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Research Article

Differential expression of glucose transporters during chick embryogenesis

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Abstract. The patterns of Glut1 and Glut3 glucose transporter protein and mRNA expression were assessed during embryogenesis of chicken brain and skeletal muscle, Glut4 protein levels were also evaluated in skeletal muscle and heart, and Glut1 was examined in the developing heart and liver. Glut1 protein expression was detectable throughout brain ontogeny but was highest during early development. Glut1 mRNA levels in the brain remained very high throughout development. Glut3 protein was highest very early and very late and mRNA was highest during the last half of development. In embryonic skeletal muscle, the levels of Glut1and Glut3 proteins and mRNA were highest very early, and declined severely by mid-development. Glut1 protein and mRNA in the heart also peaked early and then decreased steadily. Although Glut1 mRNA levels were consistently high in the embryonic liver, Glut1 protein expression was not detected. These results suggest that (1) Glut1 is developmentally regulated in chick brain, skeletal muscle, and heart, (2) Glut1 mRNA is present in liver but does not appear to be translated, (3) Glut3 in brain increases developmentally but is virtually absent in muscle, and (4) Glut4 protein and mRNA appear to be absent from chick heart and skeletal muscle.

Key words. Development; chick; Glut1; Glut3; Glut4; embryogenesis.

A high metabolic demand for fuel energy is required for the sustained growth, differentiation, and metabolism of fetal tissues [1, 2]. During fetal development, the supply of glucose to the embryo is relatively constant since it is provided by the placenta in mammals or by the egg yolk in birds. However, after birth or hatching, the glucose supply is more variable and the primary energy source may change [2] with the result that the organism must successfully adapt the glucose transport system, if it is to survive. This adjustment of the glucose transporter system must begin during fetal development.

Facilitative glucose transport across the plasma membrane of the cell is mediated by a family of membrane-bound protein isoforms which have a unique tissue-specific distribution. This distribution may be related to the differing requirements for glucose by the various adult tissues [3]. Glucose transporter (Glut1), also called erythrocyte/HepG2-type, is expressed at highest levels in brain (primarily vascular endothelium and glial cells) and placenta, is present in cultured cells and most tissues, and may be at least partially responsible for constitutive uptake [3–5]. Glut3 (neuronal) is insulin insensitive and has highest levels in neurons and neural cells, kidney, placenta, and testes [3, 5, 6]. Insulin-sensitive Glut4 appears later during mammalian development and is found primarily in muscle and fat [3]. Other members of the family are Glut2, found almost exclusively in the liver, small intestine, kidney, and insulin-positive pancreatic β cells, and Glut5, a fructose transporter located in the

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small intestine [3]. A newly identified isoform, Glut8, is found in various tissues including muscle, brain, liver, kidney, and testes, with highest expression in the latter, and appears to have a role in insulin-regulated glucose uptake in murine blastocysts [7, 8].

Despite variations in the tissue expression of Glut1 and Glut3, the two transporters play important roles with respect to the cell economy of glucose. Both transporters have a high affinity (K_m) for glucose allowing them to transport glucose efficiently even under low-glucose conditions [3, 4]. Furthermore, this high affinity for glucose means that the rate of glucose uptake is determined by the number of transporter molecules in the plasma membrane and not by the extracellular glucose concentration [4, 6]. In mammalian muscle and adipose tissue, insulin stimulation increases V_{max} of glucose uptake primarily by causing translocation of pre-formed Glut4 transporter molecules from an intracellular site to the surface of the plasma membrane [6].

The developmental patterns of the facilitative glucose transporters have been previously described during rodent embryogenesis [1, 9, 10]. Although Glut1 and Glut3 are present in adult chickens [11–13] and in chicken-derived cell lines [14, 15], glucose transporter expression during chicken embryological development has not been reported. The chicken embryo in ovo has been widely used as a model to study the effect of various growth factors [16, 17], and drugs including ethanol [11, 12, 18, 19], caffeine [20], nicotine [12, 21], and narcotics [21, 22] on various organ systems. Additionally, the chick model has been utilized to study neurogenesis [23], angiogenesis [24], oncogenic transformation [14, 15] and bacterial pathogenesis and toxicity [25–27]. Because glucose is an essential component of embryonic development and metabolism, the expression of glucose transporters during embryogenesis may be relevant to studies examining the effect of drugs, growth factors, or chemicals on embryonic metabolism and/or structure. The aim of the studies reported here was to describe the patterns of facilitative glucose transporter expression in the developing chicken embryo.

Materials and methods

Preparation of chicken embryos

Nonincubated fertilized chicken eggs (Arbor Acre/Ross) obtained from Webber's Hatchery, (Goldsboro, N.C.) were incubated in a humidified hatchery (Humidaire Incubator Company, New Madison, Ohio) maintained at 100 °F. On the day of harvest, embryos were removed and the tissues were collected, weighed, snap frozen in liquid nitrogen, and stored at –80 °C. Because chick embryonic organ systems were not sufficiently differentiated prior to developmental day 10 to permit the harvesting of distinct structures, the whole trunk and the head (minus the eyes) were harvested separately from day 5 and day 7 embryos. By day 10, distinct organ systems can be identified, by day 12, the chick appears fully formed, and by day 14, the developing chick has recognizable feathers. Brain, skeletal muscle, heart, and liver tissue were collected from 10-, 12-, 14-, 17-, and 19-day-old embryos (hatching = day 21). For some experiments, adult male Arbor Acre/Ross chickens and/or Sprague Dawley rats were anesthetized with sodium pentobarbital, tissues harvested, snap frozen, and processed in the same manner.

