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ERYTHROCYTE/HEPG2-TYPE GLUCOSE TRANSPORTER IS CONCENTRATED IN CELLS OF BLOOD-TISSUE BARRIERS

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In search of possible diverse roles of glucose transporters(GT's), we examined whether any GT's are present in blood-tissue barriers where selective flow of glucose from blood to tissue cells is critically important. We found in rat that the erythrocyte/HepG2-type GT is localized in all the limiting plasma membranes known to serve as blood-tissue barriers, whether the barriers are endothelial type(brain, iris, inner retina, peripheral nerve) or epithelial type(choroid plexus, ciliary body, outer retina, peripheral nerve, placenta), except for plasma membranes in testis and thymus where no appreciable amount of the GT was found. The erythrocyte/HepG2-type GT may play a vital role for the entry of glucose into these firmly guarded tissues.

Glucose transporters(GT's) are integral membrane proteins that transport glucose across the plasma membrane(1,2,3). Five isoforms of GT or GT-like proteins of facilitated diffusion and a single Na^+-dependent GT have been so far identified and sequenced(4,5,6). They are considered to play important roles in providing glucose to various types of cells, regulation of blood glucose levels, absorption of glucose in the intestine and kidney, and regulation of insulin secretion in the pancreas(6,7).

Various experiments with the use of dyes and electron-dense tracers have established the concept of blood-tissue barriers in brain(8,9), cerebrospinal fluid(9), peripheral nerve(10), retina and aqueous humor of the eye(11),

Abbreviations: GT,glucose transporter;PBS,phosphate-buffered saline;SDS,sodium dodecylsulfate;PAGE,polyacrylamide gel electrophoresis;PMSF,phenylmethylsulfonyl fluoride;EDTA,ethylenediaminetetraacetic acid;DAPI,4',6-diamidino-2-phenylindole.

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placenta(12), seminiferous tubules of the testis(13), and thymus(14). Electron microscopic observations revealed that endothelial or epithelial cells connected by tight junctions are the structural basis of these blood-tissue barriers. The specific environment in these specialized tissues and organs is maintained by blood-tissue barriers, where only selective flow of a limited number of substances such as glucose and amino acids is allowed(8,9,12). We show here that the erythrocyte/HepG2-type GT, one isoform of facilitated diffusion GT(4), is concentrated in the plasma membranes of the limiting endothelial or epithelial cells in the blood-tissue barriers.

MATERIALS AND METHODS

The antibody used was rabbit anti-human erythrocyte GT antibody(3,15) or anti-peptide antibody(serum) to the synthetic peptide corresponding to the C-terminal(amino acids 480-492) or amino acids 474-485 of the deduced sequence of the HepG2 GT(4). The anti-peptide antibodies were raised in rabbits by the injections of the peptide-keyhole limpet hemocyanin conjugates(16,17). All three antibodies showed no appreciable difference in immunoblotting or immunohistochemical studies.

Tissues and organs were taken from ether-anesthetized Sprague-Dawley rats 4-8 weeks old. The brain vessel enriched fraction was prepared according to the published method(18). All the samples were homogenized in phosphate-buffered saline(PBS) containing a protease inhibitor mixture(50 μg/ml aprotinin, 2 μg/ml pepstatin, 2 μg/ml leupeptin, 2 mM PMSF and 5 mM EDTA), mixed with Laemmli sampling solution, and incubated at 37°C for 30 min. SDS-PAGE, immunoblotting and the detection of the GT with [^{125}I]-protein A were performed as described(19), with minor modifications: proteins were blotted onto a polyvinylidine difluoride membrane(Immobilon P, Millipore); and autoradiography was performed with Fuji HR films and Fuji HR-16 intensifier at -80°C for 5 hrs to 2 days.

For immunofluorescence microscopy, tissues were fixed in 3% formaldehyde-PBS. Semithin frozen(0.5-1.5 μm thick) and cryostat sections(10 μm thick) were cut and incubated with anti-GT antibodies(or serum), and then with rhodamine-labeled affinity-purified goat anti-rabbit IgG(Jackson Immunoresearch, West Grove, PA, USA)(20). Some sections were further stained with fluorescein-labeled phalloidin (1:50 dilution, Molecular Probes, Eugene, OR, USA) for F-actin, and 2 ug/ml DAPI (4',6-diamidino-2-phenylindole) for DNA staining.

