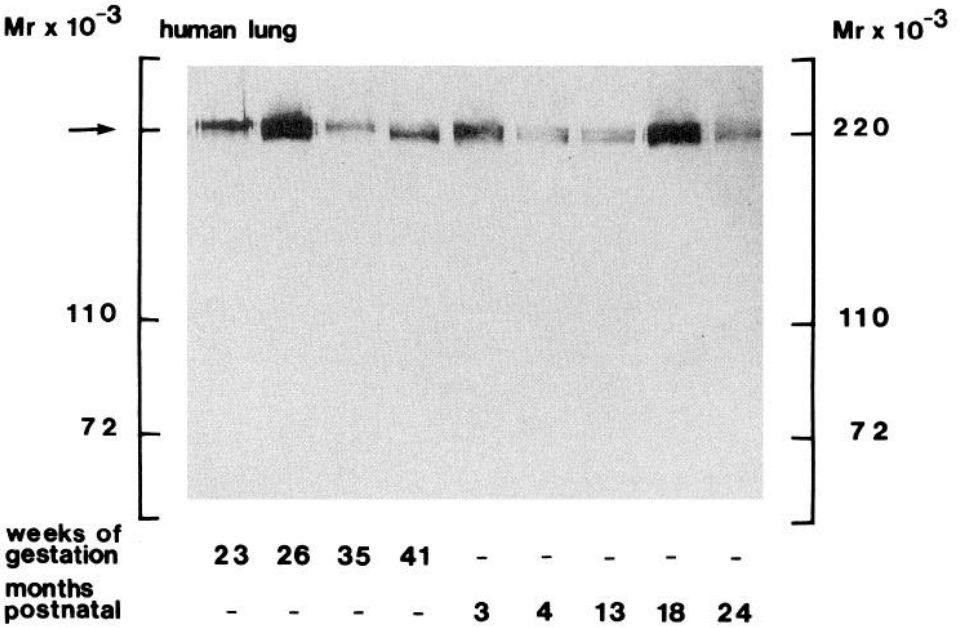

FIG. 1. Immunoblotting of protein extracts from human lung. Approximately 200 μg protein extracts were analyzed by SDS-PAGE. Proteins were transferred by electroelution to nitrocellulose sheets, and the sheets were incubated successively with anti-IGF-II/M6P receptor antiserum and an immunoperoxidase stain as described in *Subjects and Methods*. The *arrow* points to the specific 220-kDa receptor band. Lung protein extracts from four fetuses and five infants were analyzed.

present at 23, 26, 35, and 41 weeks gestational age, and 3, 4, 13, 18, and 24 months postnatal. In contrast, when protein extracts from human kidney were examined by immunoblotting, not only the 220-kilodalton (kDa) receptor band was stained but also protein bands at 140 and at 72, 77, and 82 kDa were detected (Fig. 2A): the latter species were also seen when human kidney proteins were analyzed using control rabbit serum instead of the antireceptor antiserum (Fig. 2B). These proteins were also seen on blots when only the avidin horse radish peroxidase complex was added without prior exposure of the nitrocellulose sheets to any serum and/or biotinylated second antibody (data not shown). In addition, these bands had no peroxidase activity as shown by enzymatic testing using 4-chloro-α-naphtol as staining reagent and H_2O_2 as substrate (data not shown). These results suggest that the immunostaining procedure used in these experiments detects biotinylated proteins which are present in some (kidney, liver, muscle) but not in other (lung, brain) human organs. The anti-IGF-II/M6P receptor antiserum used throughout these experiments, however, is specific for the human IGF-II/M6P receptor as has been described originally (31). As in lung tissue, the IGF-II/M6P receptor band at an approximate molecular weight of 220 kilos was present in kidney tissues at all ages examined: it was seen in kidney extracts from 23, 26, and 41 weeks gestation fetuses and also from infants of 3, 4, 13, 18, and 24 month postnatal age (Fig. 2A).

