Vol. 188, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1992 Pages 149-154

Distribution of GLUT3 Glucose Transporter Protein in Human Tissues

Peter R. Shepherd[1], Gwyn W. Gould[2], Caroline A. Colville[2], Scott C. McCoid[3], E. Michael Gibbs[3] and Barbara B. Kahn[1*]

[1] Charles A. Dana Research Institute and Harvard Thorndike Laboratory, Beth Israel Hospital and Harvard Medical School, 330 Brookline Ave, Boston, MA 02215

[2] Department of Biochemistry, University of Glasgow, Glasgow, Scotland

[3] Pfizer Central Research, Eastern Point Rd, Groton, Connecticut

Received August 24, 1992

SUMMARY: To investigate the tissue distribution of the GLUT3 glucose transporter isoform in human tissue we produced affinity purified antibodies to the COOH terminus of the human GLUT3. Both antibodies recognize a specific GLUT3 band in oocytes injected with GLUT3 mRNA but not in those injected with H_2O or GLUT1, 2, 4, 5 mRNA. This immunoreactive band in GLUT3 injected oocytes is photolabelled by cytochalasin-B in the presence of L- but not D-glucose indicating that it is a glucose transporter. A high cross reactivity between the human GLUT3 antibodies and a 43 kDa cytoskeletal actin band was identified in all oocyte lysates and many human tissues. However, the specific GLUT3 band could be distinguished from the actin band by carbonate treatment which preferentially solubilized the actin band. Using these antibodies we show that GLUT3 is present as a 45-48 kDa protein in human brain with lower levels detectable in heart, placenta, liver and a barely detectable level in kidney. No GLUT3 was detected in membranes from any of 3 skeletal muscle groups investigated. We conclude that a major role of GLUT3 in humans is as the brain neuronal glucose transporter.

Glucose is a key metabolite in all human tissues and its entry into cells occurs by facilitated diffusion through specific transmembrane glucose transporter proteins. Understanding this transport process at the molecular level has been greatly advanced by recent molecular cloning studies which reveal a family of at least five isoforms of the facilitated glucose transporter protein (GLUT1-5). These exhibit unique tissue distributions and kinetic properties (1, 2, 3); GLUT1 is widely distributed in the body, GLUT2 is a low affinity transporter expressed mainly in liver, kidney, intestine and pancreatic beta-cells; GLUT4 is the highly insulin sensitive glucose transporter located predominantly in heart, muscle and adipose tissue; and GLUT5 protein is widely distributed (4) and has recently been found to be largely a fructose transporter (5). GLUT3 has been cloned from human (6), mouse (7) and chicken (8) and expression of human GLUT3 in

*To whom correspondence should be addressed.

0006-291X/92 $4.00

Xenopus oocytes has clearly demonstrated that it is a glucose transporter (9). GLUT3 mRNA and protein appear to be confined to the brain in rodents (7, 10) but in humans the mRNA is widely distributed (3, 6) indicating GLUT3 could play a role in glucose metabolism in many tissues. Difficulties have been encountered in identifying GLUT3 protein in human tissues (11, 12) but a recent study has identified GLUT3 in human brain membranes (11). GLUT3 protein has not been identified in any other human tissues. Here we report the development of two antibodies raised to the COOH terminal of human GLUT3 sequence and we use them to demonstrate the distribution and relative abundance of GLUT3 protein in human tissues.

MATERIALS AND METHODS

Preparation of membranes: Post nuclear membrane fractions were prepared from human and rodent tissues as previously described (4). In some cases these membranes were treated with a 100 fold volume excess of 100 mM $NaCO_3$ (pH 11.5) for 15 minutes on ice following which membranes were pelleted at 100 000 rpm in a TL100.2 rotor for 30 minutes at 4 °C. Protein content was reassayed using the bichinconinic acid method (Sigma). Subcellular fractionation of rat muscle was carried out as previously described (13).

Antibodies: A peptide was synthesized corresponding to the 14 COOH terminal amino acids of the published sequences of the human isoform of GLUT3 (NH2-MNSIEPAKETTTNV-COOH). Antiserum was produced from 2 rabbits (R1672 & R1673) and antibodies were affinity purified as previously described (10). Actin antiserum was purchased from Sigma and an antibody to the COOH terminal of GLUT4 was the gift of Dr Mike Mueckler.

