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Developmental Regulation of GLUT-1 (Erythroid/Hep G2) and GLUT-4 (Muscle/Fat) Glucose Transporter Expression in Rat Heart, Skeletal Muscle, and Brown Adipose Tissue*

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ABSTRACT. The expression of GLUT-1 (erythroid/Hep G2) and GLUT-4 (muscle/fat) glucose transporters was assessed during development in rat heart, skeletal muscle, and brown adipose tissue. GLUT-4 protein expression was detectable in fetal heart by day 21 of pregnancy; it increased progressively after birth, attaining levels close to those of adults at day 15 post natal. In contrast, GLUT-4 messenger RNA (mRNA) was already present in hearts from 17 day-old fetuses. GLUT-4 mRNA stayed low during early postnatal life in heart and brown adipose tissue and only increased after day 10 post natal. The expression pattern for GLUT-4 protein in skeletal muscle during development was comparable to that observed in heart. In contrast to heart and skeletal muscle, GLUT-4 protein in brown adipose tissue was detected in high levels (30% of adult) during late fetal life.

During fetal life, GLUT-1 presented a very high expression level in brown adipose tissue, heart, and skeletal muscle. Soon after birth, GLUT-1 protein diminished progressively, attaining adult levels at day 10 in heart and skeletal muscle. GLUT-1 mRNA levels in heart followed a similar pattern to the GLUT-1 protein, being very high during fetal life and decreasing early in post natal life. GLUT-1 protein showed a complex pattern in brown adipose tissue: fetal levels were high, decreased after birth, and increased subsequently in post natal life, reaching a peak by day 9.

Progesterone-induced postmaturity protected against the decrease in GLUT-1 protein associated with post natal life in skeletal muscle and brown adipose tissue. However, GLUT-4 induction was not blocked by postmaturity in any of the tissues subjected to study.

These results indicate that: 1) during fetal and early post natal life, GLUT-1 is a predominant glucose transporter isotype expressed in heart, skeletal muscle, and brown adipose tissue; 2) during early post natal life there is a generalized GLUT-1 repression; 3) during development, there is a close correlation between protein and mRNA levels for GLUT-1, and therefore regulation at a pretranslational level plays a major regulatory role; 4) the onset of GLUT-4 protein induction occurs between days 20–21 of fetal life; based on data obtained in rat heart and brown adipose tissue, there is a dissociation during development between mRNA and protein levels for GLUT-4, suggesting modifications at translational or posttranslational steps; and 5) postmaturity blocks the decrease in GLUT-1 expression but not the induction of GLUT-4. observed soon after birth. All these findings suggest that GLUT-1 repression and GLUT-4 induction are mediated by different mechanisms. (*Endocrinology* **130:** 837–846, 1992)

GLUCOSE transport by facilitated diffusion is expressed in virtually all mammalian cells. Recent studies have established that this process is mediated, in mammalian cells, by a family of related glucose transport proteins. So far, five different complementary DNAs (cDNAs) encoding these different species have been isolated (1–11), which have been named GLUT-1 (erythroid/Hep G2), GLUT-2 (liver), GLUT-3 (brain), GLUT-4 (muscle/fat), and GLUT-5 (small intestine). These proteins exhibit considerable homology in their primary sequences, but differ in their biochemical properties and tissue distribution (3, 5, 11–14).

Insulin causes a rapid stimulation of glucose transport in brown and white adipose tissue, heart, and skeletal muscle, the only tissues that express type GLUT-4 glucose transporters (6–10, 13). Nevertheless, these tissues also express GLUT-1 isotype (15–17), although, based on studies performed with isolated rat adipocytes, GLUT-4 represents 90% of the total glucose transporters expressed in this cell type (18). In spite of the large

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differences in absolute expression, both carriers play relevant roles in respect to the cell economy of glucose. Thus, under basal conditions, glucose transport is maintained by the activity of GLUT-1 transporters present in the plasma membrane. However, after stimulation with insulin, the GLUT-4 isotype becomes the major glucose transporter in this membrane (18). In this regard, insulin plays a complex role in the regulation of glucose transport, since on one hand, it causes a rapid translocation of GLUT-4 carriers in isolated adipocytes and, on the other hand, it seems to be required to maintain GLUT-4 transporter expression, so adipocytes from diabetic or starved animals show a marked depletion of GLUT-4 transporters (19–22), concomitant with a substantial decrease in the effect of insulin-stimulating glucose transport (23–26).

Regarding the development of the effect of insulin on glucose uptake by insulin-sensitive tissues, it is known that during fetal life, tissues such as heart or diaphragm have a high rate of glucose use (27, 28), which is greater than in adult tissue. In addition, it has been reported that fetal heart responds to insulin-increasing glucose transport (27), and that the effect of insulin on glucose transport in the diaphragm increases markedly during post natal life (28), showing no correlation with the total size of intracellular glucose transporters, as assessed by cytochalasin B binding (28). However, there is a lack of information regarding the developmental regulation of glucose transporter expression in insulin-sensitive tissues. Therefore, in this report we have investigated the expression of GLUT-1 and GLUT-4 in heart, skeletal muscle, and brown adipose tissue during development in the rat. Here, we describe the high expression of GLUT-1 during fetal life and the post natal acquisition of GLUT-4.

Materials and Methods

Materials

[^{125}I]Goat anti-mouse immunoglobulin G and [^{125}I]protein A were purchased from Amersham (Amersham, UK). Hybond N was from Amersham and random priming DNA labeling kit from Boehringer (Mannheim, Germany). Immobilon was obtained from Millipore (Bedford, MA). All electrophoresis reagents and mol wt markers were obtained from Bio-Rad (Richmond, CA). γ-Globulin and most commonly used chemicals were from Sigma (St. Louis, MO).