Preparation of total membranes

Total membrane proteins were prepared from frozen tissue samples as described previously [11]. Briefly, the tissue (200 mg/ml) was disrupted in cold detergent-free Pilch's homogenization buffer (25 mM Hepes, 4 mM EDTA, 25 mM benzamidine, 57 µM phenylmethylsulfonyl fluoride, 1 µM leupeptin, and 0.15 µM aprotinin) with a Polytron tissue homogenizer (Brinkman). Following centrifugation at 150,000 g for 1 h at 4 °C, the pellet was resuspended in Pilch's and rehomogenized. Sufficient Triton-X100 was added to yield a 1% (v/v) solution and the membranes were left on ice for 1.5 h, with frequent mixing. The samples were then centrifuged again at 150,000 g for 1 h. The supernatant containing the solubilized membrane proteins was harvested and the amount of protein present was quantitated by the BCA assay (Pierce Chemical, Rockford, Ill.). The membranes were aliquoted and stored at –80 °C.

Antibodies

Rabbit anti-rat Glut1 antibody was obtained from Charles River Pharmservices, Southbridge, Mass. (formerly East Acres) and rabbit anti-chicken Glut3 antibody [15] was a kind gift of Dr. Martyn White (Thomas Jefferson University, Philadelphia, PA.). The anti-rat Glut4 antibodies were obtained from Chemicon International, Temecula, Calif., and from Charles River Pharmservices. The rabbit anti-human Glut4 antibodies were a kind gift of Dr. Lynis Dohm, East Carolina University. The peroxidase-conjugated secondary antibodies were purchased from Sigma (St. Louis, Mo.).

Immunoblot analyses

Western blot analyses were carried out as described previously [11]. Briefly, unheated total membrane proteins in reduced sample buffer (20 µg/lane) were subjected to electrophoresis on 10% SDS-acrylamide gels under reducing conditions. Proteins were transferred to Immobilon membranes in Towbin's transfer buffer and blocked in 5% powdered milk in TBS (pH 7.2) overnight at 4 °C. In some experiments, membrane proteins derived from rat skeletal and heart muscle were included as positive controls. Blots were then incubated with rabbit anti-

Glut1, anti-Glut3, or anti-Glut4 polyclonal antibody for 2 h at room temperature, washed and then labelled with peroxidase-conjugated secondary antibody for 1 h at room temperature. In control experiments, no primary antibody was used. The blots were then washed and the proteins were detected by enhanced chemiluminescence using a luminol solution and exposure to X-ray film (Kodak Biomax ML). The autoradiograms were subjected to densitometric scanning (Hewlett Packard Scanjet II) in the linear-signal range and the bands were quantitated by ImageQuant software (Molecular Dynamics). The blots were stained with Coomassie blue and used to correct for unequal loading.

RNA isolation and northern blotting

Total cellular RNA was isolated from chick embryonic tissue (at several developmental ages) and from adult rat and chicken tissues using Trizol reagent (GIBCO Life Technologies). Northern blotting was performed as described previously [11]. Briefly, chick RNA samples (20 µg) were separated on 1% agarose-formaldehyde gels, transferred to PVDF membrane (Hybond-N; Amersham, Arlington Heights, Ill.), hybridized at 48 °C with ^{32}P-labelled cDNA probes, and washed at room temperature and at 50 °C. In some experiments, 10 µg of rat muscle and liver RNA were included as positive and negative controls, respectively. Low-stringency conditions (hybridization at 42 °C and only room temperature washes) were used for the Glut4 experiments. The chicken cDNA probes were a kind gift of Dr. Martyn White, Thomas Jefferson University: a 2.0-kb *Eco*RI/*Bam*HI fragment containing chicken Glut1 cDNA [15], a 1.7-kb fragment containing chicken Glut3 [28], and a 1.1-kb *Pst*I fragment containing chicken glyceraldehyde-3-phosphate dehydrogenase (pGAD3) which was used as a loading control and to correct for unequal loading [15]. The 2.5-kb *Eco*R1 fragment containing mouse Glut4 cDNA was a kind gift of Dr. Lynis Dohm, East Carolina University [29]. The membranes were then exposed to a PhosphorImager screen and the RNA bands quantitated by ImageQuant software (Molecular Dynamics).

Results

Glut1 and Glut3 expression in the brain during embryological development

Western blotting methodology was used to estimate the levels of Glut1 and Glut3 proteins in total membrane samples of tissue from embryonic chick head (days 5 and 7) and brain (days 10–19). Representative immunoblots

