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GLUT 1-GLUCOSE TRANSPORTER PROTEIN IN ADULT AND FETAL MOUSE LUNG*#

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We observed a ~45-50 kD GLUT 1 protein in mouse lung homogenates and demonstrated a greater abundance in fetus compared to adult. In situ immunohistochemical analysis demonstrated GLUT 1 expression only in the perineural sheath of nerves. While the trapped fetal red blood cells expressed GLUT 1 abundantly, adult red blood cells were devoid of GLUT 1. No GLUT 1 was evident in fetal and adult lung alveolar and bronchiolar epithelial cells, vascular endothelial cells and the lung mesenchymal elements. Thus, GLUT 1 is not the major lung glucose transporter.

Glucose forms a substrate for mammalian lung oxidative (1) and glycogen metabolism (2,3), and is essential for the process of cellular growth and differentiation (4). Various investigators have suggested an intricate relationship between

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Abbreviations: GLUT = accepted terminology for a glucose transporter isoform. RBC = Red Blood Cell.

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lung glucose-glycogen metabolism and surfactant phospholipid synthesis (2,3). In various tissues, glucose enters cells via membrane associated glycoproteins termed glucose transporters (5). Of the five major facilitative types cloned, GLUT 1 the human erythrocyte/Hep G2/rat brain isoform (6) is expressed in variable amounts in most tissues examined, thereby suggesting an "ubiquitous distribution" for this isoform (7). In fact, developmental studies in rat indicate GLUT 1 to be prominently expressed by all fetal tissues examined including skeletal muscle and liver (7,8), which later mature to mainly express a non-GLUT 1 type of transporter isoform. Similar to all fetal tissues, fetal rat lung has previously been observed to express GLUT 1 mRNA more abundantly than the adult counterpart (7), however GLUT 1 protein in mammalian lung has not been characterized. Therefore, we undertook this study to detect the presence of GLUT 1 protein in the mammalian fetal and adult lung, and to characterize the cellular localization of GLUT 1.

MATERIALS AND METHODS

Animals: Non-pregnant adult and date pregnant (term ~19 days) C57BL/6NTacFBR mice were obtained from Taconic Laboratories, housed in individual cages at the Pediatric Research Institute, and maintained in 12 hour light and dark cycles. The NIH guidelines in the care and use of all animals were followed.

Tissue Preparation: Homogenates from non-pregnant adult lungs (n=8) or pooled lungs from fetal pups within a litter (n=8) were used.

Western Blot Analysis: 100 µg of fetal and adult lung homogenate proteins were separated by 10% discontinuous SDS-polyacrylamide gel electrophoresis. Western blot analysis was performed as previously described (9), employing 2µg/ml of a protein A affinity purified anti-rat GLUT 1 IgG (R493) raised in rabbit against the C-terminus of the GLUT 1 peptide (9) (a gift from Dr. M. Mueckler, Washington University, St. Louis, MO.), as the primary antibody. Autoradiography was undertaken for variable lengths of time until a sharp discernible protein band appeared.

Immunohistochemistry: Six micron thick paraffin and frozen sections of fetal and adult mouse lungs were subjected to immunohistochemical analysis as previously described (10).

Varying concentrations ranging from 1:100 to 1:100,000 of R493 were employed as the primary antibody. Pre-immune serum, omission of the primary antibody and pre-absorption of the primary antibody with GLUT 1 COOH-peptide were used as the appropriate controls.

RESULTS

Fetal and adult lung homogenates demonstrated the presence of a ~45 kD GLUT 1 protein by Western blot analysis (figure 1). The expression of GLUT 1 was more abundant in fetal lung (figure 1A) than in the adult (figure 1B), based on the length of autoradiographic exposure required to obtain optimal protein band clarity. Examination of adult lung sections (figure 2 E,F,G,H) revealed GLUT 1 immunoreactivity only in Schwaan cells which were located in the perineurium of peribronchiolar (figure 2 F) and perivascular (figure 2 H) nerves. Adult RBCs demonstrated no immunoreactivity for GLUT 1 (figure 2 H). In fetal lung sections (figure 2 A,B,C,D) on the other hand, in addition to the Schwaan cells, GLUT 1 was present in RBCs which were trapped in large numbers within the lungs (figure 2D). Unlike the brain (16-18), the endothelial cells lining fetal (figure 2B) and adult