Animals and tissue sampling

Female Wistar rats (150–200 g) obtained from our own colony were mated, and gestation was timed from the appearance of spermatozoids in vaginal smears. The rats were fed with Purina laboratory chow *ad libitum* and housed in animal quarters maintained at 22 C with a 12-h light, 12-h dark cycle. At different gestational times (17–21 days), mothers were anesthetized with sodium pentobarbital (5–7 mg/100 g body wt). Fetuses were removed, and interscapular brown adipose tissue, heart, and skeletal muscle (hindlimbs) were rapidly collected and frozen in liquid nitrogen. When the post natal period was studied, pups remained with their mother after delivery and were anesthetized with sodium pentobarbital at different times before tissue sampling.

Postmature fetuses were obtained on day 23 from pregnant rats treated daily with 7 mg progesterone (35 mg/ml ricine oil) from day 20 of pregnancy (29). Control pregnant rats received equal volumes of ricine oil, and their pups were studied 3 days later (day 1 post natal).

Preparation of membrane fractions from tissues

Tissues were homogenized in 10 vol ice-cold buffer containing 25 mM HEPES, 250 mM sucrose, 4 mM EDTA, 1 trypsin inhibitor unit/ml aprotinin, 25 mM benzamidine, 0.2 mM phenylmethylsulfonylfluoride, 1 μM leupeptin, and 1 μM pepstatin, pH 7.4. Homogenates from brown adipose tissue and heart were centrifuged at 5,000 × *g* for 5 min at 4 C. The supernatant was then centrifuged at 150,000 × *g* for 2 h at 4 C to obtain the membrane fractions. The homogenates from skeletal muscle were centrifuged at 15,000 × *g* for 20 min at 4 C. The supernatants were adjusted to 0.8 M KCl, incubated at 4 C for 30 min, and then centrifuged for 90 min at 200,000 × *g* at 4 C to obtain the membranes. The membrane pellets were resuspended in homogenization buffer and repeatedly passed through a 25-gauge needle before storage at −20 C. Proteins were measured by the method of Bradford (30) using γ-globulin as a standard.

Electrophoresis and immunoblotting of membranes

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed on membrane protein in accordance with the method of Laemmli (31). Proteins were transferred to Immobilon as previously reported (18) in buffer consisting of 20% methanol, 200 mM glycine, 25 mM Tris, pH 8.3. After transfer, the filters were blocked with 5% nonfat dry milk, 0.02% sodium azide in PBS for 1 h at 37 C and were incubated with antibodies. Transfer was confirmed by Coomassie blue staining of the gel after the electroblot. Antibody 1F8 purified by protein A chromatography (kindly donated by Dr. Paul F. Pilch, Boston University, Boston, MA) was used at 5–10 μg/ml in 1% nonfat dry milk, 0.02% sodium azide in PBS for 1 h at 37 C, to immunoblot GLUT-4. Detection of antibody-antigen complexes was effected with goat antimouse [^{125}I]antibody and autoradiography. Rabbit Bb antiserum raised against the purified human erythrocyte glucose transporter (a gift of Dr. Christin Carter-Su, University of Michigan, Ann Arbor, MI) was used directly at a 1:400 dilution and was incubated with transferred protein overnight at room temperature in 1% nonfat dry milk, 0.02% sodium azide in PBS. Detection of the immune complex with the rabbit antibody was accomplished using [^{125}I]protein A for 4 h at room temperature. The autoradiograms were quantified using scanning densitometry. Immunoblots were performed under conditions where autoradiographic detection was in the linear response range.

RNA isolation and Northern blot analysis

Total RNA from heart was extracted using the acid guanidinium isothiocyanate/phenol/chloroform method as described by Chomczynski and Sacchi (32). All samples had a 260/280 absorbance ratio over 1.7.

After quantification, total RNA (15 μg) was denatured at 65 C in the presence of formamide, formaldehyde, and ethidium bromide (33) to allow the visualization of RNA. RNA was separated on a 1.2% agarose/formaldehyde gel and blotted on Hybond N filters. The RNA in gels and in filters was visualized with ethidium bromide and photographed by UV transillumination to ensure the integrity of RNA, to check the loading of equivalent amounts of total RNA, and to confirm proper transfer. RNA was transferred in 10 × standard saline citrate (SSC; 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0).

Blots were initially prehybridized for 4 h at 45 C in 50% formamide, 5 × Denhardt's (1 × Denhardt's solution is 0.02% polyvinylpyrolidone, 0.02% Ficoll, 0.02% BSA), 0.5% SDS, 5 × SSPE (1 × SSPE is 0.15 M NaCl, 1 mM EDTA, and 10 mM NaH_2PO_4, pH 7.4), and 0.5 mg denatured salmon sperm DNA. The blots were then hybridized to the corresponding probes for 12 h at 42 C in 50% formamide, 5 × Denhardt's, 0.05% SDS, 5 × SSPE, 10% dextran sulfate, and 0.5 mg denatured salmon sperm DNA. The cDNA probe for GLUT-1 is a 1346-base pair *Eco*RI fragment and the cDNA probe for GLUT-4 is a 2007-base pair *Sal*I fragment. Both cDNA probes were obtained from Dr. Graeme I Bell (University of Chicago). The cDNA probes were labeled with [^{32}P]cytidine triphosphate by random oligonucleotide priming. The probes were included at 1.5×10^6 cpm/ml. Filters were washed for 15 min in 2 × SSC at room temperature, and then twice in 0.4 × SSC, 0.1% SDS (first wash for 20 min and second wash for 30 min) at 55 C. The abundance of specific glucose transporter message was quantitated by scanning densitometry of autoradiograms as described above.

