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The inner blood-retinal barrier: Cellular basis and development

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ABSTRACT

The blood-retinal barrier (BRB) regulates transport across retinal capillaries maintaining proper neural homeostasis and protecting the neural tissue from potential blood borne toxicity. Loss of the BRB contributes to the pathophysiology of a number of blinding retinal diseases including diabetic retinopathy. In this review, we address the basis of the BRB, including the molecular mechanisms that regulate flux across the retinal vascular bed. The routes of transcellular and paracellular flux are described as well as alterations in these pathways in response to permeabilizing agents in diabetes. Finally, we provide information on exciting new studies that help to elucidate the process of BRB development or barriergenesis and how understanding this process may lead to new opportunities for barrier restoration in diabetic retinopathy.

1. Introduction

1.1. Barriers of the central nervous system

The brain and retinal tissues are the highest energy-demanding systems in the body, because of neuronal activity. With 2% of the total body mass, the brain consumes 20% of the basal metabolic rate (Clarke & Sokoloff, 1999), while 8% is consumed by retina (Howard, Blakeslee, & Laughlin, 1987; Niven & Laughlin, 2008). The endothelial cells that form the blood-brain barrier (BBB) and blood-retinal barrier (BRB), provide enough oxygen and glucose for neuronal function while restricting the flux of other molecules and cells in order to protect the neuronal environment. The specific characteristics of the vasculature in these tissues provide the basis of a barrier function as described in this review.

In 1885, Paul Ehrlich published the first observations of a Central Nervous System (CNS) barrier. Ehrlich observed that water-soluble dyes injected subcutaneously stained all organs except the brain and spinal cord (Ehrlich, 1885). But it wasn't until 1900, when the concept of a CNS barrier was introduced by Lewandosky who termed it "capillary wall", and by Lina Stern in 1918, who introduced the terminology "barrier" (Saunders et al., 2014). The pioneering work performed by Ehrlich, Lewandosky, Stern and other scientists led to the recognition of the BBB.

Similarly, Schnaudigel in 1913 and Palm in 1947, recognized the BRB using intravenously injected trypan blue that stained all organs of rabbits with the exception of the CNS, including the retinal tissue (Palm, 1947), which was further supported at the ultrastructural level by Cunha-Vaz et al. (Cunha-Vaz, Shakib, & Ashton, 1966; Shakib & Cunha-Vaz, 1966). Thus, the BBB and the BRB form part of the barriers of the CNS. We now understand that the BBB and BRB provide tight control of the neuronal environment regulating the flux of blood borne material into the neural parenchyma. These barriers maintain proper neural homeostasis and protect the neural tissue from potential blood borne toxicity.

1.2. Blood-retinal barrier

Structurally, the BRB is composed of two distinct barriers; the outer BRB (oBRB), consisting of retinal pigment epithelium that regulates transport between the choriocapillaris and the retina, and the inner BRB (iBRB), which regulates transport across retinal capillaries. Loss of the iBRB contributes to the pathophysiology of a number of retinal diseases including diabetic retinopathy (Frey & Antonetti, 2011). This review will first describe the basis of the iBRB and routes of transport across retinal endothelial cells. Changes in barrier properties during diabetes and other blinding diseases will be discussed and the review will end with novel insight into factors that regulate development of the barrier.

2. Flux across the iBRB

The CNS barriers are not absolute barriers that block all transport across the endothelium. Instead, the CNS barriers are highly

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selective barriers that regulate the movement of ions, water, solutes and cells, across the vascular bed. Flux of a solute or water, describes the net movement over time across a barrier, while permeability describes the property of the barrier, in this case, of the iBRB. Changes in permeability across the vascular bed may occur through changes in transport across the cells, broadly termed transcytosis, or through changes in the junctional complex connecting cells, leading to flux around the cells or paracellular permeability. Both transcellular and paracellular routes are composed of multiple specific pathways that may act simultaneously and are not mutually exclusive, and collectively contribute to altered flux.

2.1. Transcytosis

In 1960, Dr. Palade et al. described invaginations of the plasmalemma in the capillary endothelium that "pinch off" from the surface and form intracellular vesicles (Palade, 1960). The electron microscopy techniques at the time allowed detection of intracellular vesicles and invaginations, that suggested a regulated and directional transport (Palade & Bruns, 1968). In addition, some groups have observed a preference of localization of vesicles, suggesting a unidirectional flow (Hofman, Hoyng, Van der Werf, Vrensen, & Schlingemann, 2001). However, this idea has been challenged since recent studies showed invaginations at both sides (Chow & Gu, 2017).

In 1979, Nicolae Simionescu introduced the term transcytosis. In his electron microscopy studies, he identified the appearance of highly dense structures that he called specialized plasmalemma vesicles within endothelial cells. We now know that transcellular transport across retinal endothelial cells is necessary for regulation of the retinal environment's homeostasis.

