Developmental Regulation of Insulin-Like Growth Factor II mRNA in Different Rat Tissues*

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Insulin-like growth factor II (IGF-II) is present at high levels in fetal and early neonatal rat plasma, and decreases profoundly following birth. In the present study, the levels of IGF-II RNA in different rat tissues at different ages were determined by hybridization to a rat IGF-II cDNA probe. IGF-II RNA was present in 11 of 13 fetal or neonatal tissues examined: at higher levels in muscle, skin, lung, liver, intestine, and thymus; at lower levels in brain stem, heart, cerebral cortex, kidney, and hypothalamus; and undetectable in spleen and pancreas (although the latter RNA was partially degraded). In each tissue, Northern blot hybridization revealed the presence of six IGF-II RNAs: 6, 4, 3.8, 2.2, 1.7, and 1.2 kilobase pairs, consistent with results previously observed in the BRL-3A rat liver cell line and attributed to alternative RNA processing. Although differences in the relative abundance of these RNAs were observed in different tissues, the same size species occurred in all tissues with the 4-kilobase pair RNA the most abundant species. RNAs from the different tissues were examined at six developmental ages (days 18 and 21 of gestation; days 2, 11, 22, and 75 after birth) by hybridization to slot blots and Northern blots. In lung, thymus, kidney, and brain stem, IGF-II RNA was expressed at higher levels in the fetus than after birth, whereas in muscle, skin, liver, heart, and intestine, the high fetal levels of IGF-II RNA continued through day 11 or day 22 after birth. IGF-II RNA persisted into adulthood in cerebral cortex and hypothalamus. Although the significance of these tissue-specific differences in the developmental regulation of the expression of IGF-II RNA remains to be established, they exhibit intriguing temporal correlations with major maturational events in some tissues such as lung and muscle.

IGF-II gene is expressed in several rat tissues, and that tissue IGF-II may exhibit developmental regulation similar to that seen in serum.

Recently, using a cDNA probe to IGF-II from a rat liver cell line, BRL-3A, we demonstrated that the developmental regulation of serum IGF-II also correlates with the levels of hybridizable IGF-II RNA in the liver (7, 8). The hybridization probe was derived from a 12 S (1.2 kb) fraction of BRL-3A RNA enriched in mRNA that stimulates the translation of prepro-IGF-II in a cell-free translation system. It hybridized to multiple RNA species of 6, 4, 2.2, 1.7, and 1.2 kb in Northern blots of RNA from BRL-3A cells or neonatal rat liver (9). We have shown that the predominant 4-kb IGF-II RNA is stable, cytoplasmic, polyadenylated, and co-linear with the 1.2-kb RNA, and have postulated that the different forms reflect alternative processing of a single primary RNA transcript (9). In the liver, all of the IGF-II RNAs exhibited coordinate developmental regulation: abundant in 2-, 12-, and 15-day liver, and undetectable in 25-, 35-, and 65-day liver, suggesting that a major transition in IGF-II gene expression occurs between days 15 and 25 (9).

In the present study, we examine the hybridization of this rat IGF-II probe to RNA prepared from different fetal and neonatal rat tissues, to determine the presence and size of IGF-II RNA. Tissues were examined at different ages to determine whether the developmental pattern of IGF-II and IGF-II RNA expression observed in serum and liver, respectively, is common to all rat tissues.

EXPERIMENTAL PROCEDURES

Animals—Wistar rats aged 2, 11, 22, and 75 days were killed by cervical dislocation. Brain, liver, thymus, lung, spleen, pancreas, kidney, intestine, skeletal muscle, and abdominal skin were excised and immediately frozen in liquid nitrogen. Timed pregnant rats were killed at day 16 or day 21 of gestation, and the above fetal tissues excised and frozen. Tissues were taken from individual rats of 22 and 75 days and as litter pools from younger rats. The amount of tissue ranged from 20.3 g from fetal animals to 31 g from postnatal rats.