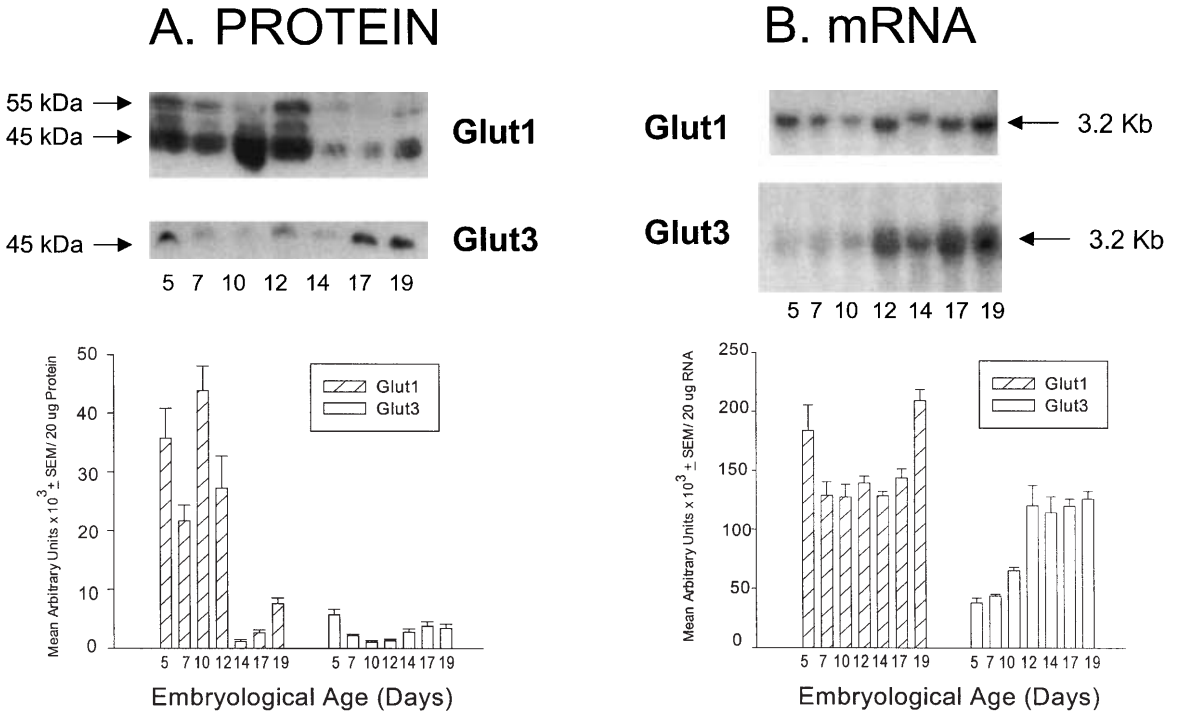

Figure 1. Changes in Glut1 and Glut3 expression during embryogenesis of chick brain. Illustrated are the changes in developmental expression of Glut1and Glut3 protein (*A*) and mRNA (*B*) levels during brain embryogenesis. Total membranes and total RNA were prepared from chick embryonic tissue (days 5 and 7 head, days 10–19 brain) as described in Materials and methods. Representative blots are shown above the histograms. The proteins (20 µg/lane) were resolved by SDS-PAGE and analyzed by Western blotting. The histogram (*A*) summarizes the quantitation of scanned immunoblots for the glucose transporter proteins. Northern blots (*B*) of Trizol-isolated chick RNA samples (20 µg/lane) were hybridized with chick-specific cDNA probes as described in Materials and methods. Phosphoimaging was used to quantitate the amount of label in each band. Data are expressed as mean arbitrary units ± SE of six experiments.

are presented above the bar charts in figure 1 A. As shown in the Glut1 immunoblot, a diffuse band in the 45 to 55-kDa range was detected and is consistent with the reported range of molecular weights for the Glut1 protein in brain and is due to heterogeneous glycosylation [4, 10]. As shown in figure 1 A, Glut1 protein (45–55 kDa) in the developing brain was highest during the first half of embryological development, and by day 19 had declined to 25% of the day 5 level. Glut3 (45-kDa) protein levels (fig. 1 A) steadily decreased after day 5, increasing again on day 19.

Northern blotting methodology was used to estimate the levels of Glut1 and Glut3 mRNA (fig. 1 B). In contrast to protein expression, Glut1 mRNA levels remained high throughout development. However, Glut3 mRNA experienced low levels early in development, then quickly increased on day 12 and remained elevated through day 19 of development.

Glut and Glut3 expression in muscle during embryological development

The levels of Glut1 and Glut3 proteins and mRNA were quantitated in day 5 and 7 trunk and day 10–19 skeletal muscle tissue. As shown in figure 2 A, the level of Glut1 protein (45–55 kDa) was ten-fold higher on day 5 than at any other developmental age examined. By day 10, the level of Glut1 in muscle had precipitously declined to a low level that remained unchanged as development progressed. The amount of Glut3 (45 kDa) was highest on day 5, after which it precipitously decreased and became nearly undetectable from day 12 onward.

In contrast to protein expression in skeletal muscle, the quantity of both Glut1 and Glut3 mRNA decreased progressively (fig. 2 B), reaching the lowest level on day 19.

Glut1 expression in heart and liver tissue

As shown in figure 3 A, Glut1 (45-kDa) protein expression in embryonic heart tissue was highest on day 10 (earliest age tested) and showed a gradual 20-fold decrease which reached a nadir on day 19. The quantity of Glut1 mRNA in the heart (fig. 3 B) also peaked early in development and then steadily declined to 50% of the peak value by day 19. Glut1 protein was undetectable in the chick liver by Western blotting methodology at any of the embryonic ages tested (representative blot in fig. 4 A). A protein sample from day 5 chick head was included as a positive control. However, as shown in fig. 4 B, Glut1 mRNA was detected at high levels in the embryonic liver.