Immunodetection of IGF-II/M6P receptor protein—variable expression of the receptor in multiple human tissues

When tissue extracts of heart, psoatic muscle, lung, kidney, liver, brain, testis, ovary, spleen, and thymus from 10 human fetuses and infants were analyzed for IGF-II/M6P receptor content, all organs examined contained detectable amounts of receptor protein (Fig. 3, A, B, C). Three representative immunoblots showing the analysis of tissue extracts from one fetus (25 weeks gestation) or infant (3- and 13-month postnatal age) respectively, are shown (Fig. 3, A, B, C). The apparent molecular weight of the receptor band varied among the organs studied: generally, the receptor in brain was of lower molecular weight than the IGF-II/M6P receptor species in the remaining eight tissues. In some organs (thymus, liver, lung, ovary, spleen) doublet bands were occasionally detected (Figs. 3 and 4). The amount of immunoreactivity measured in the different organs varied considerably. However, when the organs were grouped according to their IGF-II/M6P receptor content as estimated from 10 immunoblots, heart, thymus, and kidney consistently contained the highest amount of IGF-II/M6P receptor immunoreactivity whereas brain and muscle expressed the lowest level of IGF-II/M6P receptor immunoreactivity. The receptor content in ovary, testis, liver, lung, and spleen was intermediate (Table 1).

Immunoquantitation of IGF-II/M6P receptor expression during human fetal life and early infancy

The putative developmental regulation of IGF-II/M6P receptor expression was studied by immunoblotting tissue extracts from the same organ of donors of different ages: when protein extracts of lung, liver, kidney, thymus, and heart from human fetuses (23, 26, 35, and 41 weeks gestation) and infants (3, 4, 13, 18, and 24 months of age) were subjected to SDS-PAGE and immunoblotted using the specific anti-IGF-II/M6P receptor antiserum 66416, the receptor protein was again found in all organs and at all ages examined: there was considerable variation of the amount of receptor protein as measured by immunoblotting between ages. However, there was no clear and consistent pattern of variation which would have indicated a developmental regulation of receptor expression in the human between 23 weeks of gestation and 24 months postnatal (Fig. 4). In addition, no sex difference