Results

Expression of mRNA and protein levels of GLUT-4 and GLUT-1 glucose transporters in rat heart during development

The expression of GLUT-1 and GLUT-4 glucose transporters was initially assessed during development of rats in cardiac muscle. To that end, membrane fractions and total RNA were purified from hearts during development, and transporter protein and mRNA content was determined. No significant differences were detected regarding the yield of membrane protein per gram of heart in 21-day fetuses (9.2 ± 2.1 mg protein/g tissue), 1-day neonates (12.3 ± 1.9 mg protein/g tissue), 10-day neonates (10.2 ± 2.4 mg protein/g tissue), 15-day neonates (11.1 ± 1.7 mg protein/g tissue), or adults (13.6 ± 3.2 mg protein/g tissue). In contrast, a greater RNA yield was obtained in heart during fetal (3.2 ± 0.4 mg/g tissue) or early neonatal life (2.5 ± 0.2 mg/g tissue) compared to the adult state (1.2 ± 0.1 mg/g tissue).

GLUT-4 protein was assessed by Western blot using the specific monoclonal antibody 1F8 (13). GLUT-4 protein was barely detectable in heart membrane fractions at days 19 and 20 of fetal life (Fig. 1), and a low expression was detected by day 21 (Fig. 1). Expression of GLUT-4 at day 21 of fetal life represents 13% of adult values. GLUT-4 increased after birth, becoming progressively more abundant and attaining adult levels after day 15 of post natal life (Fig. 1). No difference in GLUT-4 electrophoretic migration was detected throughout development.

The presence of GLUT-4 mRNA was determined by Northern blot using a human cDNA probe under high stringency conditions. Thus, Northern analysis of brain tissue, which does not express GLUT-4 (13), did not reveal any labeling (data not shown). GLUT-4 mRNA levels were already detectable but low at day 17 of fetal life (Fig. 1) compared to the adult state (8% of adult levels). mRNA levels remained relatively low during early neonatal life (20% of adult levels) (Fig. 1) and substantially increased after day 10 post natal (Fig. 1). At day 15 post natal, GLUT-4 mRNA levels were still markedly lower than in the adult heart in spite of the fact that no differences were detected at the level of GLUT-4 protein. As with GLUT-4 protein, no difference in electrophoretic migration of GLUT-4 mRNA was detected during development.

GLUT-1 protein was assessed by Western blot using polyclonal antibody Bb against human erythrocyte glucose transporter, which does not cross-react with GLUT-4 transporter (18). This antiserum recognizes, in Western blot, polypeptides other than GLUT-1, showing apparent mol wts greater than the glucose carrier. However, when GLUT-1 is initially immunoprecipitated using a C-terminal-specific monoclonal antibody (34), only the immunoprecipitate shows, by Western blot using antiserum Bb, the band corresponding to GLUT-1 (data not shown). Therefore, this antiserum is useful to determine the expression of GLUT-1 protein. The expression of GLUT-1 glucose transporter protein was much greater in heart membrane extracts from days 19, 20, or 21 of fetal life (Fig. 2) compared to the adult group. In fact, GLUT-1 levels expressed in heart during the adult state only accounted for 2% of levels found during fetal life. However, soon after birth GLUT-1 protein initiated a rapid decrease, and GLUT-1 levels were already low after 6 days (22% of fetal levels) and 10 days (6% of fetal levels) of neonatal life (Fig. 2). Antibody Bb allows the detection of differences in electrophoretic mobility between GLUT-1 from rat brain and from cultured rat renal fibroblasts (data not shown). However, no difference in GLUT-1 electrophoretic migration was detected throughout development.