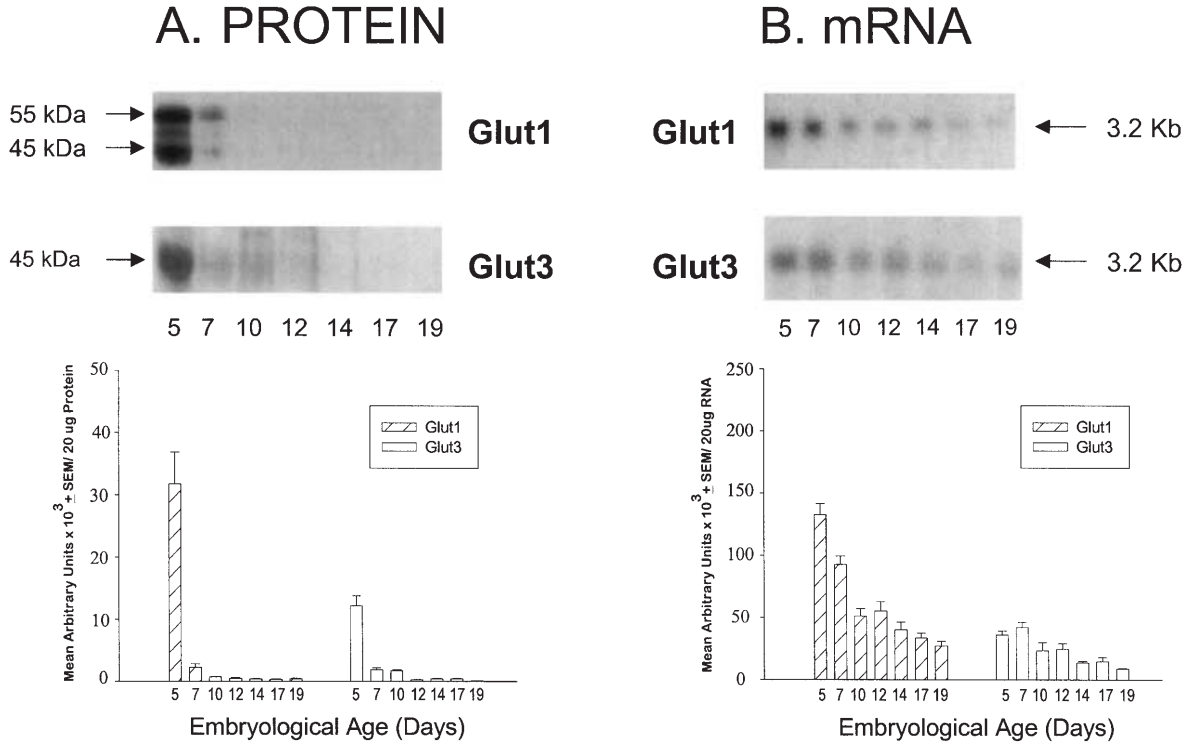

Figure 2. Changes in Glut1 and Glut3 expression during chick muscle embryogenesis. Illustrated are the developmental expression of Glut1 and Glut3 protein (*A*) and mRNA (*B*) levels in embryonic skeletal muscle. Both total membranes and total RNA were prepared from chick embryonic tissue (days 5 and 7 whole trunk, days 10–19 muscle) as described in Materials and methods. Representative blots are presented above the histograms. The proteins (20 µg/lane) were resolved by SDS-PAGE and analyzed by Western blotting. The histogram (*A*) summarizes the quantitation of scanned immunoblots for the glucose transporter proteins. Northern blots (*B*) of Trizol-isolated chick RNA samples (20 µg/lane) were hybridized with chick-specific cDNA probes as described in Materials and methods. Phosphoimaging was used to quantitate the amount of label in each band. Data are expressed as mean arbitrary units ± SE of six experiments.

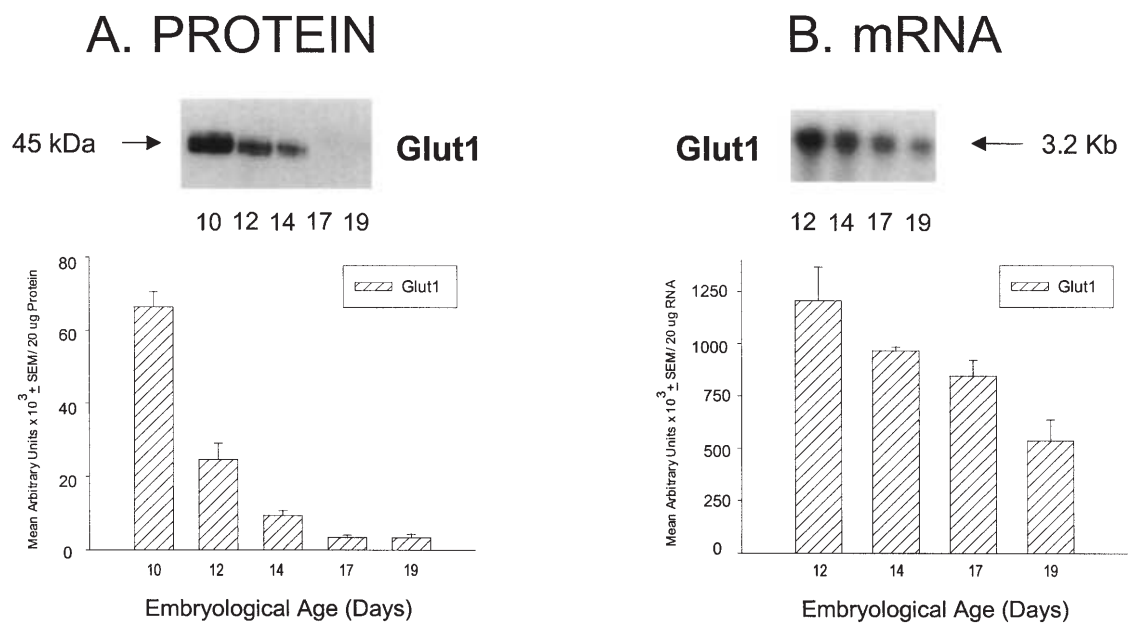

Figure 3. Changes in Glut1 expression during chick heart embryogenesis. Illustrated are the developmental expression of Glut1 protein (*A*) and mRNA (*B*) levels in embryological heart tissue. Hearts were harvested from day 10, 12, 14, 17, and 19 chick embryos and total membrane proteins and total RNA were prepared as described in Materials and methods. The proteins (20 µg/lane) were resolved by SDS-PAGE and analyzed by Western blotting. The histogram (*A*) summarizes the quantitation of densitometrically scanned immunoblots for the glucose transporter proteins. Northern blots (*B*) of Trizol-isolated chick RNA samples (20 µg/lane) were hybridized with chick-specific cDNA probes as described in Materials and methods. Phosphoimaging was used to quantitate the amount of label in each band. Data are expressed as mean arbitrary units ± SE of four experiments.

