Pericapillary permeability of the ciliary processes: role of molecular charge

Nancy S. Peress and David C. Tompkins

The pericapillary permeability of the ciliary processes to intravenously injected native (anionic) ferritin, neutral ferritin, and two cationic ferritin derivatives was studied in normal rats by electron microscopy. Anionic and neutral ferritin were largely confined to the circulatory compartment. Those particles that entered the pericapillary region of the ciliary processes were randomly scattered within the basal lamina. In contrast, cationic ferritin left the circulatory compartment and accumulated in the pericapillary region in association with the endothelium, the endothelial fenestrations, and the subendothelial basal lamina. The most cationic of the tracers used exhibited the greatest penetration and accumulation. The results indicate that the localization of tracer within the pericapillary region of the ciliary processes is directly related to the tracer's isoelectric point. The findings suggest that this region of the ciliary processes contains fixed anionic groups that influence its permeability.

Key words: cationic ferritin, ciliary processes, selective permeability, anionic probe, basal lamina

The present study utilizes cationized ferritins as specific ultrastructural probes for polyanions to determine whether there are fixed negatively charged substances in the pericapillary region of the ciliary processes that may influence its selective permeability. The investigation of fixed electrostatic forces in the ciliary processes is important in defining the factors responsible for the composition of the normal aqueous humor and in understanding the pathogenesis of disease states that damage the ciliary processes. The pericapillary region of the ciliary processes, like the choroid plexus and renal glomeruli, is a site of selective immune complex entrapment in such disorders as systemic lupus erythematosus, murine lupus, and experimental serum sickness. These disorders are associated with an alteration in the protein composition of the filtered and secretory products of these analogous structures. Intrinsic electrostatic forces appear to be important determinants of normal renal glomerular and choroid plexus filtration. A loss of glomerular polyanion has been demonstrated in experimental disorders characterized by proteinuria, i.e., immune complex glomerulonephritis. The present study, which describes the selective distribution of native (anionic), neutral, and cationic ferritin within the pericapillary region of normal rat ciliary processes, provides direct evidence that electrostatic forces influence filtration in the ciliary processes.

Materials and methods

Native cadmium-free horse spleen ferritin (polysciences) was cationized according to a modification of the original ferritin cationization proce-
dures described by Danon et al. Four ferritin tracers, native (pI 3.9 to 5.1), neutral (pI 5.9 to 8.1), and two progressively more cationic ferritin derivatives (pI 7.9 to 10.0 and 10.0 to 12.0) were utilized for intravenous injection. These were designated I through IV, respectively. The isoelectric points of each ferritin tracer were determined by isoelectric precipitation. The ferritin solutions contained between 35 and 100 mg/ml tracer in normal saline, determined spectrophotometrically at 270 nm based on a value of $E_{270}^\infty = 79.9$. Eight normal adult male Wistar rats, two for each tracer, received an injection of 0.15 mg/gm of body weight of tracer ferritin via the dorsal vein of the penis. These animals were sacrificed by decapitation 15 min after injection. A second group of four rats, two for each tracer, received 0.15 mg/gm doses of the cationic tracers III and IV. These rats were sacrificed by decapitation 2 hr after the intravenous injection of tracer ferritin. Samples of ciliary body and renal cortex were immediately obtained from all animals and fixed by immersion in 3% glutaraldehyde in 0.2M Na cacodylate overnight. They were postfixed in 1% OsO$_4$ and 0.05M potassium ferricyanide in 0.2M Na cacodylate. Tissue was then stained in block with 1% uranyl acetate and embedded in Epon 812. To obtain uniformity of thickness, only thin sections silver to grey were utilized. Unstained and stained lead-citrate sections were examined and micrographs were taken. Examination was confined to the distal portions of the ciliary processes and to the outer loops of the renal glomeruli for purposes of comparison and internal control of methods. As a measure of permeability, the concentration of injected tracer in the immediately adjacent capillary lumena was compared with that in the filtration barrier. The tracers were well tolerated in the amounts utilized and produced labeling of the glomerular filtration barrier corresponding to that reported by others.