GLUT-1 mRNA levels in heart followed a similar

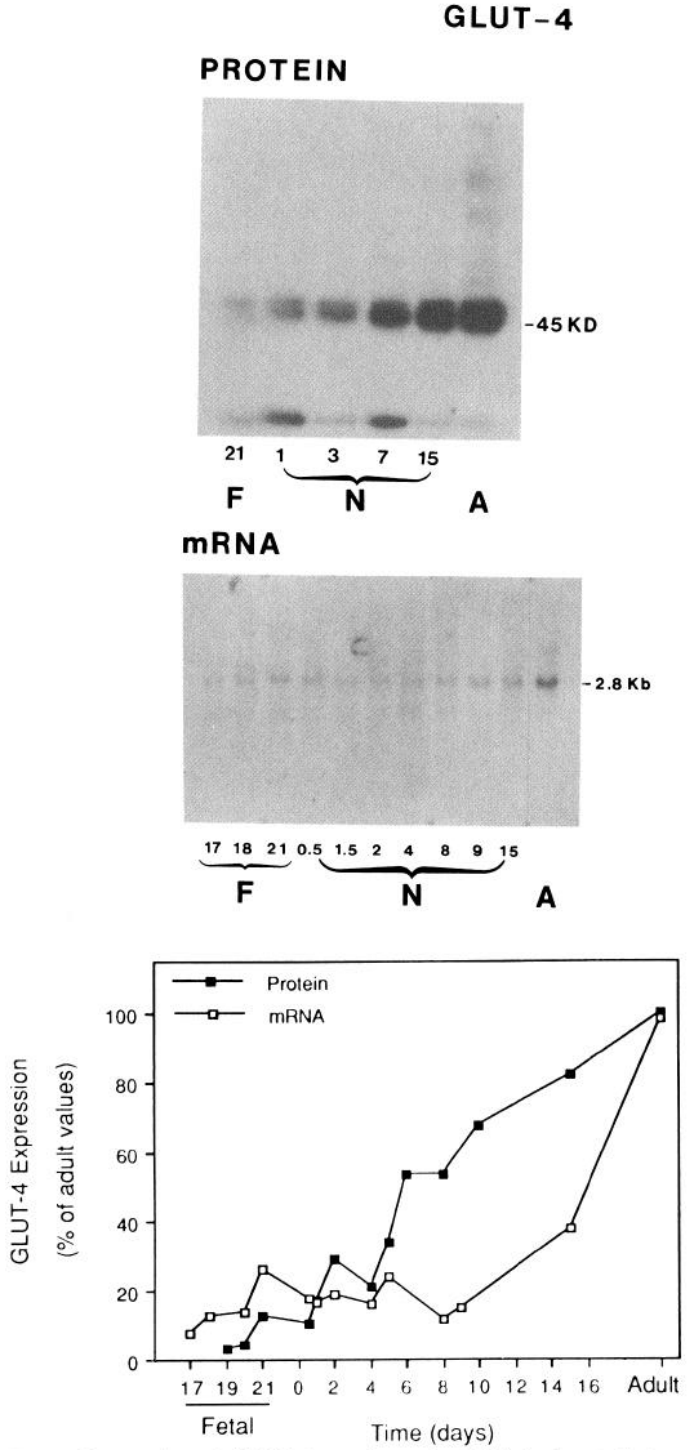

FIG. 1. Expression of GLUT-4 protein and mRNA in heart during development. Total RNA and membrane proteins were purified from pooled hearts obtained from rats (from day 19 of fetal life through 15 post natal and adult rats). Two hundred micrograms of membrane proteins or 15 μg total RNA from the different experimental groups were applied on gels. After blotting, GLUT-4 protein (*top panel*) was detected by incubation with monoclonal antibody 1F8. GLUT-4 mRNA (*middle panel*) was detected after hybridization with a 2007-base pair *Sal*I fragment as a cDNA probe (see *Materials and Methods*). Autoradiographs were subjected to scanning densitometry. The results of two to five separate experiments are shown and expressed as a percentage of adult values (*bottom panel*). SEMs ranged between 5–21% of mean values.

