Urethane-Induced Rat Retinopathy

Plasticity of the Blood–Retinal Barrier in Disease

Gary E. Korte, Roy W. Dellhorn, and Margaret S. Burns

Sodium fluorescein and fluoresceinated dextrans penetrate the blood–retinal barrier (BRB) of rats with urethane-induced retinopathy.1-3 We have extended these observations, using horseradish peroxidase (HRP) as an ultrastructural probe of the BRB. Intravenous HRP penetrated the BRB 7 weeks after urethane treatment began. This occurred where retinal capillaries invaded the retinal pigment epithelium (RPE) after photoreceptor degeneration. The penetration intensified with duration of the retinopathy, but remained localized near intraepithelial capillaries. The mechanisms by which HRP penetrated the BRB changed as the retinopathy progressed. In the earliest stages (7–10 weeks of age) vesicular transport across endothelia and/or leakage from the choriocapillaris into the pericapillary space of intraepithelial capillaries and then along this space into the retina. At later stages, two more mechanisms were at work: (1) fenestrae developed in the intraepithelial capillaries, and (2) the RPE attenuated, losing its barrier function. Except where this occurred, intercellular RPE junctional complexes remained intact and retarded HRP. We suggest that the rat urethane retinopathy models the plasticity of BRB components—RPE and endothelia—over the course of retinal disease. Invest Ophthalmol Vis Sci 25:1027-1034, 1984

When neonatal rats receive sequential injections of urethane their photoreceptors die, and retinal capillaries become embedded in the retinal pigment epithelium (RPE).1 In the late stage of retinopathy (30 or more weeks after urethane treatment begins), these capillaries have numerous fenestrae—unlike their parent retinal capillaries. The fenestrae certainly contribute to the passage of sodium fluorescein and FITC-dextrans across the blood–retinal barrier (BRB) in rats with late stage urethane retinopathy.1,2 Kritzinger and Bellhorn3 have shown that the BRB of rats in earlier stages of urethane retinopathy (10–16 weeks after urethane treatment begins) passes sodium fluorescein when and where retinal capillaries penetrate the RPE.

But does this early barrier breakdown also result from the presence of fenestrated capillaries in the RPE? There are several mechanisms by which the BRB may fail, such as opened tight junctions between RPE cells or increased vesicular transport across endothelia.4 These alternatives warranted an electron microscopic study of the BRB during the early or developing stages of urethane retinopathy, using the electron dense tracer horseradish peroxidase (HRP). This revealed several, rather than one, mechanisms by which HRP crossed the BRB. This showed us that the changes in BRB components (RPE and endothelia) during urethane retinopathy are more complex and dynamic than we previously believed.

Materials and Methods

Thirteen newborn male and female pigmented (Long-Evans) rats received weekly subcutaneous injections of urethane (1 mg/g body weight, from a 100 mg/ml solution in saline) for 8 weeks after birth. Ten control rats received only saline. We processed the eyes for electron microscopy after the rats received an intravenous injection of HRP. For this procedure, the rats were anesthetized (Nembutal, 40 mg/kg body weight) and received HRP (Sigma Chemical Corp.; St. Louis, MO: 0.3–0.4 mg/g body weight) through a cannulated femoral vein. Fifteen minutes later, the eyes were enucleated and the cornea, lens, and iris removed. The eyecups were immersed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 3–5 hr, at 4°C. Then the eyecups were rinsed overnight in buffer. We localized HRP by the deposition of diaminobenzidine reaction product in the presence of hydrogen peroxide.5

From the Eye Research Laboratory, Department of Ophthalmology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, New York.

Supported by USPHS grant EY-02038 (RWB) and Research to Prevent Blindness, Inc.

Submitted for publication: January 13, 1984.

Reprint requests: Gary E. Korte, Eye Research Laboratory, Department of Ophthalmology, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467.
Fig. 1. Intraepithelial capillaries embedded in the RPE of an 8-week-old urethane rat. Intravenous HRP. A, Black HRP reaction product fills the capillary lumen (L), pericapillary space and Bruch's membrane (BM), after penetrating the choriocapillaris (C). HRP also entered the subretinal space of the retina (R) (×5,600). B, HRP in the subretinal space (arrows) of A. Note absence of fenestrae in the intraepithelial capillary (L, lumen) but the presence of numerous tracer-filled intraendothelial vesicles. HRP also outlines RPE basal folds facing the new capillary. Portion of a macrophage in subretinal space, m (×15,200).
Fig. 2. An RPE process (curved arrow) runs along the capillary. Neuronal soma of remnant inner nuclear layer, N (the photoreceptors have died) (×8,500). Inset. Portion of the capillary seen in the main figure, adjacent section. A thin lamina of Müller cell processes (MC) covers the capillary beginning where its RPE cover stops (arrow). HRP penetrated between the Müller cell and RPE processes and is seen as black reaction product in the retinal extracellular space, especially evident to the left of the capillary (×19,200).

