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Abstract

Rat kidney endothelial cell morphology was examined after introducing iron colloid particles of positive or negative charge to investigate the relationship between the electric charge and permeation through the glomerular capillary. The kidneys were first perfused with Hanks' solution through the renal arteries and then with iron colloid particles of positive or negative charge. The iron colloid particles of positive charge were prepared with ferric chloride and cacodylate solutions, and the negative particles were prepared with iron chondroitin sulfate colloid particles. The iron colloid particles of positive charge adhered to the surface of endothelial cells of the glomerular capillaries, as well as the arterioles, capillaries and venules. Some particles were taken up by pinocytosis, accumulated in the glomerular basement membrane and appeared in the urinary spaces passing through the filtration slits of podocytes. Iron colloid particles of negative charge neither adhered to the endothelial cells nor were taken by the cells. They did not permeate into the urinary spaces. Permeation into the tubular lumen through the peritubular venules was not observed with particles of positive or negative charge.

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PERMSELECTIVITY OF THE GLOMERULAR WALL EXAMINED WITH IRON COMPOUND TRACER

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Abstract. Rat kidney endothelial cell morphology was examined after introducing iron colloid particles of positive or negative charge to investigate the relationship between the electric charge and permeation through the glomerular capillary. The kidneys were first perfused with Hanks' solution through the renal arteries and then with iron colloid particles of positive or negative charge. The iron colloid particles of positive charge were prepared with ferric chloride and cacodylate solutions, and the negative particles were prepared with iron chondroitin sulfate colloid particles. The iron colloid particles of positive charge adhered to the surface of endothelial cells of the glomerular capillaries, as well as the arterioles, capillaries and venules. Some particles were taken up by pinocytosis, accumulated in the glomerular basement membrane and appeared in the urinary spaces passing through the filtration slits of podocytes. Iron colloid particles of negative charge neither adhered to the endothelial cells nor were taken by the cells. They did not permeate into the urinary spaces. Permeation into the tubular lumen through the peritubular venules was not observed with particles of positive or negative charge.

The mechanism of translocation or permeation of substances through the blood capillary is not known in detail. Serum proteins and other macromolecules do not pass through the capillary walls in spite of the endothelial cell pores or caveolae intracellulares which are large enough to allow permeation of macromolecules through the cells. In the kidney the glomerular endothelial cells have many pores (fenestrae) but albumin and other large plasma proteins do not permeate into the urinary lumen. Tracer studies suggest that both the glomerular basement membrane (GBM) and filtration slit may play major roles in the filtration function of the kidney (1-6). The basement membrane consists mainly of collagen fibers of mesh structure that may allow the permeation of macromolecules. Glomerular epithelial cells are endowed with a thick anionic cell coat and this may function as a protein barrier (7-11). Tests using negatively charged ferritin particles showed that the particles hardly reached the pores or invaded

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the underlayer of the endothelial cells (lamina rara interna). In this paper it is reported that transfused iron colloid particles of positive charge (ICPP) adhered to the glomerular endothelial cells, passed through the endothelial cell pores and GBM and were excreted into the urinary lumen, whereas iron colloid particles of negative charge (ICPN) did not adhere to the endothelial cell surface and did not permeate the cell pores and basement membrane. ICPN were not excreted into the urinary spaces.

MATERIALS AND METHODS

Twenty-eight male albino rats of the Wistar strain weighing about 250 g were used. They were anesthesized by ether, and the peritoneal cavities were opened. The abdominal aorta was ligated at two points, one just above the branching of the arteria mesenterica superior and the other above the branching of the arteria mesenterica inferior. The vena cava inferior was severed. Then a drip effusion needle was inserted into the abdominal aorta, and the kidneys were perfused with Hanks' solution for 5 min. After perfusion, ICPP was introduced into six animals and ICPN into six other animals. In each group three animals were sacrificed immediately after completion of iron perfusion, and the remaining three animals were sacrificed 10 min later. The kidneys of 12 other animals were perfused with Hanks' solution for 5 min. They were divided into two groups of six animals each. Animals in the first group were perfused with 10 ml of 0.1% trypsin dissolved in Hanks' solution, and those of the second group were perfused with 0.4% trypsin solution. In each group three animals were further perfused with ICPP, and three others were perfused with ICPN. These latter 12 animals were sacrificed immediately after perfusion with iron colloid solution. The remaining four animals served as controls, and they were perfused with only Hanks' solution. Two control animals were sacrificed immediately after perfusion and the two other animals 10 min after perfusion. Upon sacrifice the kidneys were collected, fixed with 1.25% glutaraldehyde, dehydrated through ethanol, embedded in paraffin, sectioned and stained by the Prussian blue method of Perls with or without poststaining by Kernechtrot (12).