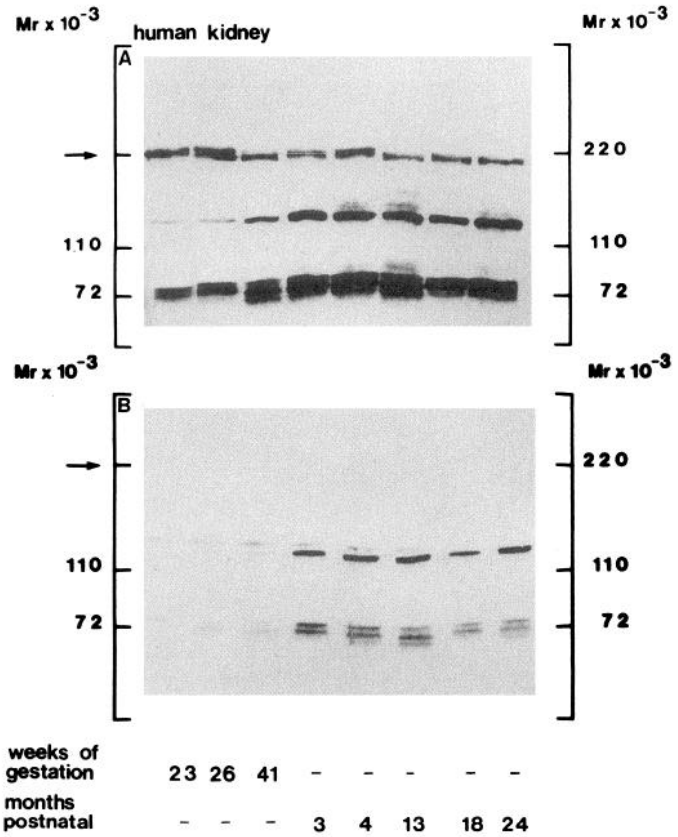

FIG. 2. Immunoblots of protein extracts from human kidney. Immunoblotting was performed as in Fig. 1. A, Anti-IGF-II/M6P receptor antiserum and an immunoperoxidase stain were applied; B, control rabbit serum and the immunoperoxidase stain were used. The *arrow* points to the IGF-II/M6P receptor band. Kidney protein extracts from three fetuses and five infants were analyzed. The nonspecific protein bands presumably represent biotinylated enzymes which are expressed in kidney.

of receptor expression was apparent (data not shown). To quantitate the relative amount of IGF-II/M6P receptor protein in human kidney and liver at different age points further, immunoblotting was performed using ^{125}I-protein A and autoradiography for visualization of receptor species. Specific receptor bands were then cut out from the nitrocellulose sheets and radioactivity counted in a γ-counter. Again, there was considerable variation in receptor content of the same sample between experiments. More importantly, receptor content of different samples also varied greatly. In kidney, receptor content was apparently higher in samples that were obtained from fetuses than in postnatal samples. However, when statistical analysis of relative receptor content at different ages was performed, comparing three prenatal samples with four postnatal samples, no statistically significant difference was found (Table 2). In addition, no significant difference of IGF-II/M6P receptor content between three fetal and five postnatal liver samples was detected (Table 2).

Detection of tissue-specific expression of IGFBPs in multiple human tissues by Western ligand blotting with ^{125}IGF-II

To compare the variable expression of the IGF-II/M6P receptor protein in different tissues with the presence of