Glut4 expression in chick tissues

Although four different polyclonal anti-Glut4 antibodies were tested, Glut4 protein was undetectable in chick embryonic skeletal and heart muscle tissue. Total membrane proteins derived from rat skeletal muscle and heart were included as positive controls. A representative blot is presented in figure 5A.

Northern blotting methodology under low-stringency conditions (hybridization at 42 °C with a mouse Glut4 cDNA probe for 18 h followed by two washes at room temperature) was used to assay for chick Glut4 mRNA. However, as shown in fig. 5B, Glut4 mRNA was not detected in chick embryonic skeletal muscle or in chick heart tissue (data not shown) at any of the developmental ages tested. Adult rat muscle served as a positive control.

Unlike mammals, Glut4 protein is possibly not expressed in the embryo but appears after hatching and, therefore, would be present in the adult chicken. To address this question, the same assay system (Northern blotting and low-stringency conditions) was used to search for Glut4 mRNA in various adult chicken tissues. As shown in figure 5C, Glut4 mRNA was not detected in any of the tissues tested (leg and breast muscle, liver, brain, heart, and adipose tissue). Rat muscle and liver RNA samples were included as positive and negative controls, respectively.

Discussion

Glucose is an essential source of fuel energy in the embryo [4, 10, 30]. As part of the developmental process, the facilitative glucose transporter proteins undergo alterations in their expression and regulation that are essential to the continued development, growth, and survival of the organism.

Glut1 is the isoform expressed in most fetal tissues [6, 31]. Because the Glut1 protein is present in varying amounts in so many tissues, it may be responsible, at least in part, for constitutive, insulin-insensitive glucose uptake [6]. In the rodent brain, Glut1 protein and mRNA levels have been shown by several investigators to be high in the fetus, transiently decreased in the neonate, and increased again as the animal matures, reaching highest levels in the adult [9, 32, 33]. In the findings reported here, the level of Glut1 protein is highest early in chick brain embryogenesis, decreases during mid development, and increases in late development, mirroring the pattern in the rat.

Due to the lack of sufficient organ differentiation prior to day 10, chick trunks and heads were harvested on developmental days 5 and 7. By day 10, distinct organ systems (muscle, brain, liver, and heart) had formed, were harvested and assayed. Therefore, the changes in transporter protein and mRNA levels observed in the early developmental period (prior to day 10) versus changes

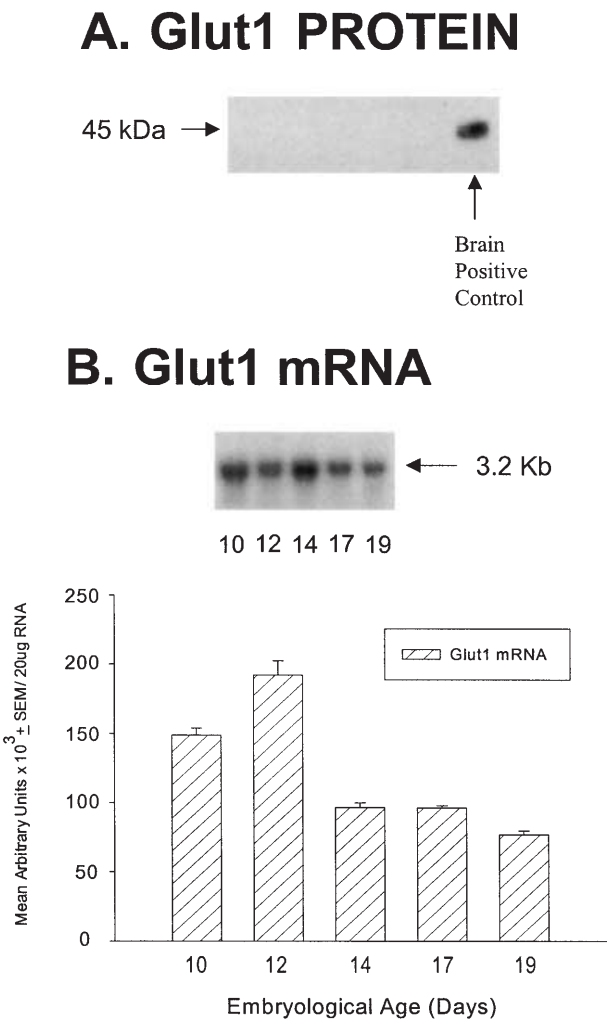

Figure 4. Changes in Glut1 mRNA expression during chick liver embryogenesis. Illustrated is the developmental expression of Glut1 in chick liver. Livers were harvested from day 10, 12, 14, 17, and 19 chick embryos. Total membrane proteins and total RNA were prepared as described in Materials and methods. (*A*) Proteins (20 µg/lane) were resolved by SDS-PAGE and analyzed by Western blotting. A representative immunoblot is presented. Brain-derived total membrane proteins (day 5 chick embryo) served as a positive control. (*B*) Northern blots of Trizol-isolated chick RNA samples (20 µg/lane) were hybridized with chick-specific cDNA probes as described in Materials and methods. Phosphoimaging was used to quantitate the amount of label in each band. A representative blot and the resulting histogram are presented. The data are expressed as mean arbitrary units ± SE of four experiments.