Oocyte expression studies: *Xenopus* oocytes were isolated and injected with H_2O or 15 ng of GLUT 1, 2, 3, 4 or 5 mRNA as described (9). 48 hours after injection oocytes were homogenized in 2% Triton-X-100 in 25mM sodium phosphate buffer (pH 7.4) containing 200 µM di-isopropylflurophosphate and 1 µg/ml pepstatin A, 20 oocytes in 1 ml. Following centrifugation at 2000 x g for 5 minutes to pellet the yolk sac, the solubilized proteins were precipitated using acetone, and resuspended in Laemelli loading buffer containing 1% SDS. Following SDS-PAGE proteins were transferred to nitrocellulose at 350 mAmps for 2-3 hours and immunoblotted as described below.

Cytochalasin-B Photoaffinity Labelling: Groups of 100 oocytes injected with GLUT3 mRNA or with H_2O were suspended in homogenization buffer (83mM NaCl, 1mM $MgCl_2$, 10 mM HEPES ph 7.9 also containing protease inhibitors) and homogenized with 20 hand driven strokes in a glass/teflon homogenizer. Yolk was pelleted by centrifuging at 1000 x g for 5 minutes and membranes were pelleted from the supernatant by centrifugation at 100 000 x g for 90 minutes. Membranes were photolabelled with [^{3}H]cytochalasin-B in the presence of cytochalasin-E and D- or L-glucose as previously described (4). Labelled membranes were run on SDS-PAGE, bands were cut from the gel and ^{3}H incorporation in each band was determined.

Immunoblotting: Membrane samples and rabbit actin (US Biochemicals) were analysed by SDS-PAGE and immunoblotted as previously described (4) .

RESULTS AND DISCUSSION

Antibody affinity purified from rabbit R1672 recognizes a broad band at about 50-65 kD in lysates from oocytes injected with GLUT3 mRNA but not those injected with H_2O or with mRNA for GLUT1, 2, 4 or 5 (Fig. 1). In lysates from all oocytes, including those injected with water, prominent bands were also seen at 43 kD and 97 kD (Fig. 1). To demonstrate that 50-65 kD band recognized by the GLUT3 antibody was a glucose transporter, plasma membranes from GLUT3 injected oocytes were photolabelled by ^{3}H-cytochalasin-B in the presence of cytochalain-E and L- or D-glucose. When the photolabelled material was run on a gel the peak of D-glucose inhibitable photolabelling (Fig. 2) corresponded exactly with the size of the immunoreactive band previously seen (Fig. 1), thus, indicating that GLUT3 protein is a facilitated glucose transporter. This is supported by previous data demonstrating that similarly injected oocytes transport D-glucose at a

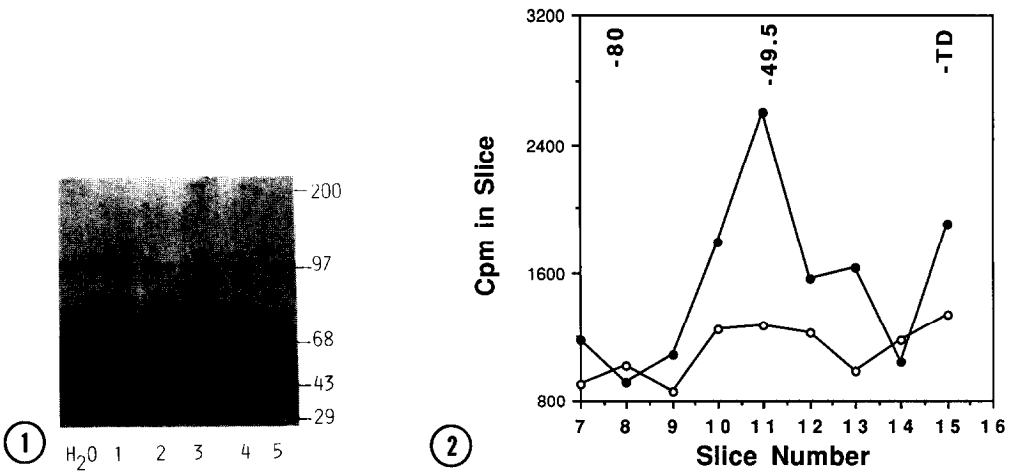

Figure 1. Western blot of protein extracts from oocytes injected with H_2O or with mRNA of 1. GLUT1, 2. GLUT2, 3. GLUT3, 4. GLUT4, 5. GLUT5 probed with anti-human GLUT3 antibody R1672. Protein was extracted and western blotted as described in Materials and Methods.