For immunoelectron microscopy, tissues were fixed in 3% formaldehyde-0.5% glutaraldehyde-PBS. Ultrathin frozen sections were cut, and stained(21) with anti-GT antibodies and affinity-purified goat anti-rabbit IgG(Jackson Immunoresearch) conjugated to 10-nm colloidal gold(22,23). Immunolabeled specimens were ultrathin-embedded in LR White(London Resin, Basingstoke, UK)(24).

RESULTS AND DISCUSSION

The erythrocyte/HepG2-type GT was detected in brain microvessels, inner portion of the retina, pigment epithelium of the retina, ciliary body of the eye, and placenta(Fig.1). The apparent molecular weight of the brain microvessel GT(50 kDa) corresponded to the reported values of 53 kDa(25), about 55 kDa(26), and 45-60 kDa(27). The molecular weights of the GT's in other tissues with blood-tissue

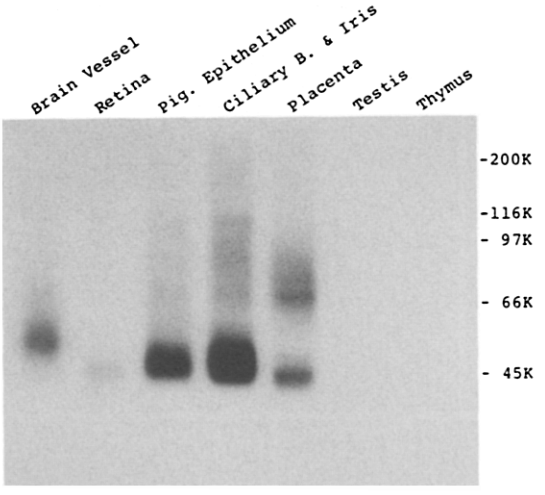

Fig.1. Immunoblotting with the anti-HepG2 GT antibody of rat tissues having blood-tissue barriers. Cell homogenates (10 μg protein each) were subjected to SDS-PAGE and immunoblotting with anti-peptide antibody raised against the C-terminal peptide of the HepG2 GT. The erythrocyte/HepG2-type GT was detected in brain microvessels (Brain Vessel, 50 kDa), inner portion of the retina (Retina, 43 kDa), pigment epithelium of the retina (Pig. Epithelium, 46 kDa), ciliary body and iris of the eye (Ciliary B. & Iris, 46 kDa), and in the placenta (44 kDa). Broad profiles of the detected bands are characteristic of the erythrocyte/HepG2-type GT's(3). No comparable amount of the GT was found in testis and thymus, other known blood-tissue barriers. The nature of a minor 67 kDa band in placenta is unknown.

barriers(43-46 kDa) are close to the values previously reported for other cells and tissues(44-55 kDa)(3). With immunoblotting, no appreciable amount of the GT was found in testis and thymus.

We examined the localization of the GT by staining frozen sections of tissues having blood-tissue barriers immunohistochemically. The erythrocyte/HepG2-type GT was concentrated in the endothelial cells of the blood vessels in the brain(Fig.2a and also see Table 1)(26,27) where the well-known blood-brain barrier exists(8,9). Both the luminal and the basal plasma membranes were positive for the GT(Fig.2a).

The eyeball is separated from the outside by the blood-ocular barrier, i.e., blood-retina and blood-aqueous barriers(11). The survey view showed that intense labeling for the GT was found in the epithelia of ciliary body and in the pigment epithelium of the retina(Fig.2b), which is consistent with the recent report by the immunoperoxidase labeling of paraffin sections(28). We found that both of the two-layered ciliary body epithelium, i.e., the superficial non-pigmented and the basal pigmented layers, was positive. The labeling was seen along the entire plasma membrane including the highly infolded plasma membrane of the epithelial cells (Fig.2c,d). Endothelial cells beneath the pigmented epithelium and ciliary body