There are a variety of routes that make up the transcellular route (Klaassen, Van Noorden, & Schlingemann, 2013). Some small lipophilic molecules are able to passively diffuse along the retinal endothelial membrane and cross the BRB (Toda, Kawazu, Oyabu, Miyazaki, & Kiuchi, 2011). Other, larger lipophilic molecules and hydrophilic molecules require ATP-dependent processes to cross the barrier including; receptor mediated vesicular transport, non-receptor mediated pinocytosis, transporters and pumps (Fig. 1). Brain and retinal endothelial cells selectively regulate the transcellular movement of molecules from the blood to the neural tissue by controlling the expression of molecules at both, luminal and abluminal sides. Retinal endothelial cells express a low number of receptors, transporters and vesicle formation mediators, in combination with a high expression of efflux pumps (Sagaties, Raviola, Schaeffer, & Miller, 1987), which collectively contributes to the BRB.

2.1.1. Caveolae

After the discovery of caveolin-1, the structural molecular component of plasmalemma vesicles (Glenney & Soppet, 1992; Kurzchalia et al., 1992; Rothberg et al., 1992), the vesicles were re-named caveolae. Caveolae appear as an electron dense structure by electron microscopy, due to the lipid rafts enriched in glycosphingolipids, sphingomyelin, cholesterol and lipoproteins. In addition to caveolin-1, cavin protein also covers caveolae surface and promotes vesicle stabilization at the plasma membrane (Hill et al., 2008; Liu & Pilch, 2008; Vinten, Johnsen, Roepstorff, Harpoth, &

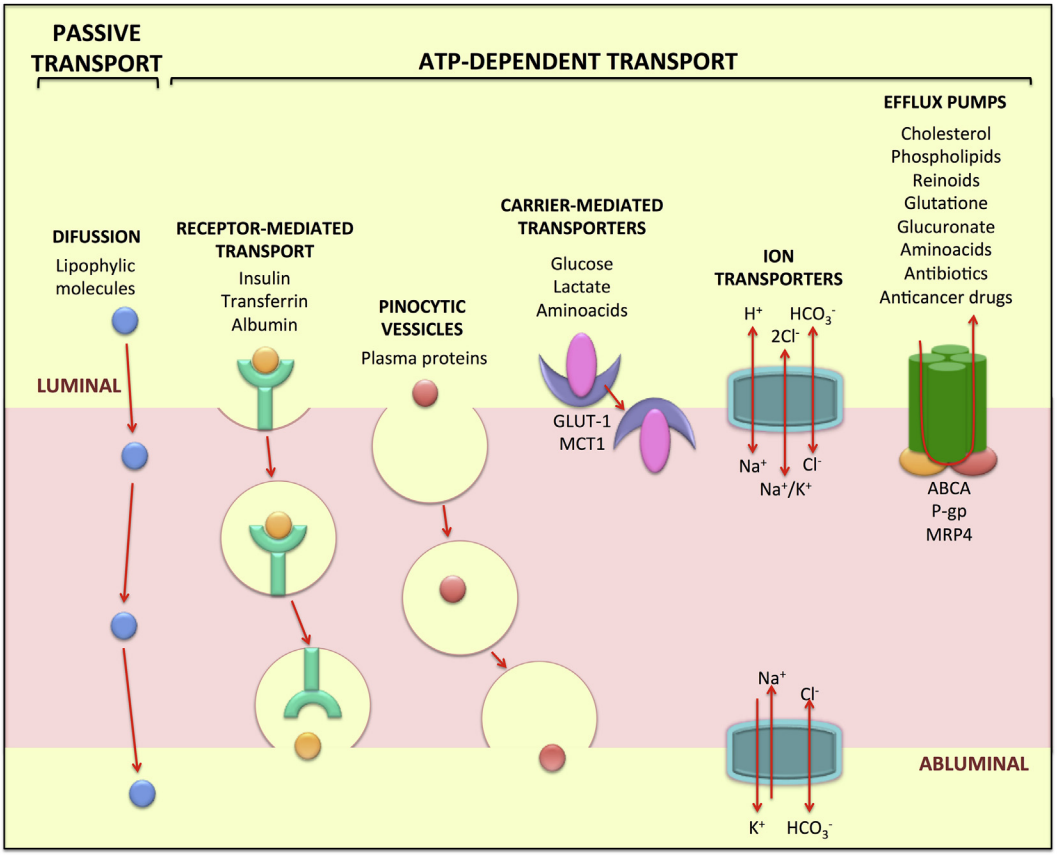

Fig. 1. Mechanisms of transcellular transport across retinal endothelial cells. Some molecules can cross by diffusion due to their lipophilic properties. Other transport mechanisms are energy-dependent processes and include receptor-mediated transport, pinocytic vesicles, carrier-mediated transporters, ion transporters and efflux pumps. The endothelial cells that constitute the BRB, express a low number of these transporter mechanisms, some of the most important are indicated.

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