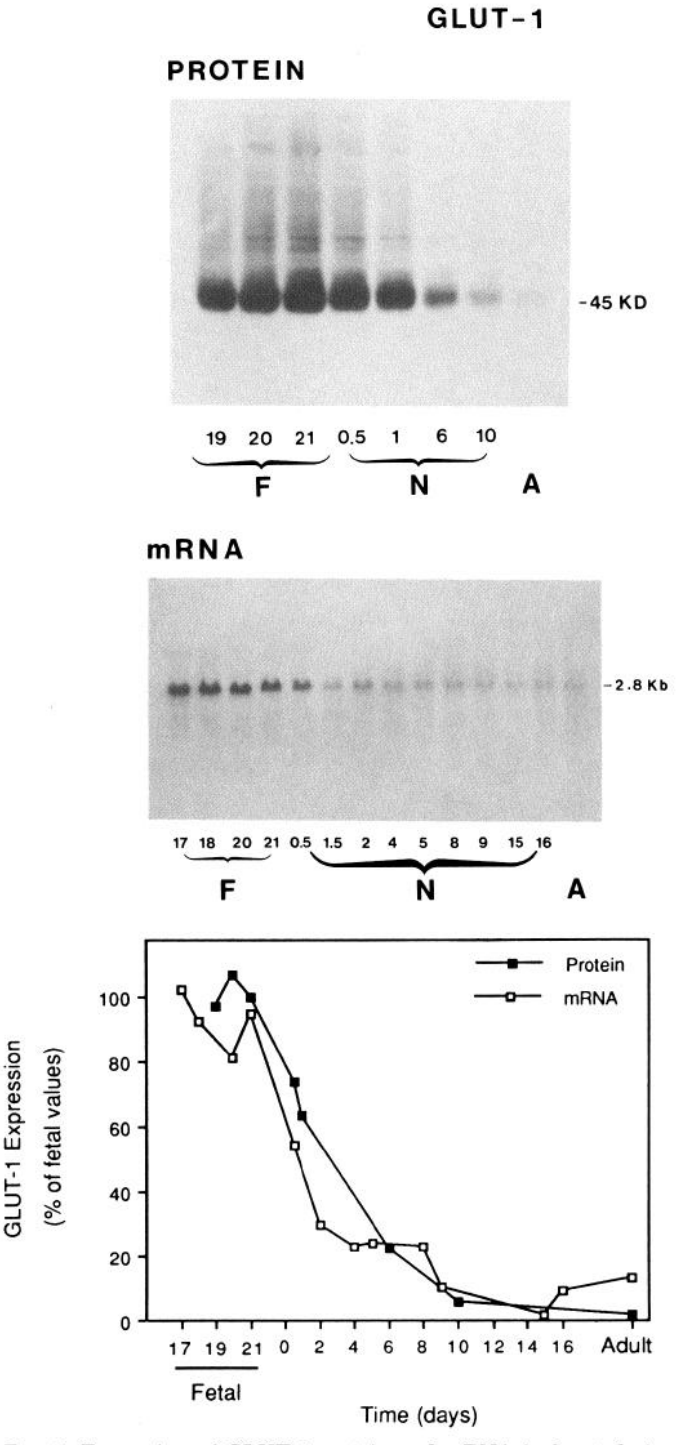

FIG. 2. Expression of GLUT-1 protein and mRNA in heart during development. Total RNA and membrane proteins were purified from pooled hearts obtained from rats (from day 19 of fetal life through 15 post natal and adult rats). One hundred micrograms of membrane proteins or 15 μg total RNA from the different experimental groups were applied on gels. After blotting, GLUT-1 protein (*top panel*) was detected by incubation with antibody Bb. GLUT-1 mRNA (*middle panel*) was detected after hybridization with a 1346-base pair *Eco*RI fragment as a cDNA probe and as described in *Materials and Methods*. Autoradiographs were subjected to scanning densitometry. The results of one to two separate experiments are shown and expressed as a percentage of fetal (day 21) values (*bottom panel*).

pattern to the GLUT-1 protein, being very high during fetal life and decreasing early post natal (Fig. 2).

Expression of GLUT-4 and GLUT-1 protein in skeletal muscle during development

In an attempt to define the existence of a general pattern, we next investigated the expression throughout development of GLUT-4 and GLUT-1 glucose transporters in skeletal muscle. To this end, membrane fractions were purified from hindlimb skeletal muscle. A low yield of membrane proteins was detected in 21-day fetuses (2.7 ± 0.6 mg protein/g tissue), 1-day neonates (2.4 ± 0.3 mg protein/g tissue), and 5-day neonates (2.5 ± 0.4 mg protein/g tissue) compared to adult levels (5.2 ± 0.7 mg protein/g tissue). Membrane protein yield increased progressively in 10-day neonates (3.8 ± 0.5 mg protein/g tissue) and 15-day neonates (4.1 ± 0.8 mg protein/g tissue).

In keeping with the observations in heart, GLUT-4 protein was initially detected in skeletal muscle by day 21 of fetal life (Fig. 3). Expression of GLUT-4 at day 21 of fetal life was low and only accounted for 6% of adult values. In addition, GLUT-4 protein substantially increased soon after birth and attained adult levels at day 15 of neonatal life (Fig. 3). No differences in electrophoretic migration pattern was detected between GLUT-4 in preparations from adult and perinatal groups.

GLUT-1 protein also presented a very high expression level during fetal life in skeletal muscle (Fig. 3). Thus, GLUT-1 levels expressed in skeletal muscle during the adult state only accounted for 3% of levels found during fetal life (day 20 or day 21). Also in keeping with the pattern found in heart, GLUT-1 protein initiated a rapid decrease soon after birth, so GLUT-1 levels were low at day 3 post natal (20% of fetal levels). Protein levels comparable with the adult state were found by day 10 of neonatal life (Fig. 3).

Expression of GLUT-4 and GLUT-1 protein in brown adipose tissue during development

The developmental regulation of GLUT-4 and GLUT-1 glucose transporter expression was also investigated in brown adipose tissue, an insulin-sensitive tissue that plays a critical thermogenic role in the newborn. To that end, membrane fractions were purified from interscapular brown adipose tissue. No difference in the yield of membrane proteins was detected in 21-day fetuses (19.8 ± 3.7 mg protein/g tissue) and 5-day neonates (14.9 ± 1.8 mg protein/g tissue) compared to adult levels (16.6 ± 2.2 mg protein/g tissue).

GLUT-4 protein was initially detected in brown adipose tissue by day 20 of fetal life (8% of adult levels) (Fig. 4), and expression of GLUT-4 by day 21 of fetal life accounted for 30% of adult values. Thus, GLUT-4