For electron microscopy small pieces of fixed tissue were refixed with 1.25% glutaraldehyde for 3 hr, postfixed with 1% osmium tetraoxide for 2 hr (12), dehydrated through an ethanol series and embedded in Epon by the conventional method (12). Thin sections were prepared with a Porter-Blum MT-1 microtome, stained with lead citrate (12) and observed under a Hitachi electron microscope, HU-11A.

ICPP were prepared by effusing 5 ml of $FeCl_3$ solution into 60 ml of boiling water using a syringe adapted with a 0.25 mm needle. After cooling, the pH of the solution was adjusted to 6.5 by adding 0.2 mol sodium cacodylate and made isotonic by adding glucose. As ICPN, iron chondroitin sulfate was suspended in cacodylate buffer solution, pH 6.5, containing glucose at an isotonic level. In all iron colloid solutions the final concentration of iron was adjusted to 2 mg Fe per ml by using isotonic glucose solution. The solutions were prepared just

before use (12). Electrophoresis was carried out on cellulose acetate membranes at 0.6 mA/cm for 15 min to determine the electric charge of the iron colloid particles in these solutions. After electrophoresis, the membranes were heat-dried and stained by the Prussian blue method of Perls.

Glucose and sodium cacodylate used were commercial products purchased from Nakarai Chemicals, Osaka. Ferric chloride and iron chondroitin sulfate used were commercial products purchased from Katayama Chemicals, Kyoto and Dainippon Seiyaku, Osaka, respectively.

RESULTS

Electrophoresis of iron colloid particles demonstrated that ICPP moved toward the cathode and ICPN toward the anode. The adsorption test of iron exchange resin particles showed that in a cacodylate buffer solution of pH 6.5 ICPP were adsorbed to particles of negative charge and ICPN to positively charged particles. These tests showed that ICPP is positively charged and ICPN is negatively charged.

Light microscope observations of tissue sections stained with Prussian blue reaction revealed that a heavy iron deposition was present in the interior vessel surfaces of animals treated with ICPP (Fig. 1), but no iron reaction was present in tissues treated with ICPN (Fig. 2). Negative iron reaction was also found in the controls. In the glomerulus Prussian blue reaction appeared stronger in tissues collected immediately after ICPP perfusion than in tissues collected 10 min after

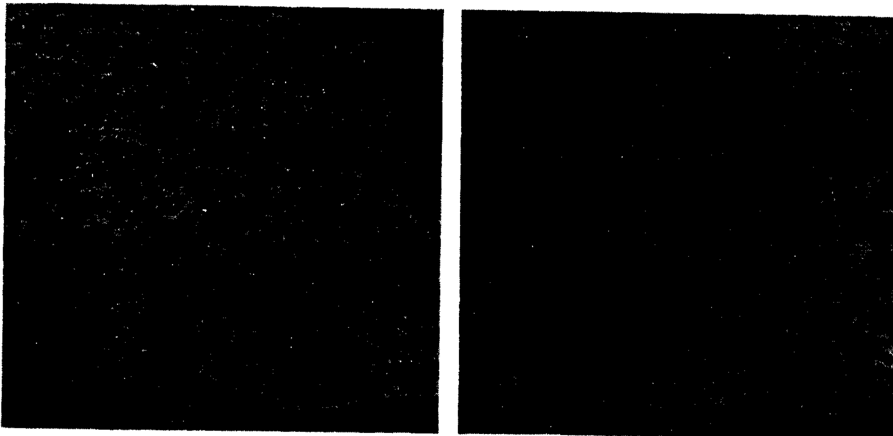

Fig. 1. A light micrograph of rat kidney. The tissue was removed 10 min after introduction of iron colloid particles of positive charge. The section was stained by the Prussian blue method of Perls. Note that the endothelial cells of vessels were stained with iron reaction, and in the tubuli, iron reaction positive casts were present. ×100.

Fig. 2. A light micrograph of rat kidney. The tissue was removed 10 min after introduction of iron colloid particles of negative charge. The section was stained by the Prussian blue method of Perls. No iron reaction was seen. ×100.

F
P
3

perfusion. In the latter case, urinary casts of positive iron reaction were markedly deposited in the renal tubuli (Fig. 1). No findings suggesting severe tissue damage were found in either case.