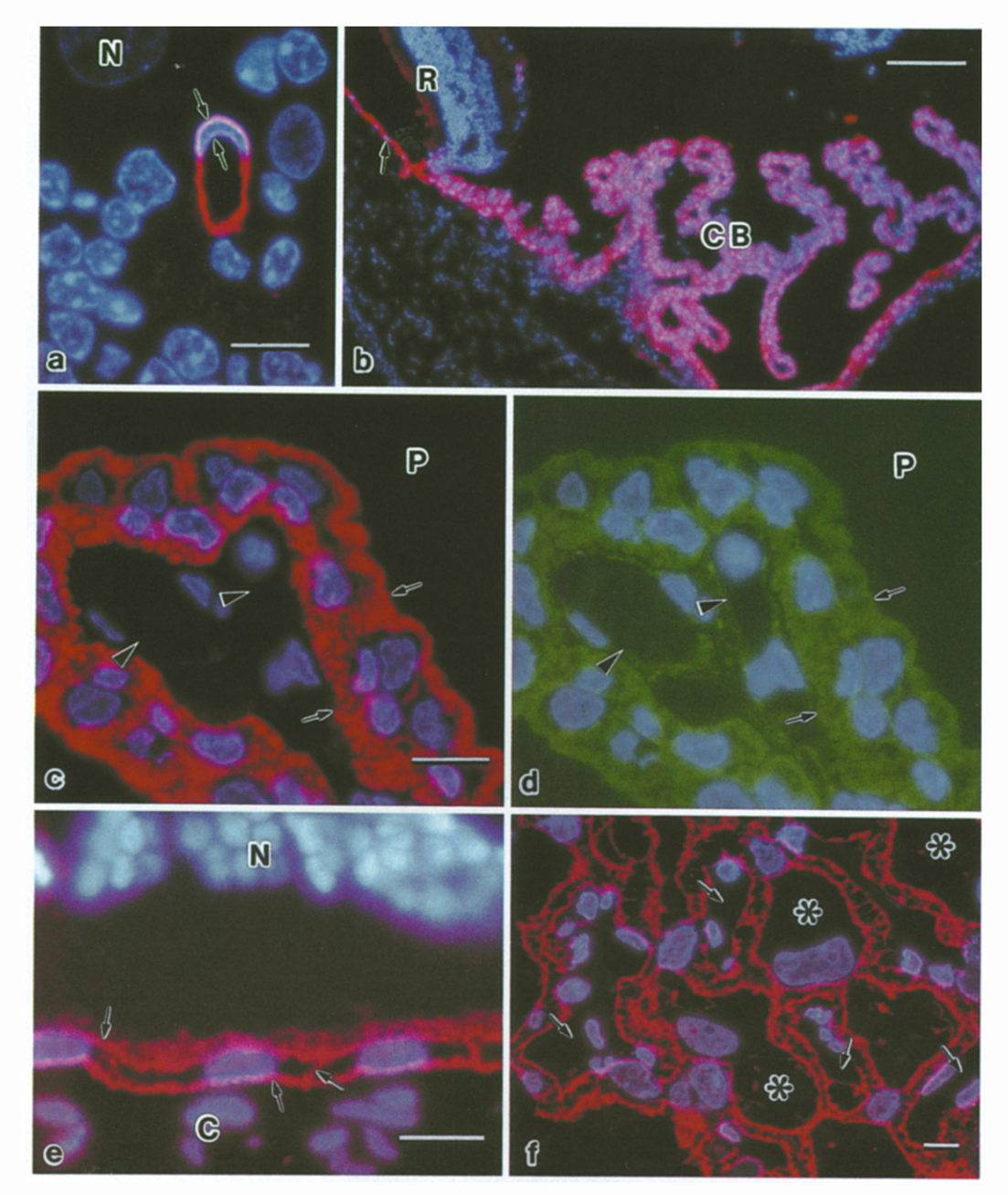

Fig.2. Immunofluorescence localization of the erythrocyte/HepG2-type GT in rat tissues. Frozen sections were stained for the GT(red), F-actin(green), and DNA(blue). Double-exposure images were photographed to facilitate cell identification. Sections were stained with either anti-human erythrocyte GT antibody (a,e), or anti-C-terminus of HepG2 GT antibody(b,c,f). (a)Brain(cerebellum). The GT is concentrated in endothelial cells. The entire plasma membrane of the endothelial cell is positively stained(arrows). N:Purkinje cell. (b)A survey view showing the retina(R) and the ciliary body(CB) of the eye. Intense labeling is seen in the ciliary body, and pigment epithelium(arrow) of the retina. (c,d)Ciliary body of the eye. The plasma membranes of both two layers of the epithelium are strongly stained for the GT. Labeling corresponding to the infoldings of plasma membrane is evident (arrows). See also Fig.3. Blood vessels are negative (arrowheads). P:posterior chamber. (e)Outer part of the retina. Positive staining is seen in the plasma

epithelium were negative. In the pigment epithelium, the whole aspects of the plasma membrane of the pigment epithelium was intensely labeled(Fig.2e). In the iris and inner part of retina, on the other hand, endothelial cells were positive. The electron microscopic examination of the immunogold-labeled ultrathin frozen sections showed the GT to be localized in the infolded plasma membrane of the cells in the ciliary body epithelium(Fig.3) and pigment epithelium as well as endothelial cells of retina.