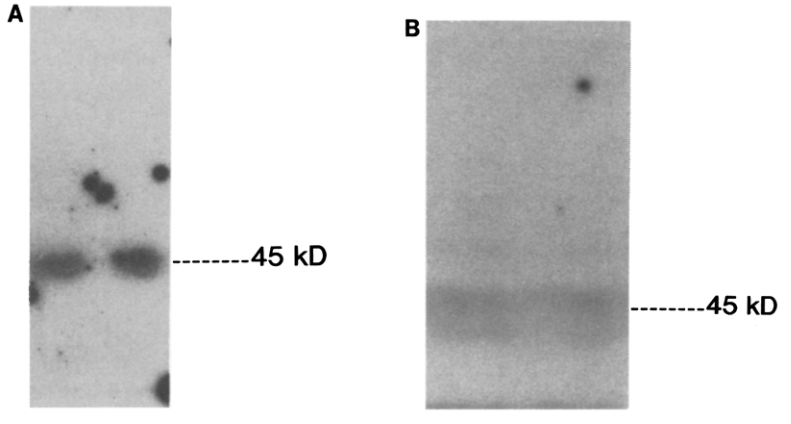


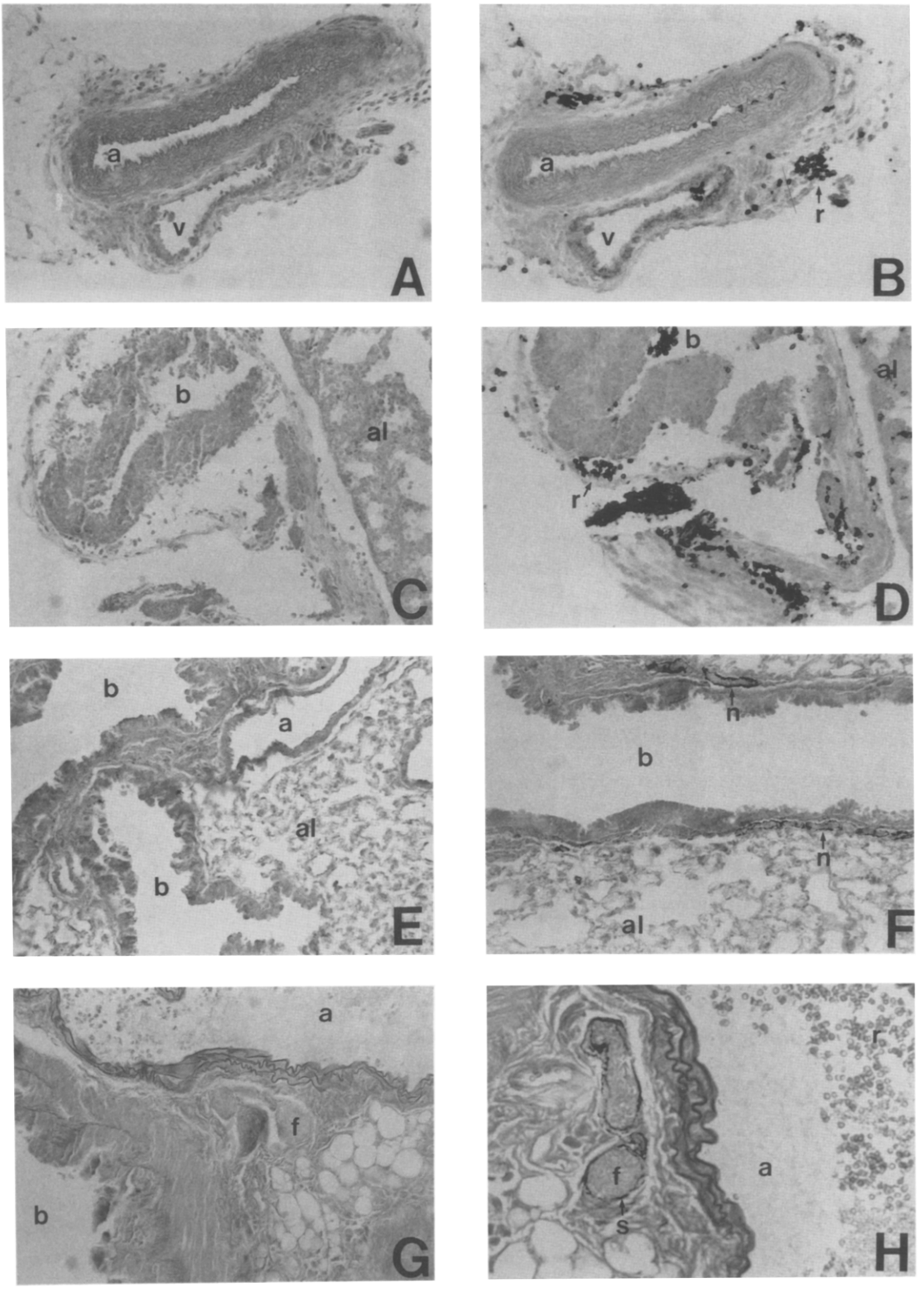
Figure 1. A representative autoradiograph of a Western blot demonstrating a ~45 kD GLUT 1 protein band in 100 µg of lung homogenates from the fetus (A) and adult (B) following two and six days respectively of exposure to X-ray film.