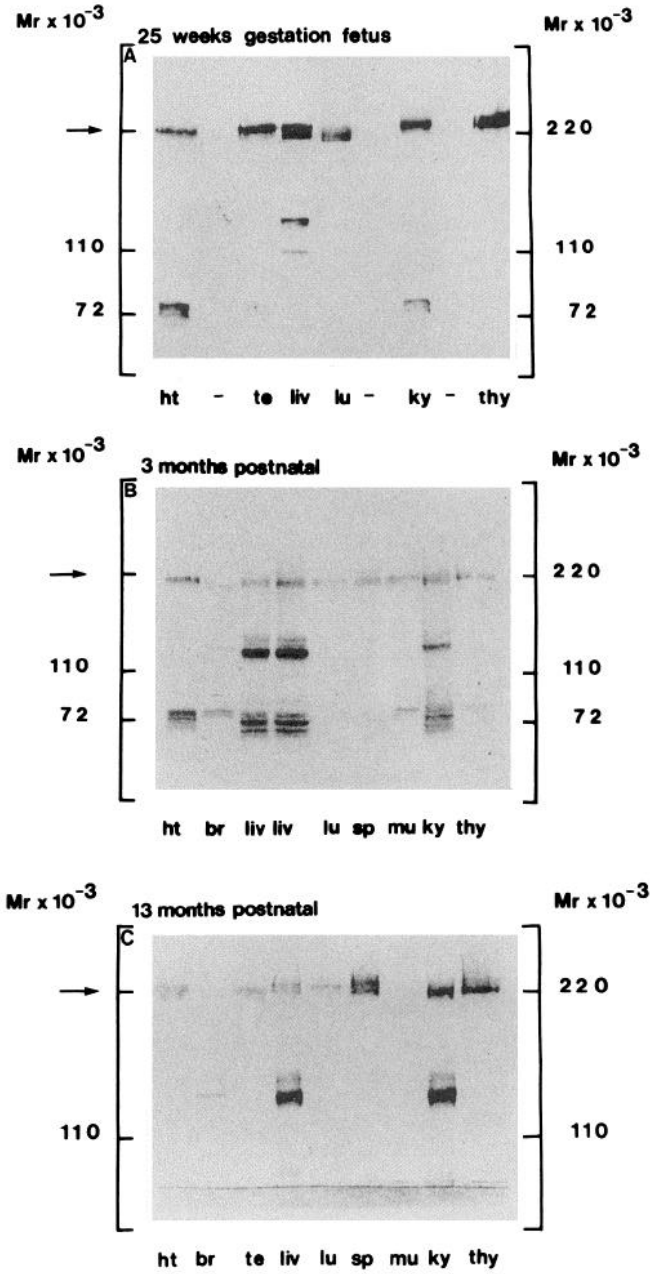

FIG. 3. Immunoblots from tissue extracts (same age, different tissues). Immunoblotting was performed as described in Fig. 1. A, 25 weeks gestation; B, 3 months postnatal; C, 13 months postnatal.

multiple IGFBP species in the same organs, protein extracts from five different human tissues (psoatic muscle, brain, liver, lung, and kidney) were analyzed by SDS-PAGE and ligand blotting using ^{125}I-IGF-II. Five distinct radiolabeled protein species with an approximate molecular weight of 43, 38, 34, 30, and 20 kilos, respectively, were detected (Fig. 5). The intensity of the radiolabeled bands varied among the tissues studied: in brain, the 34-kDa band was predominant, while in psoatic muscle, the 30-kDa species was the major band as seen in seven ligand blotting experiments using tissue samples from eight fetuses and infants. The apparent

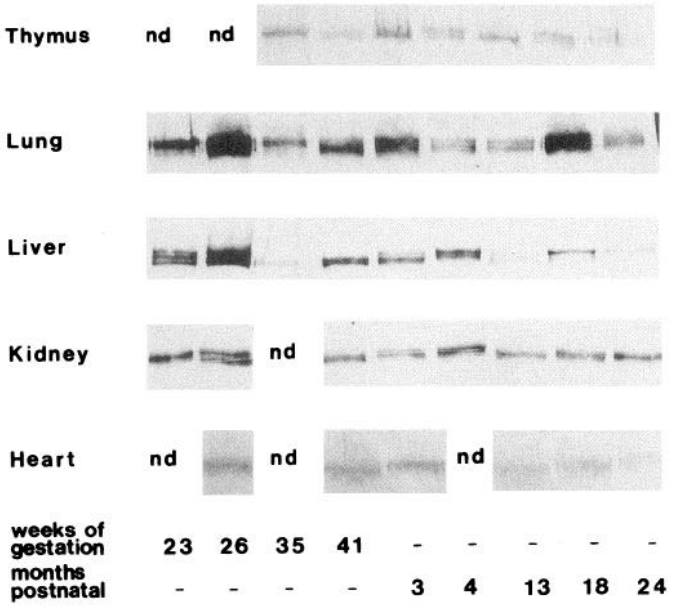

FIG. 4. Immunoblots from tissue extracts (different age, same tissue). Immunoblotting was performed as described in *Subjects and Methods*. Protein extracts (200 μg) were applied to each lane. The 220-kDa receptor bands from each organ are shown in a representative manner for four fetuses and five infants.

TABLE 1. IGF-II/M6P receptor content of human tissues (10 subjects, 23 weeks gestation to 24 months postnatal)

	Low	Intermediate	High
Organs with low receptor content			
Brain	n = 5	n = 3	0
Psoatic muscle	n = 6	n = 1	0
Organs with intermediate receptor content			
Ovary/testis	0	n = 8	0
Liver	n = 1	n = 6	n = 3
Lung	n = 3	n = 5	0
Spleen	n = 1	n = 5	n = 2
Organs with high receptor content			
Heart	n = 1	n = 3	n = 4
Kidney	n = 1	n = 2	n = 7
Thymus	n = 1	n = 1	n = 8

molecular weight of the IGFBP species found in human tissues by ligand blotting experiments corresponds well with the molecular weight of IGFBP species commonly detected in human plasma and serum. The tissue distribution of the IGFBPs cannot be explained by differential degradation in the various organs nor by contamination of the tissues by blood. A more detailed analysis of the expression of IGFBP species in human tissues has been carried out (Funk, B., U. Kessler, W. Kiess, manuscript submitted).