Slices of eye reacted in the absence of diaminobenzidine served as controls. The slices then were rinsed in buffer for 1.5 hr, postfixed in osmium tetroxide, dehydrated in alcohol, and embedded in epoxy resin. Thick (2–3 μm) sections were surveyed light microscopically for evidence of tracer leakage, seen as brown-red reaction product.
Fig. 3. RPE with no intraepithelial capillaries. Ten-week-old urethane rat, intravenous HRP. Black reaction product does not enter the subretinal space (arrow) due to junctional complexes (JC) between RPE cells. They appear intact at higher magnification. Remnant of degenerating photoreceptor, Bruch's membrane is at the top of the picture (x6,600).

product in the extravascular retina. Thin sections of these areas were stained with lead citrate and uranyl acetate and examined electron microscopically.

Two urethane and two control rats were examined at 6–8, 10, and 15 weeks of age. Three urethane rats (13, 26, and 28 weeks of age) that did not receive HRP prior to killing also were examined.

These investigations conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Bellhorn et al. and Kritzinger and Bellhorn described the histology of the rat urethane retinopathy. This report focused on the behavior of their BRB com-

Fig. 4. Fifteen-week-old urethane rat, intravenous HRP. An intraepithelial capillary (L, lumen) has some fenestrae in its endothelium (white arrows). HRP may penetrate them, enter the pericapillary space, and then the retina, along the space. RPE basal folds (f) face the capillary; those facing Bruch's membrane (BM) are reduced (x12,600).
Fig. 5. Attenuated RPE and passage of intravenous HRP in a 10-week-old urethane rat. Bruch’s membrane is at the top of each picture. A, Neuronal somata (N) of the inner nuclear layer (the photoreceptors have died) are separated from Bruch’s membrane by a thin lamina of RPE processes (arrows). Areas of RPE attenuation typically occur where intraepithelial capillaries (c) are present (×6,300). B, C, Areas of arrows B and C respectively in A. HRP reaction product (arrowheads) between RPE processes denotes passage from Bruch’s membrane into the neural retina (B) and pericapillary space (pcs) of intraepithelial capillaries (C). Lysosomes and residual bodies (open arrows) help identify the hypopigmented RPE processes (×38,000).
Fig. 6. Diagram of RPE and intraepithelial capillary changes during rat urethane retinopathy. HRP movement is indicated by arrows; its deposition by black. A, Seven to ten weeks of age. Intraepithelial capillaries have no fenestrae. HRP passage is by vesicular transport (not indicated) or by passage across the fenestrated choriocapillaris (above Bruch’s membrane) into the space between RPE cells, and then into the retina along the pericapillary space of intraepithelial capillaries. Junctional complexes (JC) prevent HRP passage between RPE cells. B, C. Beyond ten weeks of age, two changes occur: (1) intraepithelial capillaries develop fenestrae and pass HRP (B), and (2) RPE attenuates, denoted by loss of RPE cells II and III in Figure 6C. When this happens junctional complexes no longer connect RPE cells, and HRP can penetrate between them, unlike earlier stages (cf. Fig. 6A). Formation of RPE basal folds facing intraepithelial capillaries and their reduction facing Bruch’s membrane is illustrated in RPE cells I and IV in B (cf. Fig. 6A).

ponents—RPE and capillary endothelia—to intravascular HRP administered at various ages.

We first saw extravascular HRP in the retina of rats that were 7-8 weeks of age by the end of their series of urethane injections. HRP entered the extravascular space of the retina where retinal capillaries looped into the RPE (Fig. 1).

Discrete areas of HRP penetration were seen whenever one end of a capillary loop bridged the interface between RPE and neural retina. Only one such site usually was observed in section, as the ends of a capillary loop are several RPE cells apart. The lumen of these intraepithelial capillaries was filled with the black reaction product denoting the presence of HRP. Reaction product also filled endothelial vesicles, the pericapillary space, and the extracellular space between RPE cells. The junctional complexes between RPE cells and between endothelial cells appeared intact. We saw no endothelial fenestrae at this age.

Intraepithelial capillaries were more numerous by 10 weeks of age. Frequent examples of capillaries entering the RPE from the retina were seen (Fig. 2). They were invariably sites where HRP entered the extracellular space of the retina, as opposed to areas with no capillaries in the RPE (Fig. 3). Numerous tracer-filled vesicles were seen in endothelia but still no fenestrae.