lung vasculature (figure 2 H) inclusive of arteries, veins and capillaries did not express GLUT 1. Furthermore, adult and fetal lung epithelial cells lining the alveoli (figure 2 D, F) and bronchioles (figure 2 D,F) were negative for GLUT 1 immunoreactivity. Mesenchymal elements such as the smooth muscle of bronchioles and connective tissue were likewise negative.

DISCUSSION

We have demonstrated no significant GLUT 1 glucose transporter isoform expression in fetal and adult lung cells. Although lung homogenates revealed more abundant GLUT 1 in the fetus than in the adult, localization demonstrated that it was primarily expressed by fetal RBCs, with lung parenchymal cells being negative. Adult RBCs were also negative, similar to findings described in pigs (11) and rabbits (12), where increasing maturity resulted in a loss of the RBC GLUT 1 protein. Thus one can explain the higher and variable expression of GLUT 1 in fetal lung homogenates when compared to the adult, based on the large numbers of fetal RBCs trapped within the tissue. Fetal RBCs may similarly be responsible for the apparent increase in GLUT 1 expression in many other fetal tissues (7,8), thus conveying a false sense of its "ubiquitous distribution". The only cell type other than the fetal RBCs which expressed GLUT 1 in the adult and fetal lungs was the Schwaan cell. We have previously shown that GLUT 1 is mainly expressed by the central

Figure 2. Immunohistochemical Analysis of Lung Sections: Fetal (A,B,C,D) and adult (E,F,G,H) lung sections treated with either the preimmune or anti-GLUT 1 IgG preabsorbed with GLUT 1 peptide (A,C,E,G), or with a 1:10,000 dilution of anti-GLUT 1 IgG (B,D,F,H). Vasculature consisting of veins (v) and arteries (a) (B), alveolar (al) and bronchiolar (b) epithelium (D,F,H) do not express GLUT 1. While adult RBCs (H) do not, fetal RBCs (r) (B) demonstrate GLUT 1 immunoreactivity (magnification 155x). While the peribronchiolar (F) and perivascular (H) nerve (n) fibers (f) do not, Schwaan cells (s) of the perineural sheath demonstrate GLUT 1 (F: magnification 150x; H: 390x).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nervous system glial cells and not neurons (13). Schwaan cells, which are located in the peripheral nervous system are of glial origin. Hence our present observation in the peripheral nervous system is consistent with our observations in brain (13) and that of others (14), who have observed the presence of GLUT 1 in the perineural sheaths of adult skeletal muscle (14). GLUT 1 mRNA has reportedly been higher in the fetal rat lung compared with the adult (7). The absence of immunostaining for GLUT 1 in lung cells, suggests that the GLUT 1 mRNA previously described in lung homogenates (7) may be of Schwaan cell origin. While adult RBCs contain insignificant amounts of mRNA, because fetal RBCs are nucleated, they may contain higher levels of mRNA. It is therefore possible that it is the RBCs within the fetal lungs that contribute to the increased GLUT 1 mRNA levels of fetal lung homogenates (7). However, one cannot exclude a significantly lower level of GLUT 1 expression in lung cells than in Schwaan cells or fetal RBCs.

Our present studies suggest that in the lung glucose transport into alveolar and bronchiolar epithelial cells takes place largely via a non-GLUT 1 glucose transporter isoform. We have determined the absence of immunolocalizable insulin-dependent GLUT 4 (9) employing R820, the previously characterized anti-GLUT 4 antibody (9) (a gift from Dr. M. Mueckler, Washington University, St. Louis, Mo), in both the adult and fetal mouse lungs. While the presence of a sodium-glucose cotransporter system in alveolar epithelial cells has been demonstrated (15) the presence of a facilitative glucose transporter in these cells has varied, depending on whether the experiments were performed in situ or in vitro. Type II alveolar cells maintained in culture demonstrated a facilitative transporter (16), however, it is possible that this observation reflects changes induced by

culturing conditions rather than processes in-situ involving the whole lung, thus casting doubt on its physiological significance. The mechanism by which glucose enters the lung epithelial cells and contributes towards pulmonary glycogen (2,3) and thereby the glycerol backbone of disaturated phosphatidylcholine (3) remains to be determined.

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