At high magnification iron reaction appeared in the endothelial cells of the arteries, capillaries and veins of kidneys perfused with ICPP. In both elastic and muscular arteries, the elastic fibers, connective tissues and muscle fibers were free of iron reaction in contrast to the deep blue endothelial cells. In glomerular capillaries, however, the basement membrane showed positive iron reaction as well as the endothelial cells, though the glomerular epithelial cells gave negative Prussian blue reaction.

Electron microscopy of kidneys irrigated with ICPP and prepared immediately revealed that the surfaces of the endothelial cells of the blood vessels were coated with electron dense particles of 100–300Å. These were considered to be ICPP, because such particles were never seen in the controls. In the basement membrane of the arteries, veins and also the venules, only a few particles were found and no particles were obsereved in the deep areas of the vessel walls.

In the glomerular capillaries, however, the basement membrane contained masses of particles which were distributed most densely in the lamina rara interna, moderately in the lamina densa and sparsely in the lamina rara externa. Some filtration slits had dense accumulations of particles, but no particles were found on the surface of the podocytes (Fig. 3). Some tubuli had electron dense particles. In glomeruli removed 10 min after ICPP injection, the deposition of particles was much less compared with glomeruli collected immediately after injection. ICPN treatment did not result in the deposition of electric dense particles (Fig. 4) as in the control cases.

Pretreatment by perfusion of 0.1% trypsin solution resulted in no noticeable effect on adsorption of ICPP to the surface of endothelial cells of glomerular capillaries whereas treatment with 0.4% trypsin inhibited the adherence of ICPP to the surface of endothelial cells. However, ICPP was found deposited in the basement membrane most densely in the lamina rara interna, moderately in the lamina densa and sparsely in the lamina rara externa. Dense accumulations of particles were found in some filtration slits but no particles were present on the surface of the podocytes (Fig. 5). Some tubuli had electron dense particles in the

Fig. 3. An electron micrograph of rat glomerular capillary wall 10 min after perfusion of 2 mg/ml of iron colloid particles of positive charge in cacodylate buffer solution. Electron dense particles (P) adhered to the surface of the endothelial cell (E) and accumulated in the lamina rara interna (LI), lamina densa (LD) and lamina rara externa (LE). Some electron dense particles accumulated in the filtration slits (F). ×60,000.

Fig. 4. An electron micrograph of rat glomerular capillary wall 10 min after perfusion of 2 mg/ml of iron colloid particles of negative charge in cacodylate buffer solution. No electron dense particle was seen. ×60,000.

lumen.

Pretreatment with 0.4% trypsin resulted in the adherence of ICPN to the surface of endothelial cells of glomerular capillaries, but ICPN did not invade into the GBM and urinary spaces (Fig. 6)