Isolation of Total RNA—RNAs were isolated from rat tissues by a modified guanidine thiocyanate-lithium chloride precipitation procedure (10). Tissue (0.6 g) was disrupted in 7 volumes (4.2 ml) of guanidinium thiocyanate buffer (5 mM guanidinium thiocyanate (Fluka), 10 mM EDTA, 50 mM Tris-HCl, pH 7.4, 8% (v/v) β-mercaptoethanol) in a Polytron homogenizer (3-5 s, medium speed, room temperature) and was then extracted with an equal volume of isomyl alcohol/chloroform (1:24). Five volumes (21 ml) of sterile 4 M LiCl were added to the aqueous phase, mixed, and stored at −20 °C overnight. The mixture was centrifuged at 9000 rpm for 90 min (4 °C). The RNA pellet was resuspended in a one-third volume (8.4 ml) of sterile 3 M LiCl using a brief Polytron homogenization. The RNA precipitate was collected by centrifugation at 9000 rpm for 20 min at 4 °C. This pellet was resuspended in 0.63 volume (5.3 ml) of re suspension buffer (0.5% Triton X-100 in sterile 10 mM Tris-HCl, pH 7.4, 10 mM vanadyl ribonucleoside complex (Bethesda Re-

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† The abbreviations used are: IGF, insulin-like growth factor; rIGF-II, rat insulin-like growth factor II; kb, kilobase pair; MOPS, 4-morpholino propane sulfonic acid.

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search Laboratories) by brief homogenization in a Polytron homogenizer. A one-tenth volume (0.53 ml) of 6 M ammonium acetate was added, and the solution was immediately extracted twice with an equal volume of isooamylic alcohol/chloroform. Sterile 0.5 M EDTA, pH 8.0, was added to the aqueous phase to a final concentration of 0.1 M. After incubation for 15 min at room temperature, the RNA was precipitated by adding 2 volumes of 95% ethanol. The precipitated RNAs were washed with 70% ethanol to remove salt, resuspended in sterile water, and stored at -20 °C until use. All RNAs with the exception of pancreas and 21-day gestation spleen were intact based on the sizes and proportions of ribosomal RNAs on ethidium bromide-stained agarose gels. Similar results were obtained with RNAs isolated from tissues of a second group of rats using the same procedure except that vanadyl ribonucleoside was omitted.

Isolation and Labeling of the Rat IGF-II Probe—The 750-base pair insert from the rat IGF-II cDNA clone (pRIGF-II-1) (7) was isolated by PsI digestion, electrophoresed through a 1.5% agarose gel, and electroeluted. Agarose contamination was removed by Elutip (Schleicher & Schuell) chromatography. The insert (100 ng) was labeled by nick translation to a specific activity of 2-3 × 10^6 cpm/µg using procedures described in the Amersham Corp. nick translation kit and [α-³²P]dCTP and [α-³²P]dGTP (3000 Ci/mmol; Amersham Corp.) (9). The probe was isolated from the reaction mixture by spermine precipitation (5 mM spermine, 100 mM KCl, 10 mM Tris-HCl, pH 7.5, 4 °C, 15 min). After Eppendorf centrifugation (10 min, 4 °C), the DNA was vortexed in 300 mM sodium acetate, 75% ethanol, incubated for 1 h at 4 °C, and collected by Eppendorf centrifugation (15 min, 4 °C).

Hybridization to Slot Blots—Serial 3-fold dilutions of the RNA samples (undiluted (6.7 µg, unless otherwise specified), 1:3, 1:9) were loaded onto GeneScreen (New England Nuclear) using a slot blot apparatus (Schleicher & Schuell) as described previously (9, 11). Hybridizations were performed in 5 X SSPE, 10 X Denhardt's solution, 100 µg/ml sheared, denatured salmon sperm DNA, 50% (v/v) formamide-dextran sulfate solution, and 1% sodium dodecyl sulfate as described previously (9, 11). (1 X SSPE is 180 mM NaCl, 10 mM sodium phosphate buffer, pH 6.8, 1 mM EDTA. 100 X Denhardt's solution is 2 g of polyvinylpyrrolidone, 2 g of Ficoll, and 2 g of bovine serum albumin in 100 ml of solution. Formamide-dextran sulfate solution is prepared by dissolving 10 g of finely ground dextran sulfate in formamide (Fluka) to a final volume of 50 ml and filtering twice through a 0.45 µM Millex filter unit.) After hybridization overnight at 41 °C, the blots were washed twice in 2 X SSPE, 0.2% sodium dodecyl sulfate at room temperature followed by two washes in 0.1 X SSPE at 60 °C.