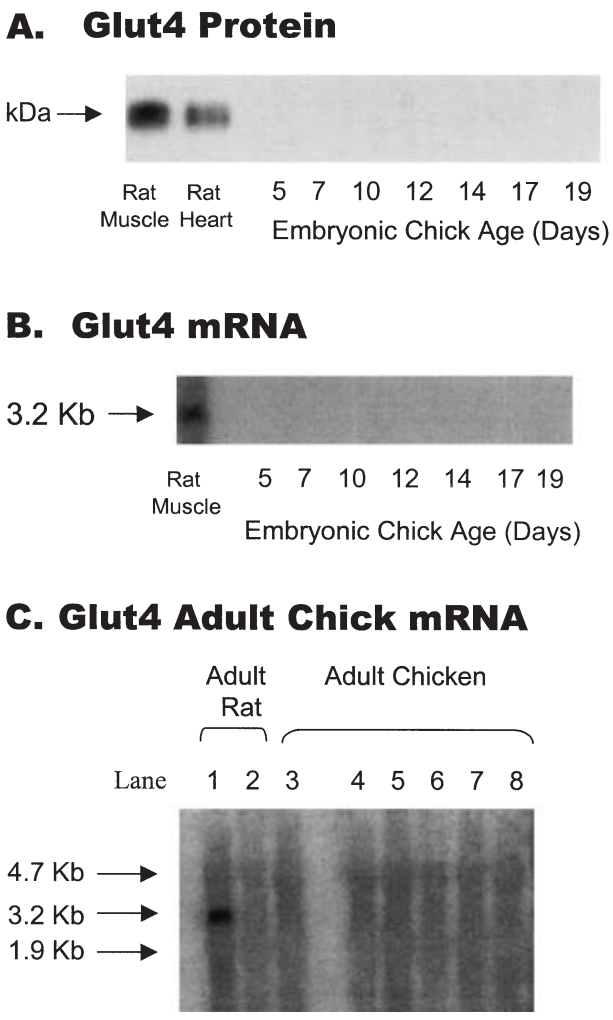

Figure 5. Absence of Glut4 protein and mRNA in chick skeletal muscle and heart. Tissues were harvested and total membrane proteins and total RNA were prepared as described in Materials and methods. (*A*) Chick embryonic skeletal (leg) muscle was harvested on day 10, 12, 14, 17, and 19, and whole-trunk tissue was harvested from day 5 and day 7 embryos. Total membrane samples from adult rat heart and skeletal muscle were included as positive controls. The proteins were resolved by SDS-PAGE and analyzed by Western blotting. A representative immunoblot is presented. (*B*) Northern blots of Trizol-isolated embryonic chick trunk/muscle-derived RNA samples (20 µg/lane) were hybridized with a mouse Glut4 cDNA probe using low-stringency conditions as described in Materials and methods. RNA derived from adult rat muscle (10 µg/lane) was included as a positive control. (*C*) Northern blots of adult chicken RNA samples (20 µg/lane) of leg muscle (lane 3); breast muscle (lane 4), brain (lane 5), liver (lane 6), heart (lane 7) and adipose (lane 8) were hybridized, under low-stringency conditions, with a mouse Glut4 cDNA probe. Adult rat heart (lane 1) and liver (lane 2) RNA samples were included as positive and negative controls, respectively.

occurring later may reflect the relative impurity or purity of the tissue that was analyzed.

In the study presented here, Glut1 protein expression decreased and Glut3 protein levels increased as brain development proceeded. In contrast, the levels of Glut1 mRNA remained high throughout chick brain development with only a slight decrease during the mid-developmental period. The high levels of Glut1 mRNA and relatively low levels of protein may suggest that the cell content of Glut1 protein is regulated at the level of translation. Alternatively, the relatively low levels may be due to a higher turnover or degradation rate of the Glut1 protein. In the chicken, Glut1 appears to be the primary glucose transporter early in brain development and Glut3 appears to become important later in development as neurons mature. Glut3 protein and mRNA levels are

lowest in the rodent embryonic brain but increase dramatically 1 day after birth [2, 33, 34].

Glut1 and Glut3 proteins and mRNA levels during chick skeletal muscle ontogeny peaked on day 5 and decreased to very low levels by day 12. Glut3 mRNA has also been found at low levels in human fetal muscle and has a low level of ubiquitous distribution in many adult human tissues [34]. Although Diamond and Carruthers [35] were unable to detect the Glut3 isoform in adult pigeon brain using an anti-human antibody, they did find the protein in leg muscle.

Glut1 protein is recognized as being heterogeneously glycosylated and the degree of glycosylation may be associated with the tissue type. In both the adult and developing rat brain, the 45- and 55-kDa forms are predominantly associated with different structures: glial cells and endothelial cells, respectively [5, 10]. The lower-molecular-weight form is most prevalent in whole-brain preparations [10]. In rat skeletal muscle and heart, Glut1 appears to be more heterogeneous in the former than the latter [1, 36]. We also observed a diffuse or heterogeneous Glut1 protein band in brain and skeletal muscle tissues at all developmental ages but only a single relatively sharp band in heart muscle tissue. The relevance of these findings is unclear.

Although Glut1 protein was undetectable in chick embryonic liver, Glut1 mRNA was present at high levels on day 10 and declined as development proceeded. Glut1 mRNA levels are highest in fetal rat tissues, decreased in the neonate and are absent in adult rodent liver [1, 6, 37]. The presence of Glut1 mRNA in the developing chick liver and the apparent absence of the protein are puzzling. Our findings might suggest that Glut1 liver mRNA is not translated. However, Glut1 protein is expressed in adult hepatocytes immediately surrounding terminal hepatic venules [38]. Therefore, the most likely explanations for our negative findings are (i) a level too low for Western blotting detection or (ii) that the liver Glut1 isoform differs from that found in other chicken tissues.