Discussion

The IGF-II/M6P-receptor is present in many tissues in rats, mice, and sheep (24, 47, 50, 54). The IGF-II/M6P receptor has also been found on many cells including cell lines from human origin (24, 37) and in human brain, liver, and placenta (1, 2, 54). In this study, we have measured the receptor in solubilized whole tissue extracts from human fetuses and infants ranging in age from 23 weeks gestation to 2 yr postnatal. IGF-II/M6P receptor immunoreactivity was present in all tissues examined (brain, kidney, liver, lung, heart, testis, ovary, thymus, and spleen). As in the rat (50), brain contained the lowest concentration of IGF-II/M6P receptor protein, and heart and kidney consistently contained large amounts of receptor immunoreactivity. In addition, thymus also contained higher amounts of the receptor. Muscle, which contained high amounts of receptor protein in the rat, had only low receptor content in the human. The receptor content in ovary, testis, lung, and spleen was intermediate. As reported for the rat (50, 55), the human IGF-II/M6P receptor varied in size. Consistent with the results in the rat, the receptor in brain was smaller in size than that in other tissues. Presumably, this variation in receptor size reflects differential glycosylation of the receptor in the different tissues as has been suggested in the literature (55). Such variation in size also occurs during receptor synthesis in cells in culture (56, 57). Immunoreactive receptor species of lower molecular weight could thus reflect receptor precursors and indicate tissues of high receptor turnover or receptor synthesis. Alternatively, lower molecular weight receptor species could represent degradation products in proteolytic tissues (37, 48).

As with the IGF-II/M6P receptor protein there was also differential expression of IGFBP species in different organs: brain predominantly contained a 34-kDa IGFBP consistent with reports in the literature of the expression of such a specific 34-kDa IGFBP in cerebrospinal fluid (52). The differential expression of IGF-II/M6P receptors and IGFBP species in different human tissues points to specific biological

TABLE 2. Immunoreactivity of IGF-II/M6P receptor in human kidney and liver as measured by immunoblotting and ^{125}I-protein A quantitation

	Gestational age (weeks)			Postnatal age (months)			
	23	35	41	3	4	18	24
Kidney							
	415	505	472	546	253	163	95
	317	202	524	415	47	112	87
	463 ± 108[a]			267 ± 75			
	Mean ± SEM						

	Gestational age (weeks)			Postnatal age (months)				
	26	35	41	3	4	13	18	24
Liver								
	93	266	476	455	374	76	364	37
	74	283	546	535	369	18	382	11
	278 ± 117[a]			262 ± 87				
	Mean ± SEM							

Values are means ± SEM of triplicate measurements of counts per min (^{125}I-protein A per 220 kDa receptor lane).

[a] Receptor content of prenatal tissues does not differ significantly from receptor content of postnatal tissues.