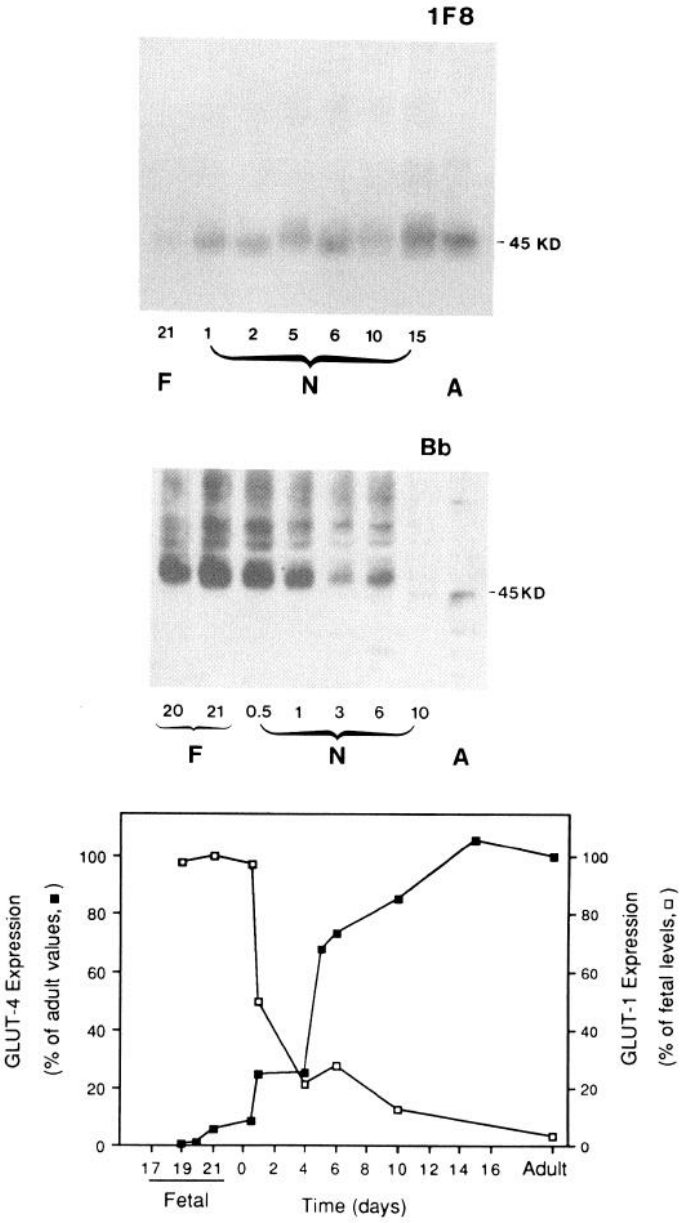

FIG. 3. Expression of GLUT-4 and GLUT-1 protein in skeletal muscle during development. Membranes were obtained from pooled hindlimb muscle from rats. Identical amounts of membrane proteins from the different experimental groups were applied on 10% acrylamide gels (200 μg for GLUT-4 and 100 μg for GLUT-1). Protein from the gels was transferred to immobilon and immunoblotted with 1F8 (GLUT-4, *top panel*) or with Bb antiserum (GLUT-1, *middle panel*). Autoradiographs were subjected to scanning densitometry, and the results of three to five separate observations are shown (*bottom panel*). Data on GLUT-1 are expressed as a percentage of fetal (day 21) values, whereas data on GLUT-4 are expressed as a percentage of adult values.

protein in brown adipose tissue during fetal life was detected at high levels compared to the adult state. This represents a substantial difference between brown adipose tissue and heart and skeletal muscle. In addition, GLUT-4 protein substantially increased soon after birth, attaining adult levels between days 5–6 post natally (Fig.

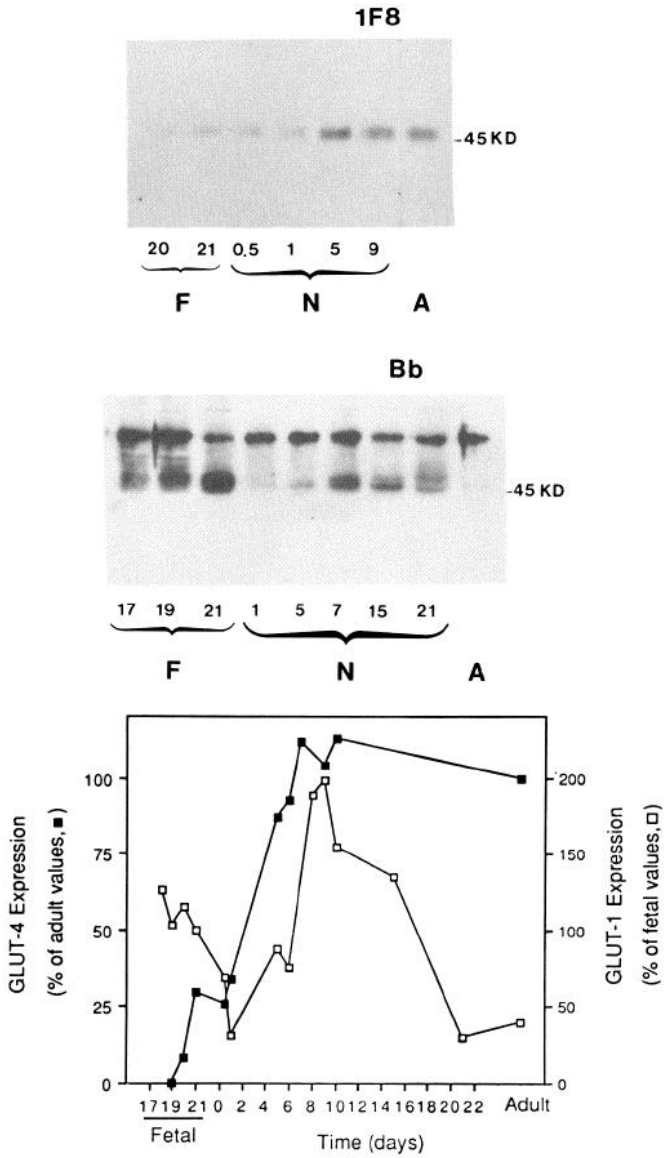

FIG. 4. Expression of GLUT-4 and GLUT-1 protein in brown adipose tissue during development. Membranes were obtained from pooled interscapular brown adipose tissue from rats. Identical amounts of membrane proteins from the different experimental groups were applied on 10% acrylamide gels (200 μg for GLUT-4 and 100 μg for GLUT-1). Protein from the gels was transferred to immobilon and immunoblotted with 1F8 (GLUT-4, *top panel*) or with Bb antiserum (GLUT-1, *middle panel*). Autoradiographs were subjected to scanning densitometry, and the results of four to five separate observations are shown (*bottom panel*). Data on GLUT-1 are expressed as a percentage of fetal (day 21) values, whereas data on GLUT-4 are expressed as a percentage of adult values.

4). Thus, adult levels for GLUT-4 protein were reached earlier in brown adipose tissue than in heart and skeletal muscle (Figs. 1 and 3). GLUT-4 mRNA was detected at low levels during fetal life (5% of adult levels at day 19) and remained relatively low during early neonatal life (6% of adult levels at day 1 post natal). GLUT-4 mRNA levels were still markedly lower by days 6, 9, 13, and 15 post natal compared to the adult brown adipose tissue (data not shown). At these times, no differences between neonatal and adult tissues had been detected at the level of GLUT-4 protein.

