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Glucose transporters are abundant in cells with "occluding" junctions at the blood–eye barriers

(eye microvessels/glucose metabolism/blood–brain barrier/immunocytochemical localization)

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ABSTRACT We studied the distribution of the "erythroid/brain" glucose transporter protein in the human and rat eye by immunocytochemistry with monoclonal and polyclonal antibodies to the C terminus of the human erythrocyte glucose transporter. We found intense immunocytochemical staining in the endothelium of microvessels of the retina, optic nerve, and iris but not in microvessels of the choroid, ciliary body, sclera, and other retro-orbital tissues. In addition, we found marked immunocytochemical staining of retinal pigment epithelium, ciliary body epithelium, and posterior epithelium of the iris. The common feature of all those endothelial and epithelial cells that stained intensely for the glucose transporter is the presence of "occluding" intercellular junctions, which constitute the anatomical bases of the blood–eye barriers. We propose that a high density of the glucose transporter is a biochemical concomitant of epithelial and endothelial cells with barrier characteristics, at least in tissues that have a high metabolic requirement for glucose.**

The physiological concept of blood–tissue barriers was formulated at the turn of this century. It is now generally accepted that such barriers are effective means by which tissues that perform specialized functions, such as the brain, can maintain a constant milieu and are shielded from the vagaries of the systemic circulation. With the advent of ultrastructural microscopy, it became evident that "occluding" junctions (zonulae occludentes) between endothelial or epithelial cells are the main anatomical features of these barriers (1–4). However, knowledge of the physiological and biochemical concomitants of tissue barriers remains largely unknown. The isolation behind barriers, preventing the simple diffusion of water-soluble substances into these tissues, necessitates transport systems in the microcirculation to ensure the steady availability of required nutrients, such as glucose.

In 1965, Crone predicted that the brain capillary endothelium, which constitutes the blood–brain barrier, is particularly enriched with a glucose transporter, similar to that of human erythrocytes, that allows the selective, stereospecific, and saturable transport of large amounts of glucose from blood to brain (5, 6). Using ligand-binding methods (7, 8) and, more recently, immunocytochemistry (9), we found that Crone's predictions were correct. Brain microvessels from several mammalian species, including man, are endowed with an unusually high density of glucose transporter protein (7–11) and messenger RNA (12, 13). In retrospect, these findings and Crone's predictions appear logical because brain capillaries, which account for <1% of the brain weight, have to transport glucose for the whole brain. In contrast, microvessels in brain regions that lack blood–brain barrier properties, such as those in the area postrema (9), and those of the heart and skeletal muscles (S.I.H., R.N.K., L.A., P.L., and G.P., unpublished observations) did not show a high density of glucose transporter.

Recent cloning and sequencing studies suggest the existence of several different glucose transporter proteins for carrier-mediated diffusion of glucose across cell membranes in various tissues (13–23). The "erythroid/brain" type of glucose transporter, which is abundant in human erythrocyte and brain endothelial membranes, exhibits major amino acid sequence differences from liver, adipose tissue, and skeletal muscle glucose transporters, for instance in the N and C termini. The tissue distribution of the different glucose transporters is not well known, and more than one transporter may be present in the same tissue (24, 25). In this report, we examined the immunocytochemical localization of erythroid/brain glucose transporter in human and rat eyes and found that only cells known to form interendothelial or interepithelial occluding junctions, thus constituting the blood–eye barriers, showed a high density of this transporter. We hypothesize that a high density of glucose transporter is a biochemical concomitant of barrier properties, at least in tissues that require glucose as an important metabolic substrate.

METHODS

Adult Wistar rats were anesthetized with pentobarbital and perfused with saline, followed by Bouins' fixative. Human eyes were obtained at autopsy and fixed in Bouins' solution. The eyes were embedded in paraffin several days later. Paraffin sections (6 μm) were hydrated and suspended in 10% normal goat serum prior to incubation with one of the primary antibody preparations. Two antibody preparations to the human erythrocyte glucose transporter were used: monoclonal antibody B315:32 that was characterized by Andersson and Lundahl (26) and found to have strong and selective binding to the C terminus of this transporter and an antiserum to a synthetic C-terminal transporter peptide that was characterized by Haspel *et al.* (27). As a blood vessel marker, we used an antiserum to type IV collagen to immunostain the vascular basal lamina (28).