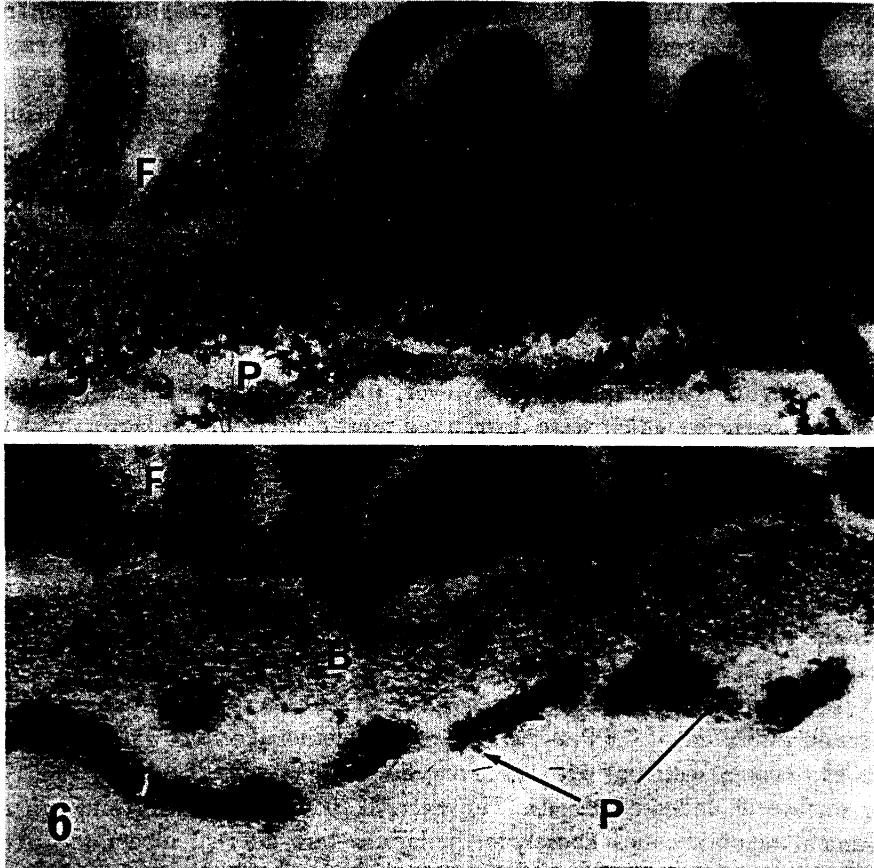

Fig. 5. An electron micrograph of glomerular capillary wall of a rat pretreated with 0.4% trypsin and perfused 10 min later with 2 mg/ml of iron colloid particles of positive charge in cacodylate buffer solution. Electron dense particles (P) did not adhere to the surface of the endothelial cell (E), but accumulated in the glomerular basement membrane (B) and filtration slits (F). ×60,000.

Fig. 6. An electron micrograph of glomerular capillary wall of a rat pretreated with 0.4% trypsin and perfused 10 min later with 2 mg/ml of iron colloid particles of negative charge in cacodylate buffer solution. Electron dense particles (P) adhered to the surface of the endothelial cell (E), but did not accumulated in the glomerular basement membrane (B) nor in the filtration slits (F). Electron dense particles did not adhere to the surface of the podocyte (PC). ×60,000.

DISCUSSION

The present observations revealed that positively charged iron colloid particles adhered to the surface of rat endothelial cells when such colloid particles were introduced into blood-depleted kidneys through the renal artery. In glomeruli the iron particles showed an affinity to the endothelial cells, passed through their cytoplasmic pores and reached the basement membrane, showing heavy depositions there. Some of these particles accumulated in the filtration slits of the podocyte and were translocated into the urinary space. But particles of negative charge neither adhered to the endothelial cells nor transferred to the basement membrane. Similar results have been reported by Rennke, Cotran and Venkatachalam (5) in observations of the translocation of ferritin particles of positive and negative charge from blood to the urinary spaces through the glomerular capillary.

The phenomenon seems to explain the mechanism of negatively charged serum proteins being kept in the blood vessels without being lost by filtration through the capillaries. The negatively charged groups on the endothelial cell surface and basement membrane may be responsible for the repulsion of negatively charged macromolecules from the endothelial surface, where they show affinity to positively charged particles allowing them to reach the urinary space by passing through the fenestrae and basement membrane.

Preperfusion with trypsin resulted in the loss of affinity of endothelial cells to positively charged iron colloid particles and reduced the rejection activity against negatively charged particles. Therefore, the groups responsible for the adhesion of positive colloid particles and rejection of negative particles are mainly the negative groups bound to the surface proteins, probably carbohydrates of negative charge. In the GBM sialoprotein also serve in the invasion of positively charged particles to the basement membrane (8, 13, 14, 15) and the rejection of negatively charged particles. The existence of neuraminic acids and hyaluronic acids can be demonstrated histochemically on the cell surface of the podocyte and capillary endothelial cells (7, 8), and it has also been demonstrated that sialoprotein is reduced in the glomeruli of the kidney of experimental glomerulonephritis (16, 17). The fact that preperfusion of kidney with trypsin induced adhesion of the negatively charged particles to the endothelial cells but did not allow the invasion of particles to the basement membrane indicates that the negatively charged glycoprotein in the GBM may be working with endothelial cells to keep the negatively charged particles or serum protein molecules in the vessels.

Glomerular filtration mechanism studies using dextran and other agents (1–4, 16, 17) may favor the molecular sieve theory. These experiments proved that particles like dextran, larger than 78 Å and 125,000 in MW, are not permeable

through the capillary wall of the glomeruli. The dextran may originally be uncharged but if the particles are introduced into blood they then become negatively charged particles acquiring a lipid monolayer coat (18). Dextran sulfate particles with negative charge are less permeable through glomerular capillaries than dextran particles of the same size with no electric charge (6). The present experiment showed that ICPP of 100-300 Å permeated easily through the GBM into the urinary space, indicating clearly that the glomerular capillary is not a simple molecular sieve, but a membrane of permselectivity. Albumin is smaller than ICPP in size, but does not permeate through the normal glomerular capillaries. The capillary wall is actually a barrier against serum proteins.

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