GLUT-1 protein was also highly expressed in brown adipose tissue during fetal life (Fig. 4), however, its developmental pattern was more complex than in heart or skeletal muscle. Thus, GLUT-1 levels diminished soon after birth (Fig. 4). This was followed by a rapid recovery period which led to a high expression level during days 8–15 post natal. Later on, GLUT-1 protein content decreased to adult control levels (Fig. 4). Adult levels accounted for 40% of fetal values.

Effect of postmaturity on GLUT-1 and GLUT-4 expression in brown adipose tissue, heart, and skeletal muscle

GLUT-4 and GLUT-1 proteins show an inverse pattern of changes in insulin-sensitive tissues in the perinatal period (Figs. 1–4); thus, whereas GLUT-1 rapidly decreases soon after birth, GLUT-4 increases during that time. To determine whether these effects are a consequence of environmental changes associated with delivery, the effect of postmaturity was next investigated. To this end, pregnant rats were treated for 3 days with progesterone (from days 20–23), and GLUT-1 and GLUT-4 protein content was assessed in brown adipose tissue, skeletal, and cardiac muscle (Fig. 5).

The content of GLUT-1 protein was greater in the postmature group than in the control group (1-day post natal) in skeletal muscle and brown adipose tissue (Fig. 5). In skeletal muscle, GLUT-1 protein levels from postmature fetuses remained between levels detected in 21-day-old fetuses and 1-day-old neonates (control group), indicating a partial protection by post maturity on GLUT-1 repression associated with early neonatal life. GLUT-1 protein in brown adipose tissue from the postmature group was similar to the levels detected in 21-day normal fetuses (data not shown), evidence that post maturity totally prevented the decrease in GLUT-1 associated with early post natal life in brown adipose tissue.

Regarding GLUT-4 expression, no differences in protein content were detected in heart from control and postmature groups (Fig. 5). Furthermore, GLUT-4 protein was increased in skeletal muscle and brown adipose tissue as a result of progesterone-induced postmaturity (Fig. 5). In conclusion, GLUT-4 induction was never blocked by post maturity.

Discussion

In this study we have demonstrated the existence of a high expression of GLUT-1 glucose transporters in in-

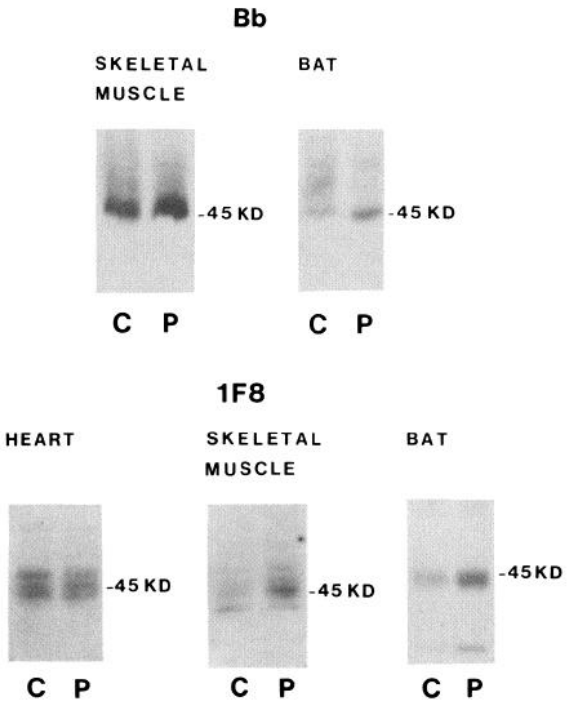

FIG. 5. Effect of post maturity on the expression of GLUT-1 and GLUT-4 protein in insulin-sensitive tissues. Postmature fetuses were obtained on day 23 from pregnant rats treated daily with 7 mg progesterone (in ricine oil) from day 20 of pregnancy. Control rats received equal volumes of ricine oil and were studied 3 days later (day 1 post natal). Membranes were obtained from heart, hindlimb muscle, and interscapular brown adipose tissue from rats. Two hundred micrograms of membrane proteins from the different experimental groups were applied on gels. Protein from the gels was transferred to immobilon and immunoblotted with Bb antiserum to detect GLUT-1 (*top panel*) or antibody 1F8 to visualize GLUT-4 (*bottom panel*). Autoradiographs were also subjected to scanning densitometry. The autoradiograms presented are representative from three separate observations.

sulin-sensitive tissues during fetal life in the virtual absence of GLUT-4 carriers. Thus, we have found that GLUT-1 carriers in insulin-sensitive tissues are around 30- to 50-fold more abundant during fetal life than in adulthood in heart and skeletal muscle. GLUT-3 mRNA levels were undetectable in heart tissue from 21-day-old fetuses and from neonates, under conditions in which expression was detected in some tissues from adult rats (Castelló, A., M. Furrias, M. Camps, X. Testar, M. Palacin, and A. Zorzano, unpublished results). A corollary of all these findings is that during fetal life, glucose uptake in peripheral tissues must be performed by GLUT-1 carriers. Bearing in mind the role of GLUT-1 on basal glucose uptake in isolated rat adipocytes (18), the high expression of GLUT-1 glucose transporters in peripheral tissues during fetal and early neonatal life explains the high rates of glucose uptake described in rat fetal heart (27) and in the incubated intact diaphragm during development (28).