Northern Blots—RNAs (15 µg) were brought up to a volume of 7 µl with water. To each sample was added 10 µl of formamide and 3 µl of 5 X MOPS buffer (0.2 M MOPS, 0.05 M sodium acetate, 0.01 M EDTA), pH 6.5. The samples were heated at 60 °C for 10 min. As each sample was removed from the water bath, 4 µl of formamide was added and the sample vortexed and placed on ice. Loading solution (5% Ficoll, 0.01% bromphenol blue, 3 µl) was added to each sample. RNAs were electrophoresed on 1.5% agarose gels containing 2.2 M formaldehyde (9, 12) in a horizontal gel apparatus (International Biotechnologies, Inc., New Haven, CT) using a 10 slot comb (17 × 1 mm teeth). Electrophoresis was at 65 V in 1 X MOPS buffer containing 0.5 µg/ml ethidium bromide with recirculation of the buffer and cooling of the gel bed. The gel was photographed against a ruler and then blotted to GeneScreen using a rapid blotting procedure. The gel was soaked twice for 15 min in 1 M ammonium acetate and blotted to GeneScreen (pre-wetted for 15 min in 1 M ammonium acetate) using a stack consisting of three pieces of dry Whatman 3 MM paper, a 1-inch stack of thick blotter paper, a glass plate, and a weight. No buffer reservoir was used. Hybridization conditions were as described for the slot blots, except that the temperature was higher (50 °C).

Densitometry—Autoradiograms were scanned using a LKB 2202 UltraScan laser densitometer controlled by an Apple IIe computer.

RESULTS

Occurrence and Relative Abundance of IGF-II RNA in Different Fetal and Neonatal Rat Tissues

IGF-II RNA was detected in 11 of 13 fetal (16 or 21 days gestation) or neonatal (2 days postpartum) rat tissues after hybridization of slot blots with the rat IGF-II probe (Figs. 1 and 2). High levels were observed in muscle, skin, lung, liver, intestine, and thymus. Lesser concentrations of IGF-II RNA were observed in brain stem, heart, cerebral cortex, kidney, and hypothalamus. No significant hybridization was observed to RNA from spleen and pancreas. Although faint hybridization was seen to slot blots of total RNA from spleen and pancreas of 2-day-old rats, no signal was seen in Northern blot analysis of RNA isolated from these tissues (Fig. 3) even after prolonged autoradiographic exposure (not shown). RNA isolated from 2-day spleen appeared intact by ethidium bromide staining of the agarose gels, but RNAs isolated from 2-day pancreas were partially degraded by this criterion. The pancreatic RNA did, however, give a positive signal when slot blots were hybridized with a rat insulin probe.²

² C. Roberts, unpublished results.
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FIG. 2. Relative abundance of IGF-II RNA in different fetal and neonatal rat tissues. The relative amount of IGF-II RNA present in each fetal or neonatal rat tissue was quantitated by densitometry of the slot blot hybridizations shown in Fig. 1. The mean ± 1 S.D. is shown. Three different RNA concentrations (designated 9x, 3x, and 1x) and two different autoradiographic exposures (3-day and 5-day) were scanned at two absorbance ranges (2.0 and 1.0 absorbance units full scale). The mean and standard error of the measurements, adjusted for the amount of total RNA applied, were normalized to values obtained with liver RNA at the same concentration, exposure, and absorbance setting. Values that were out of the linear range or undetectable on some autoradiographic exposures were excluded from the calculation.

FIG. 3. Distribution of IGF-II RNA species in different fetal or neonatal rat tissues. Northern blots of total RNA isolated from the indicated fetal and neonatal rat tissues were hybridized with the rat IGF-II probe (0.6–1.0 × 10^6 cpm/ml) at 50 °C. Representative autoradiograms are presented to illustrate the different RNA species. The presence of the less intense bands was confirmed by longer exposures (not shown). Autoradiographic exposure time varied from 2 h to 4 days. Muscle, skin, lung, liver, and brain stem RNAs were obtained from 16-day fetuses; thymus, heart, kidney, and hypothalamus from 21-day fetuses; intestine, cerebral cortex, and spleen from newborn rats (2 days postpartum). The following symbols identify the different IGF-II RNA species: solid arrow, 4 kb; open arrow, 1.2 kb; solid circle, 6, 2.2, and 1.7 kb.