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Immunocytochemical staining was performed by the peroxidase-antiperoxidase procedure (29). The primary antibody, diluted in 1% normal goat serum in Tris-buffered saline (pH 7.6), was incubated with tissue sections at 4°C for 18 hr. After removal of unbound antibody, sections were incubated at 22°C for 30 min with goat serum (1:50) directed to IgG of the primary antibody species. The final immunoreagent, peroxidase-antiperoxidase (1:200), produced in the same species (rabbit, Cappel Laboratories; mouse, Sternberger-Meyer, Jarrettsville, MD) as the primary antibody, was applied for 1 hr at 22°C. Immunoreaction was developed for maximum contrast by using diaminobenzidine (0.75 mg/ml) and 0.015% H_2O_2 in 50 mM Tris·HCl (pH 7.6). Sections were then mounted with Permount, covered with coverslips, and viewed under bright-field or phase-contrast illumination. In preliminary experiments, we found that optimum staining was achieved at a 1:2 dilution of the monoclonal antibody cell culture supernatant and at a 1:100 dilution of the antiserum. The immunostaining patterns obtained with the mouse monoclonal and the rabbit polyclonal antibodies to the glucose transporter were essentially identical, thus ruling out the possibility that the observed staining of a given structure is a cross-reaction artifact.

In control incubations to show the specificity of immunostaining, we used irrelevant antibodies, omitted the primary antibody, and in the case of antibodies to glucose transporter, adsorbed them with human erythrocyte membrane fragments prior to immunostaining. The irrelevant antibodies were mouse monoclonal and rabbit polyclonal antibodies to neurofilaments and microtubule-associated protein tau. In these controls, faint or no specific staining was found, or in the case of antibodies to the above-mentioned neuronal markers, only neurons and their processes were stained. Adjacent sections were also stained with hematoxylin/eosin to appreciate histological details.

RESULTS AND DISCUSSION

Human and rat eyes were strikingly similar in their cellular distribution of the glucose transporter, with only one substantial difference. Neural elements, especially photoreceptors, of the human retina, but not of the rat retina, stained with monoclonal and polyclonal antibodies to the glucose transporter (Fig. 1 *B* and *C*). Our preliminary results indicate similar staining in the fetal rat retina, which disappears with maturation. Because of the similarity between the human and rat eyes and in view of better tissue preservation and lack of melanin in the eyes of albino Wistar rats (which makes it easier to see the brown peroxidase reaction product), we chose most of our illustrations from the rat.

Endothelial cells of human and rat retinal and optic nerve microvessels showed intense staining with either monoclonal or polyclonal antibodies to the glucose transporter (Fig. 1 *B–D*). These vessels were morphologically similar to brain blood vessels (9). All microvessels in the retina and optic nerve stained intensely for the glucose transporter, as evidenced by the congruency in immunostained microvessels by antibodies to the glucose transporter and collagen. The endothelium of the larger vessels of the optic nerve did not stain (Fig. 1*B*). Microvessels of the iris, which were larger than those of the retina and optic nerve and often irregular, also stained intensely for the glucose transporter (Fig. 1 *E* and *F*). Yet, the abundant vessels of the choroid (Fig. 1*D*) and ciliary body (Fig. 1*E*) did not stain for the glucose transporter. All other blood vessels of the eye and retro-orbital tissue, including those of the extraocular muscles, lacrimal glands, sclera, and conjunctiva failed to stain for the glucose transporter, but they were stained with the collagen antibody (see Fig. 1*G* as an example).

In view of the similarity in barrier characteristics between brain vessels and retinal vessels (2, 30–32), the presence of a high density of the glucose transporter in microvessels of the retina and optic nerve is consistent with our previous findings in brain microvessels (9). The weak glucose transporter staining in microvessels of the ciliary body (Fig. 1*E*) is also consistent with the "leaky" endothelium of its capillaries (32). The lack of staining of the endothelium of larger vessels found in the optic nerve (Fig. 1*B*) is plausible because the endothelium of large vessels does not have major transport functions. In this respect, these results are similar to our findings in the endothelium of larger brain arteries, which also do not immunostain with antibodies to the glucose transporter (S.I.H., R.N.K., L.A., P.L., and G.P., unpublished observations).