It also should be mentioned that insulin causes stimulation of glucose uptake in fetal rat heart (27) and in diaphragm during early post natal life (28), that is, under conditions in which GLUT-4 is either absent or only scarce. These observations suggest that GLUT-1 is an insulin target during fetal life, which agrees with data obtained in several cell types (18, 35), but which is in contrast to data obtained in skeletal muscle from adult rats where insulin promotes translocation of GLUT-4 but not of GLUT-1 carriers (36). In any event, whether the effect of insulin during fetal life is related to carrier translocation as described in heart or skeletal muscle (37–39) or is a consequence of alterations in intrinsic activity of carriers remains to be determined.

The high expression of GLUT-1 during fetal life is common to heart, skeletal muscle, and brown adipose tissue, and all these tissues also undergo a decrease soon after birth which is protected, to a different extent, in postmature fetuses. Ontogenic studies performed in rat and rabbit brain also have shown that GLUT-1 protein and mRNA expression is high in fetal life, decreasing after birth (40–42). Furthermore, high GLUT-1 mRNA levels have been substantiated in rat lung, liver, and kidney during fetal life (40, 43) and they also rapidly diminish after birth. All these findings favor the existence of a circulating factor responsible for the enhanced expression of GLUT-1 during fetal life and which disappears rapidly after delivery but not in postmaturity.

In fetal heart, the high expression of GLUT-1 carriers is paralleled by high levels of GLUT-1 mRNA, suggesting the activation of a pretranslational step during fetal life. Whether the enhanced mRNA abundance is due to an increased rate of gene transcription or to an increased mRNA stability has not yet been determined.

Induction of GLUT-4 protein was detected by day 20 in brown adipose tissue (8% of adult values), and it was substantial by day 21 of gestation in brown adipose tissue (30% of adult values), heart (13% of adult values), and skeletal muscle (6% of adult values). This induction occurred later than the onset of induction of mRNA levels which, at least in fetal heart, were already detectable by day 17. After the onset of the expression in late pregnancy, all tissues investigated showed a progressive increase in GLUT-4 protein content, and this induction was not blocked by postmaturity. GLUT-4 protein levels increased up to adult values in all tissues investigated; however, adult levels were attained earlier in brown adipose tissue than in heart and skeletal muscle.

Recent observations have reported the localization of GLUT-4 glucose transporters in transverse tubule membranes in human skeletal muscle (44); in this regard, it is significant that the triads appear in rat skeletal muscle in significant numbers only post natally (45, 46), which coincides with the appearance of substantial GLUT-4 expression. Whether the expression of GLUT-4 and pro-

tein components of transverse tubules is regulated during development with an identical time-dependence and by common regulatory factors deserves further study.

We have observed in rat heart and brown adipose tissue a dissociation between mRNA and protein regarding GLUT-4 expression during development. Thus, whereas GLUT-4 protein increases progressively during post natal life, attaining adult values at day 6 or day 10, mRNA levels remain rather stable. This dissociation also has been reported for hepatic β-F1-ATPase in early post natal life (47), and it suggests the existence of GLUT-4 regulation either at a translational level or to an increased stability of GLUT-4 protein during development. Based on our data, we propose two different phases in heart and brown adipose tissue during development: an initial phase characterized by modifications at a translational or posttranslational step for GLUT-4, and a later phase in which GLUT-4 mRNA increases and the prior modifications return to levels present in adult state.

The overall development pattern for GLUT-4 and GLUT-1 in heart and skeletal muscle somewhat resembles the pattern previously described in 3T3 adipocytes with differentiation (48, 49). Thus, when 3T3 fibroblasts differentiate, GLUT-1 content decreases to some degree or remains unaltered, and GLUT-4 initiates its expression, and this is concomitant with a decrease in basal glucose uptake and an enhanced effect of insulin on glucose transport (48, 49). However, ontogenic development of muscle and adipose tissue broadly differ in quantitative terms from differentiation of 3T3 cells. Thus, whereas differentiation of 3T3 cells leads to variable changes on GLUT-1 expression—from 20–100% of levels found in fibroblasts (48, 49), rat adult tissues present GLUT-1 expression which is only about 2–3% of fetal levels. Furthermore, whereas in isolated rat adipocytes GLUT-4 represents more than 90% of total glucose carriers (18), in 3T3-L1 adipocytes GLUT-1 and GLUT-4 are present at a 3:1 molar ratio (50).

In the present study, we have reported the triggering of GLUT-4 induction and GLUT-1 repression during perinatal life. These two events seem to differ in various aspects: 1) whereas the mechanisms for GLUT-1 repression seem to be at a pretranslational level, the onset of GLUT-4 induction is dependent on translational or posttranslational activation; 2) the timing of the two events is different, the onset of GLUT-4 mRNA induction being earlier than the repression of GLUT-1; and 3) whereas GLUT-1 repression is inhibited by post maturity, GLUT-4 protein induction is not blocked. All these findings strongly suggest that the two events are mediated by different mechanisms.

The nature of the signals involved in the onset of GLUT-4 expression and GLUT-1 repression is unknown. Insulin might be involved in the regulation of glucose transporter expression during perinatal life. In this regard, it should be pointed out that circulating insulin rises to high concentrations over the last 3 days of gestation in the rat, decreasing to low levels during early post natal development (51–54) and there is a substantial expression of insulin receptors, displaying high affinity binding sites, in rat fetal skeletal muscle (55). Regarding neural activity, it has been reported that sympathetic innervation is functionally active in rat muscle and brown adipose tissue near term (56–58) and also might be proposed as a modulator of glucose carrier expression. At present, studies are being conducted to test the participation of insulin or neural activity on the developmental regulation of GLUT-1 and GLUT-4 expression in insulin-sensitive tissues.

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