Multiple IGF-II RNA Species Are Present in All Positive Rat Tissues

Multiple IGF-II RNA species (6, 4.0, 2.2, 1.7, and 1.2 kb) have been observed in BRL-3A cells and neonatal rat liver, and are thought to arise by alternative processing of a single primary RNA transcript (9). These RNA species and also a 3.8-kb RNA not previously resolved were observed in each of the 11 tissues that gave positive hybridization signals for IGF-II RNA (Fig. 3 and longer autoradiographic exposures (not
Developmental Regulation of IGF-II RNA in Different Tissues

Previous results have suggested that IGF-II and IGF-II RNAs are more abundant in the fetus and early neonate than in the adult rat (2, 5, 8, 9). This appears to be true in serum (2), liver (8, 9), and fibroblasts (5). Preliminary results (13) indicated that this fetal predominance also was true in other tissues. In the following sections, we examine the developmental regulation of IGF-II RNA in detail. In hybridization to slot blots and Northern blots of RNAs obtained at 16 and 21 days of gestation and 2, 11, 22, and 75 days after birth, significant tissue-specific differences were observed in the developmental pattern of IGF-II RNA abundance.

Tissues in Which IGF-II RNA Is Expressed Predominantly before Birth—IGF-II RNA was present at appreciable levels in lung, thymus, and kidney before birth, but decreased markedly after birth (Figs. 4 and 5). In the earliest postnatal sample examined (day 2), IGF-II RNA in lung and thymus was decreased by ~90%, and in kidney by ~70% (Fig. 5). IGF-II RNA was virtually undetectable in these tissues at 11, 22, and 75 days of age.

A similar decrease in IGF-II RNA abundance appeared to occur earlier (between 16 and 21 days of gestation) in the lung than in thymus and kidney (between 21 days of gestation and 2 days after birth). Each of the five IGF-II RNA species showed the same developmental regulation in these tissues. The faint persistence of the 4-kb RNA in 21-day fetal lung and 2-day postnatal kidney reflects the greater abundance of this species.

Tissues in Which IGF-II RNA Continues to Be Expressed at Appreciable Levels after Birth—In muscle, heart, skin, intestine, and liver, IGF-II RNA was present at high concentrations before birth, and remained at high levels for 2 or 11 days after birth, as determined by Northern blot (Fig. 6) and slot blot (Fig. 7) hybridization. IGF-II RNA was virtually undetectable by 11 days in muscle, heart, skin, and intestine and by 22 days in liver, and remained so in 75-day tissues.

Detailed examination of the hybridization results suggests that subtle differences in developmental patterns also occur in this group of tissues. Slot blot hybridization indicated that IGF-II RNA in heart and skin was reduced to ~60% of maximum at 2 days after birth, whereas levels were maximal at 2 days in muscle, intestine, and liver. By 11 days, in heart, skin, muscle, and intestine, IGF-II RNA was decreased by >80%. In liver, ~50% of maximal levels were observed at day 11 but IGF-II RNA became undetectable by day 22.

Results of Northern blot hybridizations were generally consistent with these interpretations. Significant decreases in IGF-II RNA species were seen in 2-day heart. A major transition occurred between day 2 and day 11 in skin and intestine, and between day 11 and day 22 in liver. Once again, no significant differences in the developmental regulation of the species were observed.

For kidney, IGF-II RNA was undetectable in Northern blots at days 11, 22, and 75, but appeared to persist at 20–30% levels in slot blots (Fig. 5). The apparent persistence may reflect the fact that these hybridization results have been normalized to the low levels of IGF-II RNA present in kidney at 21 days gestation.
Fig. 7. Hybridization to slot blots prepared from RNA from heart, skin, muscle, intestine, and liver at different ages. Hybridization, autoradiography, and densitometry are as described in the legend to Fig. 5. The highest concentration of RNA was 6.7 μg except for 16f skin, 8.4 μg; 2-day muscle, 4.8 μg; 11-day muscle, 3.4 μg; 22-day intestine, 13.4 μg; and 22-day liver, 11.2 μg.

Fig. 8. Hybridization of the rat IGF-II probe to Northern blots of RNA prepared from brain stem, cortex, and hypothalamus at different ages. Northern blots of total RNA (15 μg) isolated from brain stem, cortex, and hypothalamus of rats of different ages were hybridized at 50 °C with 32P-labeled rat IGF-II probe (1.0 × 10^6 cpm/ml, brain stem and cerebral cortex; 6 × 10^5 cpm/ml, hypothalamus) as in Fig. 4. Autoradiography was for 18 h (brain stem and cortex) or 4 days (hypothalamus). The right half of the 4-kb region of the 75-day hypothalamus sample is obscured by a transfer artifact.

different IGF-II RNA species were observed.