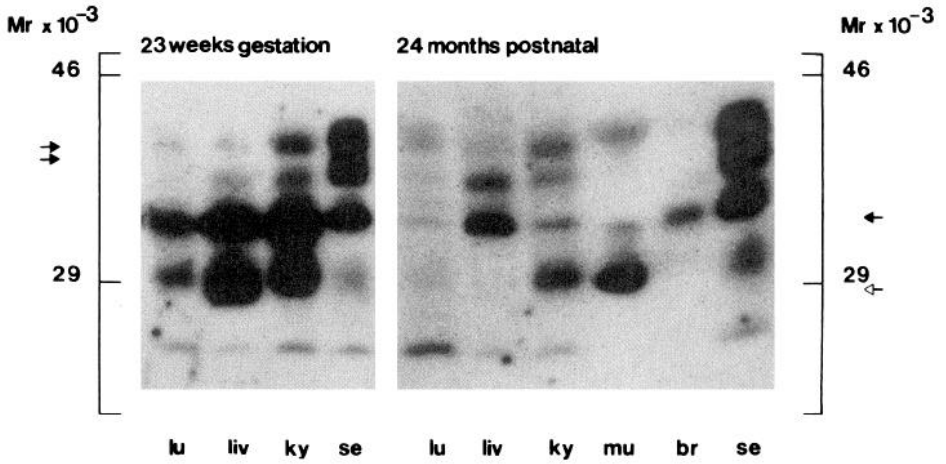

FIG. 5. Analysis of IGFBPs in protein extracts from human tissues. Proteins were extracted and analyzed by SDS-PAGE and ligand-blotting using ^{125}I-IGF-II as described in *Subjects and Methods*. For comparison, 2 μL of human serum were also analyzed on each blot. The *double arrow head* points to the 43-kDa region, presumably representing the IGFBP-3 species, whereas the *open arrow* indicates the position of the 30-kDa IGFBP predominantly expressed in muscle. The 34-kDa IGFBP in brain is indicated by a *filled arrow head*.

roles of the IGF binding moieties in the respective tissues and allows for modulation of IGF mediated biological effects in these organs.

In the rat, a dramatic (up to 100-fold) difference in the amount of IGF-II/M6P receptor between fetal and postnatal serum (46) and tissues (50) has been reported. In sheep and monkey serum, developmental regulation of IGF-II/M6P receptor expression has also been suggested (47, 51). In the human, we have found only a minimal difference of receptor content between prenatal and postnatal kidney and no such difference in liver tissues (between 23 weeks gestation and 24 months postnatal age). It is possible that the failure to detect a significant developmental regulation of receptor expression in the human was due to 1) autolytic processes which might affect different tissues at different ages in a differential manner. However, no signs of degradation (protein smear, *etc.*) appeared on any of the immunoblots, and other nonreceptor related proteins (*i.e.* biotinylated enzymes) expressed a clear developmental pattern (Fig. 2). 2) Alternatively, a more pronounced developmental regulation of IGF-II/M6P receptor expression might occur much earlier (before 23 weeks of gestation) in human life than in embryologically comparable stages of development in the rat. It is interesting to note that whereas in the rat not only the IGF-II/M6P receptor but also the ligand, *i.e.* IGF-II itself, are developmentally regulated, in the human both the amount of IGF-II (1–3) and of the IGF-II/M6P receptor (this study) are barely or only to a small extent regulated during fetal and early postnatal development. 3) Failure to detect a significant difference of receptor content between fetal and postnatal tissues might also be due to the large variation caused by the detection technique (immunoblots) (Table 2). However, when the receptor content of different organs was compared, a clear grouping of tissues with organs of high, intermediate, and low receptor content was evident. If a developmental pattern (fetal *vs.* postnatal) was as pronounced in the human as in other species (rat, sheep) (100-fold difference!), the relatively large variability of the technique and potential intersample variations would still not mask such a large difference.

Since the IGF-II/M6P receptor is present in all tissues examined and at all ages tested we hypothesize that this receptor type is involved in general functions of development and metabolism. The variable amounts of receptor protein present in different organs at different times of development make the IGF-II/M6P receptor a likely candidate of regulatory processes that might be involved in organ development, growth, and tissue remodeling. The receptor would add to the variability of IGF binding capacity in different tissues caused by the differential expression of IGFBP classes in the various organs. In addition, the IGF-II/M6P receptor binds two classes of ligands, namely, a growth factor, IGF-II, and a number of lysosomal enzymes bearing the mannose-6-phosphate recognition marker (29, 30). This latter function might also be of biological importance during development and in different tissues. The fact that the IGF-II/M6P receptor maintains—as demonstrated by receptor-ligand blotting (data not shown)—its function of binding IGF-II in all tissues examined, further points to an interconnected network of tissue degradation and growth processes that meet at the cellular level, *i.e.* at a common receptor site.

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