Strong labeling for the GT was seen in the syncytiotrophoblast plasma membrane in placenta(Fig.2f), which serves as a barrier against the mixing of fetal and maternal blood(12). High level of the GT in the syncytiotrophoblasts may be essential for providing the fetus with glucose. The GT was found in the choroid plexus epithelium, and perineural sheath cells and endothelial cells in peripheral nerves, which confirmed the previous reports(26,29). In testis, the GT was not found in Sertoli cells where a blood-tissue barrier had been assumed(13). Weak staining was found in the endothelial cells. We did not detect positive GT labeling in the thymus, another tissue known to have a blood-tissue barrier(14). Our failure of detection of the GT in these blood-tissue barriers may be due to either low levels of expression of the GT or the existence of another type(s) of GT(s).

As summarized in Table 1, the endothelial or epithelial cell layer sealed by tight junctions or syncytial in nature serves as a blood-tissue barrier. The erythrocyte/HepG2-type GT in the plasma membrane of these blood-tissue barriers may be responsible for the transport of glucose through the barrier cell layers to feed the cells inside the barriers.

In addition to the erythrocyte/HepG2-type GT, we raised anti-peptide antibodies to insulin-sensitive adipocyte-type GT(30) and to the GT of Na^+-dependent active GT(5). In using these antibodies, we did not find any positive staining in the blood-tissue barriers except in choroid plexus epithelium, where Na^+-dependent active GT was detected(K.T.,T.K.,M.K.,O.E. and H.H., unpublished observation). It is not entirely clear at present why the erythrocyte/HepG2-isoform presents in blood-tissue barriers. One reason may be that Km of several mM for glucose is

membrane of the cells of the pigment epithelium(arrows). C:choroid. N:nuclei of photoreceptor cells. (f)Placenta. Plasma membranes of syncytiotrophoblast layers are intensely positive for the GT. *:maternal blood space. Arrows:fetal capillary. Bars:10 μm in a,c,e,f, or 100 μm in b.

Table 1. Localization of erythrocyte/HepG2-type glucose transporter in the blood-tissue barrier

barrier	organ/tissue	localization of glucose transporter	location of the barrier
Endothelial type			
blood-brain barrier	brain	endothelial cell	endothelial cell(8,9)
blood-aqueous barrier	iris	endothelial cell	endothelial cell(11)
blood-retina barrier	retina, inner	endothelial cell	endothelial cell(11)
blood-nerve barrier	peripheral nerve	endothelial cell	endothelial cell(10)
blood-thymus barrier	thymus, cortex	not detected	endothelial cell (14)
Epithelial type			
blood-cerebrospinal fluid barrier	choroid plexus	epithelial cell	epithelial cell(9)
blood-aqueous barrier	ciliary body	epithelial cell	epithelial cell(11)
blood-retina barrier	retina, outer	pigment epithelial cell	pigment epithelial cell(11)
blood-nerve barrier	peripheral nerve	perineural sheath cell	perineural sheath cell(10)
placental barrier	placental labyrinth	syncytiotrophoblast	syncytiotrophoblast(12)
blood-testis barrier	testis	not detected*	Sertoli cell(13)

*Weak staining for the GT was found in the endothelial cells.

Fig.3. Immunogold detection of the erythrocyte/HepG2-type GT in the epithelial cells of the ciliary body. Ultrathin sections were stained with anti-human erythrocyte GT antibody. Gold particles representing the GT are seen along the infoldings of the plasma membrane. N:nucleus. Bar:0.5 μm.

adequate for its transport from blood to tissues, since glucose concentration in blood is also the order of several mM(7). The further physiological and immunohistochemical studies of other types of GT's(6) such as brain-type and liver-type will clarify the roles of GT's in animal cells.

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