Figure 2. Membranes were prepared from oocytes injected with GLUT3 mRNA. These membranes were photolabelled with [^{3}H]cytochalasin-B in the presence of cytochalasin-E and D-(-o-) or L- (-•-) glucose as described in Materials and Methods. Labelled membranes were run on SDS-PAGE, bands were cut from the gel and ^{3}H incorporation in each band was determined and plotted against molecular weight markers as indicated.

much higher rate than water injected controls (9) and this glucose transport is inhibitable by cytochalasin-B (6).

In human tissues both the R1672 and R1673 antibodies recognize a ~45-48 kD band in post nuclear membranes from human brain. Similar but weaker bands are seen in post nuclear membranes from liver, placenta and heart while a very faint band is seen in post nuclear membranes from kidney (Fig. 3 Panel A). Both R1672 and R1673 also recognize a prominent

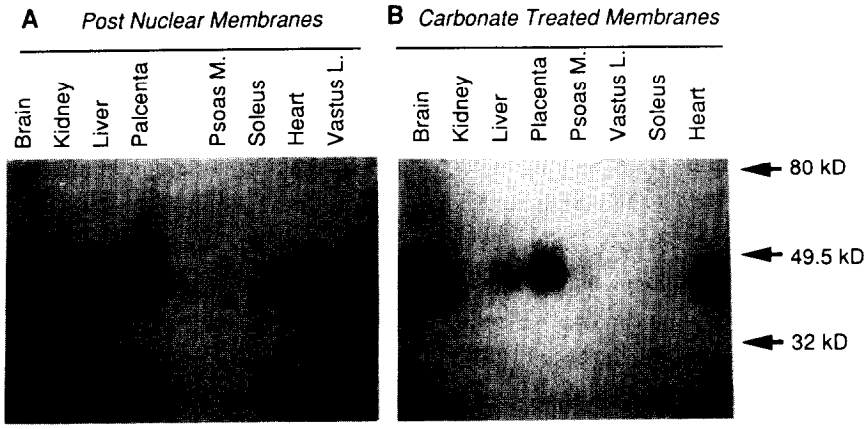

Figure 3. GLUT3 protein distribution in human tissue determined by western blotting using anti-human GLUT3 antibody R1673. **A.** 100 µg of post nuclear membranes from the indicated human tissues (blank lane is molecular weight markers lane) **B.** 60 µg protein of carbonate treated post nuclear membranes from the indicated human tissues.

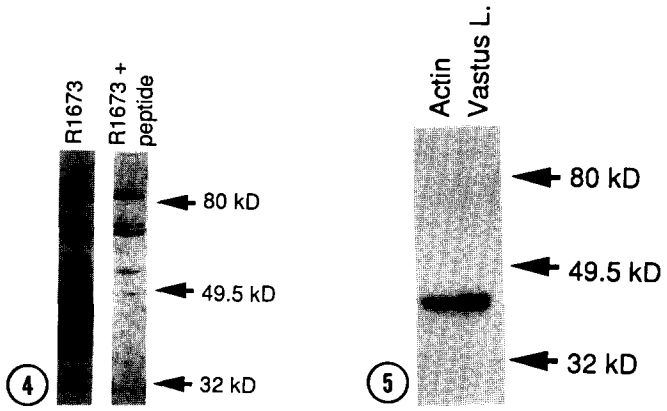

Figure 4. Western blot of 100 µg human brain post nuclear membranes with anti-human GLUT3 antibody R1673 or with R1673 preincubated with 100 µg/ml GLUT3 peptide as described in Materials and Methods.

Figure 5. Western blot of 25 µg rabbit actin and 100 µg human vastus lateralis muscle homogenate probed with R1672 anti-human GLUT3 antibody .

band of 43 kD in post nuclear membranes from brain, kidney, liver, placenta, skeletal muscle and heart. No 45-48 kD immunoreactive bands were observed in post nuclear membranes from human vastus lateralis muscle, rectus abdominus muscle or psoas major muscle (Fig. 3). The ~45-48 kD bands become more prominent when the post nuclear membranes are stripped of non integral membrane proteins using pH 11.5 $NaCO_3$ treatment while the 43 kD band is greatly reduced following this treatment (Fig. 3 Panel B). In competition studies in human brain membranes GLUT3 antibodies were preincubated with 100 µg/ml of the GLUT3 peptide and both the 45-48 kD band and the 43 kD band were ablated (Fig. 4).