In rat heart, skeletal muscle, and brown adipose tissue, Glut1 is the predominant isoform expressed during fetal and early neonatal life but is gradually replaced with insulin-regulatable Glut4 [36, 39]. There are contradictory reports of Glut4 expression in avian tissues. Diamond and Carruthers [35] reported small amounts of Glut4 in adult pigeon leg muscle when large amounts of membrane protein were loaded in the gel. Thomas-Delloye et al. [40] identified Glut4 protein by Western blotting and functional studies in skeletal muscle of 5-week-old ducklings. However, Duclos et al. [13] used tissue from 5-week-old chicks and failed to find Glut4 protein in any tissue tested, including adipose and muscle, despite using two distinct anti-Glut4 antibodies. We were also unable to detect Glut4 protein in embryonic chick muscle or heart tissue. The structure of chicken Glut4 protein may be sufficiently different from that of rodents, humans and other avian species, and, depending on the specificity of the antibody used, chick Glut4 may elude detection by Western blotting.

Alternatively, if Glut4 protein is expressed developmentally late in the chicken, perhaps during the first few weeks post-hatching, then nontranslated Glut4 mRNA might be present in the embryo. Using low-stringency conditions and a mouse Glut4 cDNA, we were unable to detect Glut4 mRNA in chick embryonic heart or trunk/skeletal muscle. Furthermore, Glut4 mRNA could not be found in any of the adult chicken tissues examined (including muscle, adipose, and heart). Although not conclusive, these findings supply further evidence that Glut4 is not present in chickens. Glut4 mRNA sequences for humans and many mammalian species including dogs, pigs, goats, rodents, cows, and sheep are listed in the NCBI GenBank. However, there is currently no listing of an avian Glut4 cDNA or expressed sequence tag.

If Glut4 is absent from chickens, Glut1 might function as the primary heart and skeletal muscle glucose transporter. Recently, a new transporter, Glut8, has been described which appears to be insulin regulatable in rodent blastocysts [8]. If present in chickens, this protein and/or another unidentified Glut-X may be responsible for insulin-regulated glucose transport in chick tissues.

In summary, Glut1 appears to play an important role as the primary transporter in chick brain, skeletal muscle, and heart early in embryological development. Although Glut3 becomes a predominant glucose carrier in the mature brain, its importance, if any, in mature muscle is unclear. The role of Glut1 mRNA in the fetal liver is unknown. Glut4 protein and mRNA appear to be either absent from chick skeletal muscle and heart tissue, are below detection levels, or are sufficiently different structurally from the mammalian transporter as to elude detection. Therefore, the identity of the primary glucose transporter and/or the insulin-sensitive transporter in maturing chick muscle and heart remains an unresolved issue.

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1 Postic C., Leturque A., Printz R. L., Maulard P., Loizeau M., Granner D. K. et al. (1994) Development and regulation of glucose transporter and hexokinase expression in rat. Am. J. Physiol. **266:** E548–E559