Results

The ciliary processes had normal ultrastructure. A definite consistent distribution for each of the ferritin species was easily observed in the pericapillary region of the ciliary processes. Native ferritin (tracer I, pI 3.9 to 5.1) was most concentrated within the capillary lumen. Isolated particles were seen in an apparently random distribution in the basal lamina and free within the epithelial cytoplasm (Fig. 1, A). A similar random distribution was observed with neutral ferritin (tracer II, pI 5.9 to 8.1). There was, however, slightly more neutral ferritin (tracer II) within the basal lamina.

With the cationic ferritin (tracer III, pI 7.9 to 10.0), a selective distribution of tracer ferritin was observed in the pericapillary region of the ciliary processes within 15 min after intravenous injection, which consisted of tracer aggregates along the endothelium often adjacent to the fenestrations. In addition, single isolated particles were noted within the inner aspect of the basal lamina and in association with bundles of collagen (Fig. 1, B). With the more cationic ferritin (tracer IV, pI 10.0 to 12.0), aggregates of tracer were seen adjacent to the endothelium and its fenestrations, within the fenestrations, and immediately beyond in the subfenestration region of the basal lamina (Fig. 1, C and D). Scattered particles were observed within the subendothelial regions of the basal lamina. Only rarely were particles of cationized ferritins seen in the outer region of the basal lamina. The epithelium was essentially free of tracer. The concentration of cationized tracer within the pericapillary region was equal to or greater than that seen free in the corresponding capillary lumen. The basal lamina distribution of cationic ferritin, predominantly restricted to the subendothelial region, was not altered by circulation time. With prolonged circulation, proportionally more cationized tracer was present within the inner aspect of the basal lamina but not in its outer region (Fig. 2).

The glomerular basal lamina distribution of both native (anionic) and neutral ferritin was similar to that observed in the basal lamina of the ciliary processes. The only perceptible difference was a slight increase in randomly scattered tracer particles within the glomerular basal lamina relative to lumen concentration. With both cationized ferritins (tracers III and IV) glomeruli not only exhibited an inner, endothelial, and subendothelial aggre-
Fig. 1. A and B. Pericapillary region of the ciliary processes of Wistar white rat, showing the distribution of tracer ferritin 15 min after intravenous injection. Micrographs are oriented with the capillary lumen at the bottom and the epithelial layer at the top. (Both x25,000.) A, Neutral ferritin, tracer I (pI 3.9 to 5.1), is concentrated in the capillary lumen. Occasional particles are randomly distributed within the basal lamina. B, Cationic ferritin, tracer III (pI 7.9 to 10.0), exhibits a selective distribution within the pericapillary region. There are aggregates of tracer along the endothelium adjacent to the luminal side of the endothelial fenestrations. Single particles are present within the inner basal lamina and in association with bundles of collagen. A rare particle is present in the outer subepithelial region of the basal lamina.
Fig. 1, C and D. Pericapillary region of the ciliary processes of Wistar white rat, showing the distribution of tracer ferritin 15 min after intravenous injection. C, Cationic ferritin, tracer IV (pI 10.0 to 12.0), is concentrated within the inner subendothelial basal lamina. Particles of tracer can be seen on the luminal side of the endothelial fenestrations, within the fenestrations, and beyond in the subendothelial basal lamina. No particles are present in the outer regions of the basal lamina. (×25,000.) D, Electron micrograph of tracer IV at higher magnification showing its passage through an endothelial fenestration. (×70,000.)
Fig. 2. Pericapillary region of the ciliary processes of a Wistar white rat 2 hr after intravenous injection of tracer IV (pI 10.0 to 12.0), showing selective endothelial and subendothelial distribution of tracer similar to that observed within 15 min (Fig. 1, C). Electron micrograph oriented as in Fig. 1, with capillary lumen at bottom and epithelium at top. (x25,000.)

gation of tracer similar to that seen in the ciliary processes but also showed striking subepithelial concentration of tracer, with particular aggregation in relationship to the epithelial slits (Fig. 3). Similar dense subepithelial tracer aggregation was never observed in the basal lamina of the ciliary processes.