**Expression of IGF-II RNA in Neural Tissues**—IGF-II RNA was detected in hybridizations to slot blots from brain stem, cerebral cortex, and hypothalamus (Figs. 1 and 2). Compared with most non-neural tissues, the abundance of IGF-II RNA in neural tissue was lower (Fig. 2). Despite the low amounts, the six IGF-II RNA species were detected in fetal brain stem, cerebral cortex, and hypothalamus (Fig. 8 and longer autoradiographic exposures (not shown)). As in non-neural tissues, IGF-II RNA levels were higher in fetal (16-day gestation) than in adult (75-day) samples. In brain stem, a major decrease occurred between day 16 and day 21 of gestation (similar to the pattern observed in lung), with almost complete elimination of the hybridization signal by day 2.

Different and more complex results were observed in cerebral cortex and hypothalamus. Although some decrease in hybridization occurred between fetal days 16 and 21, IGF-II RNA levels persisted at appreciable levels through day 11. In
fact, with prolonged autoradiographic exposure (not shown), a distinct signal persisted at 75 days. Thus, although the levels of IGF-II RNA are significantly greater in liver than in cerebral cortex and hypothalamus at 16 days gestation, in adult (75-day) rats IGF-II RNA is as abundant in cerebral cortex and more abundant in hypothalamus than in liver (results of slot blot hybridizations not shown).

**DISCUSSION**

IGF-II is a mitogenic polypeptide present in the plasma of rats (14), man (15), sheep (16), and other species (17), and in human amniotic fluid (18) and cerebrospinal fluid (19). Although IGF-II exhibits rather minimal regulation by growth hormone (14) and placental lactogen (5), plasma IGF-II exhibits a profound decrease with increasing age (2). In rat plasma, immunoreactive IGF-II is elevated at birth, and decreases 20-100-fold by 21 days of age (2). Qualitatively similar results have been obtained in rat (20) and sheep (16) using a radioreceptor assay that preferentially measures IGF-II.

Since IGF-II is not present in any tissue at a concentration higher than in serum, the major site(s) of IGF-II biosynthesis are not known. It generally has been assumed that liver is a quantitatively important site of synthesis, as is the case for the related peptide IGF-I (21). Indeed, short-term explants of term fetal rat liver synthesize IGF-II polypeptides (3), and RNA from fetal rat liver directs the synthesis of prepro-IGF-II in a cell free translation system (22). However, IGF-II also is synthesized by myoblasts cultured from 21-day fetuses (6), and by fibroblasts cultured from 11-21-day gestation rat embryos, 16-day gestation lung and skin, and 2- and 3-day neonatal lung and skin, but not by fibroblasts cultured from lung and skin of 5-50-day-old rats (5). Thus, because of its mitogenicity, IGF-II synthesis in fetal and neonatal rats (IGF-II is not limited to acting as a circulating growth factor, but is fully capable of local autocrine or paracrine actions (23).

The demonstration of IGF-II synthesis in fetal or neonatal liver, fibroblasts, and myoblasts, and the decreased IGF-II synthesis in fibroblast cultures from older animals are consistent with the developmental pattern seen in serum. However, synthesis of IGF-I but not IGF-II was demonstrated in pancreatic islet cell cultures from 3-day-old rats (24). The fact that IGF-II is synthesized by fibroblast cultures but not by islet cell cultures at day 3 suggests that the developmental pattern of IGF-II expression varies in different tissues.

Recently, using a cDNA clone encoding rat IGF-II isolated in our laboratory (7) as a probe, we demonstrated that hybridizable IGF-II RNA was abundant in livers of rats 2, 12, and 15 days of age, but was undetectable in livers of 25-, 35-, and 65-day-old rats (9). The present study utilized this hybridization probe to examine IGF-II gene expression in different tissues at different developmental ages.

(i) IGF-II RNA was detected in 8 of 10 non-neural fetal or neonatal rat tissues by slot blot and Northern blot hybridization. Tissue concentrations of IGF-II RNA varied, being higher in muscle, skin, liver, lung, thymus, and intestine, and lower in heart and kidney. No IGF-II RNA was detected in spleen, or pancreas.1 The results in pancreas are consistent with the lack of IGF-II synthesis in 3-day pancreatic islet cell cultures (24) but also may reflect partial degradation of pancreatic RNA. Soares et al. (25) reported that IGF-II RNA was present in liver, lung, heart, and muscle from 2-day-old rats.