The results in iris microvessels were interesting because they may shed light on the conflicting reports on whether or not they possess barrier properties. Whereas ultrastructural studies in monkeys (33, 34) and in mice (35) show that interendothelial junctions of iridial microvessels are impermeable to circulating horseradish peroxidase, Shakib and Cunha-Vaz (2) described discontinuous zonulae occludentes in interendothelial junctions of the iris and observed that, unlike retinal microvessels, iridial microvessels "opened" following the local application of histamine. Janzer and Raff (36) recently implied that iris microvessels do not have barrier properties. Our finding of intense staining with glucose transporter antibodies in endothelial cells of the iris (Fig. 1 *E* and *F*) suggests that these vessels do not allow the simple diffusion of glucose and indicates the existence of barrier properties in these vessels.

In addition to the positive staining for the glucose transporter in the endothelium of optic nerve, retinal, and iridial blood vessels, intense staining was also present in the following nonvascular tissues: (*i*) retinal pigment epithelium (Fig. 1 *B–D*), (*ii*) ciliary body epithelium, particularly the outer epithelium (Fig. 1*E*), and (*iii*) posterior epithelium of the iris (Fig. 1 *E* and *F*). The ciliary body stained in a similar manner as that in the brain's choroid plexus, where the epithelium, but not the vascular endothelium, stained well for the glucose transporter (9). The intense staining of these epithelia was unexpected in view of our prior experience in the brain where only the microvascular endothelium stained intensely positive. From a teleological viewpoint, the high density of glucose transporter in the retinal pigment epithelium is understandable because of the paucity of capillaries in the outer layers of the neural retina (Fig. 1*D*). Thus, glucose needed by the highly active metabolic, yet avascular, layer of cones and rods would have to diffuse over relatively long distances from capillaries in the inner retinal layers anteriorly and from the retinal pigment epithelium posteriorly. We suspect that the high density of glucose transporter in the retinal pigment epithelium plays an important role in ensuring adequate supply of glucose from choroid vessels to the outer retinal layers.

Review of ocular ultrastructure data revealed that all of the eye's endothelial and epithelial cells that stained intensely for glucose transporter are known to possess occluding intercellular junctions and are considered important components of the blood-eye barriers (32). These results were the basis for our hypothesis predicting the existence of a high density of the glucose transporter in cells joined by occluding junctions. Such cells are likely to have many unique proteins, not the least important of which are those that make up the structure of these junctions. Indeed, immunocytochemical studies with monoclonal and polyclonal antibodies have documented the existence of proteins that may be unique to brain capillaries (37, 38). However, unlike the glucose transporter, the functions of these proteins remain unknown.