2 Nagamatsu S., Sawa H., Nakamichi Y., Katahira H. and Inoue, N. (1994) Developmental expression of GLUT3 glucose transporter in the rat brain. FEBS Lett. **346:** 161–164
3 Bell G. I., Burant C. F., Takeda J. and Gould G. W. (1993) Structure and function of mammalian facilitative sugar transporters. J. Biol. Chem. **268:** 19161–19164
4 Pessin J. E. and Bell G. I. (1992) Mammalian facilitative glucose transporter family: structure and molecular regulation. Annu. Rev. Physiol. **54:** 911–930
5 Vannucci S. J., Maher F. and Simpson I. A. (1997) Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia **21:** 2–21
6 Burant C. F., Sivitz W. I., Fukumoto H., Kayano T., Nagamatsu S., Seino S. et al. (1991) Mammalian glucose transporters: structure and molecular regulation. Recent Prog. Horm. Res. **47:** 349–388
7 Doege H., Schurmann A., Bahrenberg G., Brauers A. and Joost H.-G. (2000) GLUT8, a novel member of the sugar transport facilitator family with glucose transport activity. J. Biol. Chem. **275:** 16275–16280
8 Carayannopoulos M. O., Chi M. M. Y., Cui Y., Pingsterhaus J. M., McKnight R. A., Mueckler M. et al. (2000) GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. Proc. Natl. Acad. Sci. USA **97:** 7313–7318
9 Matsumoto K., Akazawa S., Ishibashi M., Trocino R., Matsuo H., Yamasaki H. et al. (1995) Abundant expression of Glut1 and Glut3 in rat embryo during the early organogenesis period. Biochem. Biophys. Res. Commun. **209:** 95–102
10 Vannucci S. J., Seaman L. B., Brucklacher R. M. and Vannucci R. C. (1994) Glucose transport in developing rat brain: glucose transporter proteins, rate constants and cerebral glucose untilization. Mol. Cell. Biochem. **140:** 177–184
11 Carver F. M., Shibley I. A., Miles D. S., Pennington J. S. and Pennington S. N. (1999) Increased intracellular localization of brain GLUT-1 transporter in response to ethanol during chick embryogenesis. Am. J. Physiol. **277:** E750–E759
12 Eckstein L. W., Shibley I. A., Pennington J. S., Carver F. M. and Pennington S. N. (1997) Changes in brain glucose levels and glucose transporter protein isoforms in alcohol- or nicotine-treated chick embryos. Dev. Brain Res. **103:** 59–65
13 Duclos M. J., Chevalier B., Le Marchand-Brustel Y., Tanti J. F., Goddard C. and Simon J. (1993) Insulin-like growth factor-I-stimulated glucose transport in myotubes derived from chicken muscle satellite cells. J. Endocr. **137:** 465–472
14 White M. K. and Weber M. J. (1988) Transformation by the src oncogene alters glucose transport into rat and chicken cells by different mechanisms. Mol. Cell. Biol. **8:** 138–144
15 Wagstaff P., Kang H. Y., Mylott D., Robbins P. J. and White, M. K. (1995) Characterization of the avian GLUT1 glucose transporter: differential regulation of GLUT1 and GLUT3 in chicken embryo fibroblasts. Mol. Biol. Cell **6:** 1575–1589
16 De Pablo F., Scott L. A. and Roth J. (1990) Insulin and insulin-like growth factors in early development: peptides, receptors and biological events. Endocr. Rev. **11:** 558–577
17 McMurtry J. P. (1998) Nutritional and development roles of insulin-like growth factors in poultry. J. Nutr. **128:** 302S–305S
18 Swanson D. J., Daniels H., Meyer E. M., Walker D. W. and Heaton M. B. (1994) Chronic ethanol alters CNS cholinergic and cerebellar development in chick embryos. Alcohol **11:** 187–194
19 Heaton M. B., Swanson D. J., Paiva M. and Walker D. W. (1993) Ethanol exposure affects trophic factor activity and responsiveness in chick embryo. Alcohol **9:** 161–166
20 Gilani S. H. and Persaud T. V. N. (1986) Chick embryo development following exposure to caffeine and nicotine. Anat. Anz. Jena **161:** 23–26
21 Beeker K., Smith C. P. Jr and Pennington S. N. (1992) Effect of cocaine, ethanol or nicotine on ornithine decarboxylase activity in early chick embryo brain. Dev. Brain Res. **69:** 51–57
22 Seran G. F. and Sparber S. B. (1988) Metabolism of methadone by chicken embryos prevents induction of chronic opioid-type dependence after a single injection: use of osmotic pumps for continuous infusion. Pharmacol. Biochem. Behav. **30:** 357–363
23 Rohrer H. (1991) The role of growth factors in the control of neurogenesis. Eur. J. Neurosci. **2:** 1005–1015
24 Oikawa T., Ogasawara H., Sano H., Shibata K. and Omura S. (1994) Possible functional groups responsible for inhibition of in vivo angiogenesis by herbimycin A. Biol. Pharm. Bull. **17:** 1430–1432
25 Hlywka J. J., Beck M. M. and Bullerman L. B. (1997) The use of the chicken embryo screening test and brine shrimp *(Artemia salina)* bioassays to assess the toxicity of fumonisin B1 mycotoxin. Food Chem. Toxicol. **35:** 991–999
26 Calver G. A., Burton W. W. and Gardell C. Y. (1993) Chick embryo, a model to study the lethal activity of pertussis toxin, infectivity of *Bordetella pertussis,* and their neutralization by immune sera. Can. J. Microbiol. **39:** 759–766
27 Nishigori H., Mizumura M. and Iwatsuru M. (1992) The hen's fertile egg screening test (HEST): a comparison between the acute toxicity for chick embryos and rodents of 20 drugs. Cell Biol. Toxicol. **8:** 255–265
28 White M. K., Rall T. B. and Weber M. J. (1991) Differential regulation of glucose transporter isoforms by the src oncogene in chicken embryo fibroblasts. Mol. Cellular Biol. **11:** 4448–4454
29 Neuffer P. D., Carey J. O. and Dohm G. L. (1993) Transcriptional regulation of the gene for glucose transporter GLUT4 in skeletal muscle: effects of diabetes and fasting. J. Biol. Chem. **268:** 13824–13829
30 Girard J., Ferre P., Pegorier J. P. and Duee P. H. (1992) Adapatations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiol. Rev. **72:** 507–562
31 Mueckler M. (1994) Facilitative glucose transporters. Eur. J. Biochem. **219:** 713–725
32 Devaskar S., Zahm D. S., Holtzclaw L., Chundu K. and Wadzinski B. E. (1991) Developmental regulation of the distribution of rat brain insulin-insensitive (glut 1) glucose transporter. Endocrinology **129:** 1530–1540
33 Sadiq F., Holtzclaw L., Chundu K., Muzzafar A. and Devaskar S. (1990) The ontogeny of the rabbit brain glucose transporter. Endocrinology **126:** 2417–2424
34 Kayano T., Fukomoto H., Eddy R. L., Fan Y.-S., Byers M. G., Shows T. B. et al. (1988) Evidence for a family of human glucose-like proteins. J. Biol. Chem. **263:** 15245–15248
35 Diamond D. and Carruthers A. (1993) Metabolic control of sugar transport by derepression of cell surface glucose transporters. J. Biol. Chem. **268:** 6437–6444
36 Santalucia T., Camps M., Castello A., Munoz P., Nuel A., Testar X. et al. (1992) Developmental regulation of GLUT-1 (erythroid/hepG2) and GLUT-4 (muscle/fat) glucose transporter expression in rat heart, skeletal muscle, and brown adipose tissue. Endocrinology **130:** 837–846
37 Hogan A., Heyner S., Charron M. J., Copeland N. G., Gilbert D. J., Jenkins N. A. et al. (1991) Glucose transporter gene expression in early mouse embryos. Development **113:** 363–372
38 Tal M., Schneider D. L., Thorens B. and Lodish H. F. (1990) Restricted expression of the erythroid/brain glucose transporter isoform to perivenous hepatocytes in rats. J. Clin. Invest. **86:** 986–992
39 Wang C. and Hu S.-M. (1991) Developmental regulation in the expression of rat heart glucose transporters. Biochem. Biophys. Res. Commun **177:** 1095–1100
40 Thomas-Delloye V., Marmonier F., Duchamp C., Pichon-Georges B., Lachuer J., Barre H. et al. (1999) Biochemical and functional evidences for a Glut4 homologous protein in avian skeletal muscle. Am. J. Physiol. **277:** R1733–R1740