The observations that ~45-48 kD band is competable, is an integral membrane protein and corresponds to a similar band seen in oocytes only when injected with GLUT3 mRNA argues strongly that this band represents GLUT3 protein. In contrast, the fact that the 43 kD competable band was greatly diminished when human brain, kidney, liver, placenta, heart and muscle post nuclear membrane fractions were treated with carbonate indicates that this smaller band is not an integral membrane protein and is unlikely to represent GLUT3. Further evidence for this was the fact that R1672 recognized a similar intense 43 kD band in rat muscle homogenates. This was not expected as there is only a 54 % amino acid sequence homology between human and rodent GLUT3 isoforms in the COOH terminal region to which the antibody was raised (6, 7) and in rodent brain the human GLUT3 antibodies do not cross react with the specific GLUT3 bands previously identified in rodent brain using a mouse GLUT3 antibody (10) (data not shown). Furthermore, using these mouse GLUT3 antibodies, we and others have previously reported that GLUT3 is absent from muscle in rodents (10, 11). On investigation of subcellular fractions from rat muscle the 43 kD band was localized to the cytosol and none was seen in plasma membranes, whereas in the same preparation GLUT4 was found solely in membrane fractions (data not shown). This combined with the observations that the 43 kD band was present in all oocyte lysates, regardless of whether oocytes had been injected with GLUT3 mRNA, and that in plasma

membranes of GLUT3 injected oocytes the 53 kD band was seen but the 43 kD band was not (data not shown), was further evidence that the 43 kD band was a cytosolic protein and not GLUT3.

Actin is a primarily cytosolic protein of 43 kD which is present in many tissues and has a high interspecies sequence homology. Thus it was a candidate for the 43 kD immunoreactive band detected by the GLUT3 antiserum (Fig 1 & 3). When rabbit actin was western blotted with the GLUT3 antibodies a 43 kD band is detected that is identical to that seen in human muscle (Fig. 5). Further, actin antibodies detect a 43 kD band identical to that recognized by the GLUT3 antibodies in human muscle (data not shown). These data indicate that the GLUT3 antibodies cross react with actin. The extraordinary cross reactivity of actin with these antibodies and the fact that this reaction is competed by the GLUT3 peptide could be explained by a highly homologous 5 amino acid sequence shared by the human GLUT3 COOH terminal antigenic peptide (ETTIN) and a highly conserved region in different actin isoforms (ETTYN) (14, 15). This finding has important implications for two recent investigations of GLUT3 tissue distribution which have used antibodies raised to similar human GLUT3 COOH terminal peptides (11, 12). In both these studies immunoreactive bands of about 43 kD are seen in western blots using these antibodies raising the possibility that this 43 kD band also represents cross reactivity with actin. Such cross reactivity would be particularly misleading in immunolocalization studies of GLUT3 such as those performed in rat and dog brain (12) where actin labelling may have been mistaken for GLUT3 resulting in erroneous conclusions about the distribution of GLUT3 protein in brain.

We conclude that the ~45-48 kD competable band recognized by the GLUT3 antibodies is in fact GLUT3 and we demonstrate that GLUT3 protein is predominantly found in brain in humans, with lower amounts found in heart, liver, placenta and kidney. These results are somewhat in discordance with the relative abundance of GLUT3 mRNA in different tissues. For example it has been reported that the GLUT3 mRNA levels in placenta and kidney are both approximately 50% that of brain (3, 6, 16) but in the current study we find the level of GLUT3 protein in brain to be much greater than that in placenta which in turn has far more than kidney. Also detectable levels of GLUT3 mRNA were previously reported in human muscle although in the current study we were unable to detect GLUT3 protein in post nuclear membranes from any of 3 human skeletal muscle groups. Further highlighting the discordance between GLUT3 mRNA and protein levels is a recent study which failed to locate GLUT3 protein in human adipose tissue (11) despite significant levels of GLUT3 mRNA previously being reported in that tissue. This raises the possibility of significant post transcriptional regulation of GLUT3 expression or alternately of widely differing levels of GLUT3 expression between individuals. As GLUT3 is abundant in neurons of the brain it is possible that it is also in peripheral neurons and variations in levels of contamination of other tissues by peripheral neurons could contribute to discrepencies seen between GLUT3 mRNA and protein data.