Since the probe used for their studies corresponds to the 5' non-coding region, and since their nucleotide sequence for the 5'-noncoding region differs almost completely from that reported by Dull et al. (26), the specificity of these hybridization results is uncertain. Recently, Scott et al. (27) reported that IGF-II RNA was present in 7-10-week-old human fetal liver, lung, heart, muscle, kidney, and adrenal.

(ii) IGF-II RNA also was detected at low but significant levels in three neural tissues in fetal or neonatal rats: brain stem, cerebral cortex, and hypothalamus. IGF-II RNA previously has been observed in total neonatal rat brain (25) and in adult human brain (27). However, in contrast to our positive results in the fetal rat, IGF-II RNA was not observed in human fetal brain (27). The integrity of the human fetal brain RNA was not commented upon. Expression of IGF-II RNA in brain is consistent with the results of immunocytoassays of extracts of various regions of adult human brain including anterior pituitary (28).

(iii) In all 11 tissues positive for IGF-II RNA, multiple species of IGF-II RNA were detected, with the 4.0-kb RNA the most abundant. No consistent differences were observed in the distribution of IGF-II RNA species in different tissues.

We have proposed that the different IGF-II RNA species arise by alternative processing of a primary RNA transcript (9). The significance of the different forms (e.g., whether they encode proteins other than IGF-II) is not presently known. In the case of the gene for calcitonin/calcitonin gene-related peptide, the primary transcript is processed to a 1050-nucleotide RNA encoding calcitonin in the thyroid gland and in medullary thyroid carcinomas, and a 1150-1300-base RNA encoding calcitonin gene-related peptide in the same medullary thyroid carcinomas after passing and in the brain (29). We have not detected any unique IGF-II RNA species in any tissue. This contrasts with the results of Soares et al. (25), who reported that 2-day rat muscle contained a 1.85-kb RNA not present in liver, heart, or lung. In view of the uncertainties concerning the cDNA probe used for their analysis, it remains to be established whether this represents an authentic IGF-II RNA and indicates tissue-specific processing of IGF-II RNA.

(iv) A detailed examination of IGF-II RNA levels in different tissues at six different ages confirms the general pattern of IGF-II regulation seen in serum, liver, and fibroblasts, namely, that IGF-II RNA is higher in the fetus and neonate than in older adult animals. These results are consistent with our previous results on IGF-II RNA levels in rat liver (8), and with preliminary results of Soares et al. (25) for rat liver and muscle, and Scott et al. (27) for human liver and kidney. In the present study we observed that in each tissue, highest concentrations of IGF-II RNA were present at the youngest age (15 or 21 days gestation, or 2-day neonatal) and decreased with age. However, superimposed on this general pattern, tissue-specific differences in the developmental pattern of IGF-II gene expression also were discerned.

In most tissues, an abrupt transition (50% decrease) in IGF-II RNA was observed between successive ages of examination. In one group (lung, thymus, and kidney), IGF-II RNA appeared to be expressed predominantly before birth. Within this group, the major decrease in IGF-II RNA in the lung occurred between 16 and 21 days of gestation, whereas the major decrease in IGF-II RNA in thymus and kidney occurred between 21 days of gestation and 2 days after birth.

In a second group of tissues (muscle, skin, intestine, liver, and probably heart), expression of IGF-II RNA persisted at a high level through the 2nd or 11th day of life. The results of slot blot and Northern blot hybridizations suggest that the major transition in muscle, skin, intestine, and heart occurred...
prior to day 11, whereas in liver, the major transition occurred between day 11 and day 22. The same profile was observed in liver RNA extracted from a different rat strain (Sprague-Dawley) using different extraction procedures (8, 9).

Neural tissues appear to represent more complex and distinct patterns. In cerebral cortex and hypothalamus, although some decrease in IGF-II RNA occurs between day 16 and day 21 of gestation, IGF-II RNA clearly was detectable up to 75 days. The greater relative persistence of IGF-II RNA in adult rat brain may suggest a unique role of IGF-II in the adult nervous system.