Thus, in summary, the concentration of tracer within the pericapillary region of the ciliary processes depended directly on the pI of the tracer. Anionic and neutral ferritin tended to stay in the circulation; only a small proportion penetrated the endothelial lining. In contrast, within 15 min of injection, cationized ferritin readily passed out of the capillary lumen into the pericapillary region of the ciliary processes. Focal aggregates of tracer particles were seen in relationship to the endothelial fenestrations and a pattern of particle distribution was observed within the inner subendothelial region of the basal lamina. The findings were similar 2 hr after tracer injection.

Discussion

This study of the permeability of the pericapillary region of the ciliary processes is part of a broader investigation of factors influencing the selective vulnerability of the ciliary processes and choroid plexus in systemic immune disorders characterized by the deposition of immune complexes within the pericapillary region of these filtration barriers. The permeability of the choroid plexus and the ciliary processes to markers of varying molecular size has been partially delineated. The present article probes the permeability of the pericapillary region of the ciliary processes with cationized ferritins of varying molecular charge. The study is the first to define the presence of fixed anionic groups in the pericapillary region of this filtration barrier, which appear to be electrostatic determinants of its permeability.

The patterns of ferritin distribution observed in the ciliary processes are similar to those recently described in the choroid...
Fig. 3. Renal glomerulus of a Wistar white rat, showing the distribution of cationic ferritin, tracer IV (pH 10.0 to 12.0) 15 min after injection. In contrast to the distribution of this tracer in the ciliary processes (Fig. 1, C), the glomerulus exhibits an extensive subepithelial concentration of cationic tracer. As in the ciliary processes, this tracer is also distributed in the inner aspect of the pericapillary region. Electron micrograph oriented as in Figs. 1 and 2, with capillary lumen at bottom and epithelium at top. (×25,000.)

Both these neuroepithelial structures concentrate cationic ferritin in relationship to the endothelium, the endothelial fenestrations, and the subendothelial region of the basal lamina. In contrast to the glomerulus, they both fail to concentrate this anionic probe in the subepithelial regions of the basal lamina. In this respect they are more closely similar to each other than to the nephron, with which they share many structural, physiologic, and immunologic properties. This disparity between these three analogous secretory and filtering structures is perhaps caused by differences in the morphology of their epithelial layers. The epithelium of the choroid plexus and the nonpigmented epithelium of the ciliary processes are joined by restricting zonulae occludentes, which are not present in the epithelial layer of the renal glomeruli. These are restrictive to very small proteins not retained by the barrier of the glomerular epithelium, i.e., horseradish peroxidase (mol. wt. 40,000 daltons, molecular radius 25 Å). Thus, unless there is significant transepithelial macromolecular vesicular transport, as has been suggested by certain tracer studies, the zonulae occludentes of the epithelium of the choroid plexus and the nonpigmented epithelium of the ciliary processes are the major filtration barriers that separate the blood from the fluids of the cerebral ventricles and posterior chamber.

Our failure to observe an outer, subepithelial basal lamina distribution of cationized ferritin, as seen in the glomerulus, suggests an absence of fixed polyanion in the subepithelial region of the basal lamina of the ciliary processes. Because of the consistency of the observation in all animals studied, this explanation is preferred over the alternative of enhanced tracer avidity in the inner por-
tion of the pericapillary region preventing deeper penetration of tracer. That the observed distribution of cationized ferritin in the pericapillary region of the ciliary processes reflects the localization of fixed polyanions in its structure is based on the demonstration that (1) variations in fixation, including immersion fixation without prior perfusion as used in this study, (2) prolonged prefixation perfusion, and (3) alterations in renal blood flow do not affect the glomerular distribution of tracer ferritin, which has been used to define glomerular polyanion. 3, 9

The biologic significance of electrostatic determinants of permeability is evident from the results of studies on immune disease. It has been recently shown that immune complexes with varying antigen charge have different abilities to produce glomerular immune deposits in the pathogenesis of experimental glomerular immune complex disease. 31-33 In addition to electrostatic charge, it seems clear that other intrinsic factors also influence the selectivity of immune entrapment within a capillary bed. These include the local capillary hydrostatic pressure, the sensitivity of the capillary bed to the vasoactive substances released in association with circulating immune complexes, and the presence of immune component receptors in the filtration barrier.

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REFERENCES