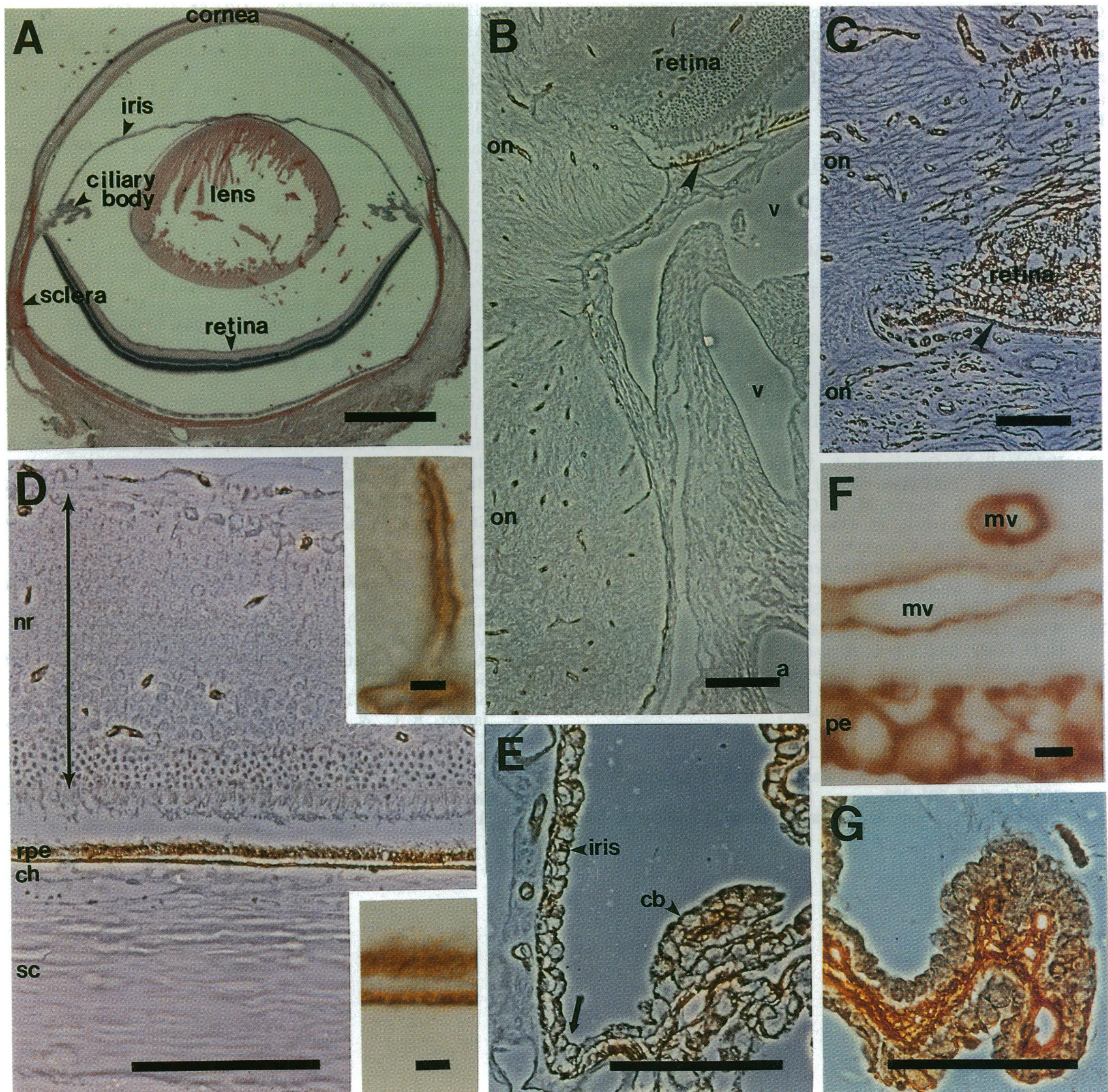

FIG. 1. (*A*) Cross section of a rat eye that was perfused-fixed *in situ* and stained with hematoxylin/eosin. This off-center section does not pass through the pupil or optic nerve. The separation of the neural retina from the retinal pigment epithelium and the underlying choroid and sclera is a fixation artifact. (Bar = 1 mm.) (*B*) Cross section through the head and intraorbital portion of the rat optic nerve immunostained with the monoclonal glucose transporter antibody. Dense brown reaction product is seen in retinal pigment epithelium (arrowhead) and in microvessels of the retina and throughout the length of the optic nerve (on). The endothelium of a larger artery (a) and veins (v) did not stain. (Phase contrast; bar = 100 μm.) (*C*) Section through the head of the optic nerve of the human eye immunostained with the monoclonal glucose transporter antibody. Brown reaction product is seen in retinal and optic nerve (on) microvessels, in the retinal pigment epithelium (arrowhead), and in other elements of the neural retina. (Phase contrast; bar = 100 μm.) (*D*) Cross section of the posterior part of the rat eye with the neural retina (nr) overlying the retinal pigment epithelium (rpe), choroid (ch), and sclera (sc). Intense immunostaining for the glucose transporter by the polyclonal antibody is seen in retinal microvessels and the retinal pigment epithelium but not in choroidal or scleral vessels. Note the lack of vascularity in the outer retina. (Phase contrast; bar = 100 μm.) (*Upper Inset*) High-power view of a retinal capillary. (*Lower Inset*) High-power view of the retinal pigment epithelium. (*Insets*, bar = 5 μm.) (*E*) Immunostaining of the ciliary body (cb) at its junction with the iris (arrow) with monoclonal antibody reveals a high density of the glucose transporter in the ciliary body epithelium but not in the endothelium of its microvessels. The iris is seen on the left with a high density of the glucose transporter in its vascular endothelium and posterior epithelium. (Phase contrast; bar = 100 μm.) (*F*) High-power view of the rat iris showing positive immunostaining with the monoclonal antibody to the glucose transporter in iridial microvessels (mv) and in the underlying posterior epithelium (pe). (Bar = 5 μm.) (*G*) Immunostaining of the ciliary body in an adjacent section to that represented in *E* with collagen IV antibody shows dense reaction product in the basal laminae of blood vessels and ciliary blood vessels epithelium but not in epithelial cells. (Phase contrast; bar = 100 μm.)

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