(v) In each tissue, the highest expression of the IGF-II gene was observed at the youngest ages examined (16 or 21 days gestation, or 2 days after birth). Although these experiments were not conducted at earlier times of fetal life, other evidence suggests that IGF-II is expressed at early times post-implantation. IGF-II is synthesized by fibroblast cultures established from whole rat embryos at the 11th day of gestation (5), and by mouse cell lines with properties characteristic of visceral endoderm (30, 31). In the human fetus, IGF-II gene expression may occur principally in the first trimester. IGF-II RNA was present in 7-10-week-old fetal tissues (27), but immunoreactive IGF-II was low in cord blood from 22-36-week-old fetuses (32).

The differential decline in IGF-II mRNA over the perinatal and neonatal period in various tissues raises the possibility that the process may be linked to major events of tissue maturation which occur throughout the body at this time. Although it is wise to extrapolate from superficial temporal associations, the correlation of IGF-II mRNA decline with functional change is particularly striking for some tissues. The fetal lung matures shortly before birth by a thinning of the intra-alveolar septa, the appearance of type II pneumocytes in the alveolar epithelium, and the synthesis of surfactant (33). Of the tissues studied, only in fetal lung was there appearance of two separate classes of fibers, and a sharp decline in the population of proliferating myoblasts (34).

In summary, IGF-II RNA is expressed in nearly all fetal and neonatal tissues. Although it is generally true that IGF-II RNA levels are higher in fetal or neonatal animals than in adult rats, clear-cut differences were observed in the age at which the most abrupt decrease in RNA levels occurred in different tissues. The precise age of onset of IGF-II RNA synthesis is unknown, since tissues were not examined before day 16 of gestation. The same six IGF-II RNA species observed in liver were present in other tissues, with the 4-kb species most prominent. It remains to be determined whether the IGF-II RNA species observed in the different tissues are translated into pro-rIGF-II. The mechanism by which the presumably single IGF-II gene (35) is differentially expressed in different tissues, and how this may correlate with possible tissue-specific functions of IGF-II, remains to be determined.

REFERENCES

1. Humbel, R. E. (1984) In Hormonal Proteins and Peptides Vol. XII (Li, C. S., ed) pp. 57-79, Academic Press, New York.

2. Moss, A. C., Nisalas, S. P., Short, F. A., Rechler, M. M., White, R. M., Knaig, A. B., and Higa, O. Z. (1986) Proc. Natl. Acad. Sci. U. S. A. 74, 34-38.

3. Rechler, M. M., Eisen, H. J., Higa, O. Z., Nisalas, S. P., Moss, A. C., Schilling, E. E., Fernoy, I., Brun, C. B., Phillips, L. S., and Baird, K. L. (1979) J. Biol. Chem. 254, 7927-7930.

4. Adams, S. O., Nisalas, S. P., Greenstein, L. A., Yang, Y.-W.-H., and Rechler, M. M. (1983) Endocrinology 112, 979-987.

5. Adams, S. O., Nisalas, S. P., Handaerger, S., and Rechler, M. M. (1983) Nature 302, 150-153.

6. Hildemann, C., Crone, C. J., Nisalas, S. P., Morrel, D., Holter, A. T., and Milner, R. D. G. (1985) Endocrinology 117, 2067-2072.

7. Whittington, H. J., Brun, C. B., Frunzio, R., Terrell, J. E., Nisalas, S. P., and Rechler, M. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4518-4523.

8. Cathala, G., Savourout, J.-P., Mendez, B., West, B. L., Marin, M. J., Du Bois, and Baxter, J. D. (1985) Endocrinology 115, 2523-2535.

9. Heath, J. K., and Isacke, S. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 131-135.

10. Dubowitz, V., and Dubowitz, V. (1979) In The Human Newborn Baby, p. 216, Edward Arnold, London.

11. Dubowitz, V., and Dubowitz, V. (1980) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

12. Dubowitz, V., and Dubowitz, V. (1982) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

13. Dubowitz, V., and Dubowitz, V. (1985) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

14. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

15. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

16. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

17. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

18. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

19. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

20. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

21. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

22. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

23. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

24. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

25. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

26. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

27. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

28. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

29. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

30. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

31. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

32. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

33. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

34. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.

35. Dubowitz, V., and Dubowitz, V. (1986) In Myology, ed. M. F. Bartlett, p. 244, Edward Arnold, London.