We first saw endothelial fenestrae in intraepithelial capillaries at 13 weeks of age, well after HRP penetrated the BRB. By the later ages we sampled (15, 26, and 28 weeks of age), numerous fenestrated capillaries existed in the RPE (Fig. 4). They apparently leaked HRP, as the tracer reaction product “connected” the capillary lumen and pericapillary space.

We saw no evidence of vesicular transport across retinal capillaries, though numerous tracer-laden endothelial vesicles occurred. Tracer entered the pericapillary space only of capillaries near or leading into the RPE. Interendothelial junctional complexes appeared intact.
We noted two changes in the RPE relevant to their barrier and transport functions in specimens 10 weeks and older: (1) The cells often formed basal folds facing the new capillary and lost those facing Bruch's membrane (Fig. 4). (2) The cell attenuated, so that Bruch's membrane was covered only by a thin lamina of RPE processes (Fig. 5). These processes were not connected by junctional complexes, so intravascular HRP entered the retina directly from the choroid at these sites (Fig. 5). We assumed the attenuated RPE resulted from cell death (as some degenerate RPE cells were observed) and migration of RPE cells into the retina, leaving behind a gap along Bruch's membrane.

Discussion

Our observations indicate that the BRB passes HRP when and where retinal capillaries penetrate the BRB, supporting Kritzinger and Bellhorn's observations using sodium fluorescein. Furthermore, the entry of capillaries into the RPE is just the first step in a process of RPE and endothelial remodelling that affects the distribution of intravascular tracers. Figure 6 summarizes our concept of these changes. Kritzinger and Bellhorn assume that tracer passage across intraepithelial capillaries in the early stages of urethane retinopathy occurred via their fenestrae, which are numerous in the later stages of the retinopathy (eg, Fig. 4; see Bellhorn et al1). But in the present study, endothelial fenestrae were rare in the intraepithelial capillaries until about 13 weeks of age—after the earliest leakage, at 7 weeks of age. What mechanisms other than fenestrae may permit this leakage?

Vesicular transport across endothelia is a likely mechanism for the passage of tracer across the BRB. The presence of many HRP-filled vesicles in the endothelia of intraepithelial capillaries suggested this. However, tracer in their pericapillary space also could have reached that point by a mechanism we documented in rat phototoxic retinopathy: passage into the pericapillary space of intraepithelial capillaries after crossing the fenestrated choriocapillaris and then into the retina along this space. This route would be facilitated by the proximity of the intraepithelial capillaries to Bruch's membrane; some even impinge on it directly (as we illustrate in rat phototoxic retinopathy).

During the later stages of the urethane retinopathy, BRB breakdown to HRP became most obvious. This was due to the presence of more fenestrated intraepithelial capillaries and to changes in a major arm of the BRB—the RPE. Starting at 10–13 weeks of age, the RPE started to attenuate due to migration of the cells into the retina and/or degeneration. Tracer penetrating the choriocapillaris then could enter the retina even more readily, as these slender RPE processes had no junctional complexes.

Our observations illustrate the dynamic role the RPE can play in BRB changes during retinal diseases. Changes in these cells, like attenuation with loss of tight junctions, contributed to HRP passage across the BRB in urethane retinopathy. More subtle influences by RPE on endothelium probably elicited the development of fenestrae in normally nonfenestrated retinal capillaries and, thus, influenced transport across endothelia. The development of RPE basal folds facing the new intraepithelial capillaries, and their loss facing the choriocapillaris, was probably accompanied by changes in transport across RPE cells themselves. This may occur in diabetic retinopathy, where the basal folds increase.

It is well known that the two major components of the BRB—the RPE and capillary endothelia—react during retinal disease. Responses similar to those occurring in the urethane retinopathy are seen in rats with phototoxic retinopathy, in several hereditary retinal degenerations of mice and rats, and in normal aged rats. Striking changes in BRB permeability have been found in these animals. Although retinal capillaries have not been found invading the RPE in any human disease, the RPE response is similar to that seen in urethane retinopathy, eg, RPE attenuation and migration in retinitis pigmentosa. These similarities lead us to propose rat urethane retinopathy as a model of RPE and endothelial plasticity in retinal disease. Urethane retinopathy presents the opportunity to study changes in cellular processes, such as transport across membranes, in a milieu mimicking that seen in several animal and human diseases.

Key words: urethane, retinopathy, blood–retinal barrier, retina, pathology

Acknowledgments

The authors thank Noel Roa and Judith Channer for excellent technical assistance and Patricia Lynch for fine secretarial help.

References