The tissue distribution of GLUT3 suggests a constitutive role for this transporter isoform, especially in tissues with a constant demand for glucose. The absence of GLUT3 from the tissues responsible for insulin stimulated glucose disposal (i.e. muscle and fat) suggests that it is not an insulin responsive glucose transporter. GLUT3 is seen in heart which is an insulin responsive tissue but heart has a constant high demand for energy which may explain the presence of significant amounts of this constitutive glucose transporter as well as GLUT1 and the highly

insulin responsive isoform, GLUT4. The low level of GLUT3 expression in liver and kidney could indicate a specialized localization for GLUT3 in these tissues. A similar case exists for GLUT1 in liver where the high K_m transporter, GLUT2, is expressed in all hepatocytes while expression of the low K_m GLUT1 is restricted to a perivenous hepatocytes (17).

In summary our data show that in humans GLUT3 protein is predominantly expressed in the brain. In brain GLUT3 is mainly localized in neurons (7, 10, 11) which have a constant demand for glucose. Importantly other glucose transporter isoforms appear to be absent from brain neurons (18) suggesting that in humans GLUT 3 is the major transporter responsible for constitutive glucose transport in neurons of the brain.

ACKNOWLEDGMENTS

We thank Dr Peter Arvan for helpful discussions and Dr Mike Mueckler for GLUT4 antiserum. This work was supported by NIDDK grant # DK43051 (BBK), JDF grant # 189833 (BBK), the Science and Engineering Research Council Biological Membranes Initiative (GWG), The Scottish Hospitals Endowment Research Trust (GWG); GWG is a Lister Institute of Preventative Medicine Research Fellow; BBK is the recipient of a Capps Scholar Award from Harvard Medical School.

REFERENCES

1. Kahn, B. B. (1992) J. Cell. Biochem. **48**, 122-128
2. Gould, G. W. and Bell, G. I. (1990) TIBS **15**, 18-23
3. Bell, G. I., Kayano, T., Buse, J. B., Burant, C. F., Takeda, J., Lin, D., Fukumoto, H. and Seino, S. (1990) Diabetes Care **13**, 198-208
4. Shepherd, P. R., Gould, G. W., Wesslau, C., Gibbs, E. M. and Kahn, B. B. (1992) Diabetes, **In Press**
5. Burant, C. F., Takeda, J., Brot-Laroche, E., Bell, G. I. and Davidson, N. O. (1992) J. Biol. Chem. **267**, 14523-14526
6. Kayano, T., Fukumoto, H., Eddy, R. L., Fan, Y., Byers, M. G., Shows, T. B. and Bell, G. I. (1988) J. Biol. Chem **263**, 15245-15248
7. Nagamatsu, S., Kornhauser, J. M., Burant, C. F., Seino, S., Mayo, K. E. and Bell, G. I. (1992) J. Biol. Chem **267**, 467-472
8. White, M. K., Rall, T. B. and Weber, M. J. (1991) Mol. Cell. Biol. **11**, 4448-4454
9. Gould, G. W., Thomas, H. M., Jess, T. J. and Bell, G. I. (1991) Biochemistry **30**, 5139-5145
10. Gould, G. W., Brant, A. M., Kahn, B. B., Shepherd, P. R., McCoid, S. C. and Gibbs, E. M. (1992) Diabetalogia **35**, 304-309
11. Maher, F., Vannucci, S., Takeda, J. and Simpson, I. A. (1992) Biochem. Biophys. Res Comm. **132**, 703-711
12. Gerhart, D. Z., Broderius, M. A., Borson, N. D. and Drewes, L. R. (1992) Proc. Natl. Acad. sci. USA **89**, 733-737
13. Hirshman, M. F., Goodyear, L. J., Wardzala, L. J., Horton, E. D. and Horton, E. S. (1990) J. Biol. Chem. **265**, 987-991
14. Vandekerckove, J. and Weber, K. (1979) Differentiation **14**, 123-133
15. McHugh, K. M. and Lessard, J. L. (1988) Nucl. Acids. Res. **16**, 4167
16. Yano, H., Seino, Y., Inagaki, N., Hinokio, Y., Yamamoto, T., Yasuda, K., Masuda, K., Someya, Y. and Imura, H. (1991) Biochem. Biophys. Res. Comm. **174**, 470-477
17. Tal, M., Kahn, B. B. and Lodish, H. F. (1991) Endocrinology **129**, 1933-1941
18. Bagley, P. R., Tucker, S. P., Nolan, C., Lindsay, J. G., Davies, A., Baldwin, S. A., Cremer, J. E. and Cunningham, V. J. (1989) Brain Res. **499**, 214-224