Uptake of Ferritin in Rat Kidney Stimulated by Renal and DOCA-Induced Hypertension

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Ferritin, a mammalian protein of high iron content, can function as a labile tissue depot for this mineral.1–4 Because of its iron content, as well as its convenient size and characteristic ultrastructure,5–8 ferritin has been used as an electron microscopic probe in intact9–11 and pathologically altered tissue.12–14

Phagocytosis by reticuloendothelial cells has been investigated by using the particulate properties of ferritin.15–18 A phagocytic function, demonstrated by the light microscopic uptake of ferritin as stainable iron, has recently been ascribed to the renomedullary interstitial cells.20 These cells are believed to be secretory in nature, possibly elaborating vasoactive lipids belonging to the class of compounds known as prosta-
glandins. The characteristic osmiophilic granularity of these renal cells has been shown to vary inversely with the development of both de-
oxycorticosterone acetate (DOCA)21 and renal hypertension.22–24

The purpose of this paper is to describe the hypertension-dependent single-dose differences in exogenous renal uptake of ferritin.

Materials and Methods

Male Sprague-Dawley rats (Zivic-Miller, Allison Park, Pennsylvania), weighing between 140 and 160 g each, were used to study DOCA-stimulated uptake of ferritin. Mineralocorticoid hypertension was induced in 10 intact animals by the subcutaneous injection, for 21 consecutive days, of DOCA (30 mg/kg) dissolved in sesame oil (0.5%). These animals received a solution of 15% NaCl ad libitum as their drinking water. The control group for this series consisted of 6 intact rats injected subcutaneously, for a similar period of time, with proportional quantities of the sesame oil vehicle. The controls were provided with tap water rather than saline.

Male Sprague-Dawley rats, weighing between 180 and 200 g each, were used to study the uptake of ferritin stimulated by renal hypertension. The hypertension was established in 6 rats by coarctating the abdominal aorta just above the junction of the left renal artery. A ligature of No. 00 surgical silk was tied snugly around the aorta and a 23-gauge stylet; the latter was removed immediately, leaving a constriction around the vessel. Four sham-operated rats were used as controls. All animals in these studies were fed a standard diet of pelleted Purina rat chow.

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After 3 weeks, blood pressure in the femoral arteries of rats made hypertensive with DOCA and their controls was measured, in animals lightly anesthetized with ether, by cannulating the artery while recording the systolic blood pressure directly from an open-arm mercury manometer. In the rats with renal hypertension and their controls, blood pressure in the right brachial arteries was measured similarly 5 weeks after the operation.

A 10% solution of horse spleen ferritin (General Biochemicals, Chagrin Falls, Ohio) was rendered free of cadmium using the method described by Farquhar and Palade. The preparation was dialyzed in the cold for 48 hours against 0.1 M disodium ethylenediaminetetraacetate (EDTA) and then for 36 hours in 0.07 M potassium phosphate buffer (pH 7.2). All animals were anesthetized with ether and a femoral vein was exposed. The cadmium-free preparation of ferritin was slowly infused into this vessel at a constant dosage of 80 mg/100 g of body weight. The rats were sacrificed by decapitation 10 hours after ferritin was administered and the left kidneys of the rats treated with DOCA and salt or sesame oil were quickly removed for histologic study. In the unilateral renal hypertensive group, the hypertrophic right kidney rather than the contralateral atrophic organ was examined histologically. Thin slices of the kidney were fixed in 10% formalin and embedded in paraffin. Sections were stained by the Golgi iron reaction (Prussian blue) with Kernechtrot (nuclear fast red) counterstaining.

For the electron microscopic studies, small blocks of the outer medullary region of the kidneys were immediately placed in ice-cold fixative where they were allowed to remain overnight. The fixative solution was composed of 2.5% glutaraldehyde, 0.05 M sodium phosphate buffer (pH 7.4) and 4% sucrose. The tissues were washed for 1 hour with several changes of a solution of 0.05 M sodium phosphate buffer (pH 7.4) and 4% sucrose and then postfixed in a solution of 1% osmium tetroxide, 0.05 M sodium phosphate buffer (pH 7.4), and 4% sucrose for 1 hour. They were then dehydrated in an ascending series of ethyl alcohols and finally embedded in Epon 812. Thin sections were cut with diamond knives and a MT-2 Porter-Blum ultramicrotome. They were mounted on unsupported copper grids (300 mesh) and were stained with 1% uranyl acetate and lead citrate. The sections were examined with a Siemens-Elmiskop 101 electron microscope.

**Results**

The blood pressures of the animals made hypertensive with DOCA ranged from 160 to 180 mmHg while their controls were 100–110 mmHg. Blood pressures of rats with renal hypertension were 180–220 and their sham-operated controls 110–125 mmHg.

**Light Microscopic Observations**

The arterioles in the renal parenchyma of the rats with renal and DOCA-induced hypertension exhibited hyalinized thickened walls with resultant narrowing of their lumens (Fig 1). Many of these arterioles were labeled with ferritin, which was observed as a blue-staining amorphous material in their lumens and as zones of discrete reaction product in the intima. These changes were of somewhat greater magnitude in the animals with renal hypertension. The arterioles from both control groups were morphologically unremarkable and free of demonstrable deposition of ferritin.
The small renal arteries in both types of hypertension often appeared occluded, especially those with renal hypertension (Fig 2). Subendothelial aggregation of ferritin was evident and was especially prominent in the animals with renal hypertension. Perivascular accumulation of ferritin was occasionally noted in both of the control groups (Fig 3).

The glomeruli of the rats with renal hypertension frequently displayed proliferative changes with partial adhesion of the glomerular tuft to Bowman’s capsule (Fig 4). A blue-staining exudate was often present, filling most of the remaining Bowman’s space. The capsule was thickened. Heavy infiltration of ferritin was evident throughout the tuft, especially in the hilar region, and within the mesangial cells (Fig 4).

The glomeruli of the rats made hypertensive with DOCA were often enlarged and hypercellular (Fig 5). The tuft frequently adhered to Bowman’s capsule and often occupied most of Bowman’s space. A blue-staining exudate partially filled the remainder of this area. Dense foci of ferritin deposition were apparent throughout the hypercellular tuft and within the mesangial cells (Fig 5). The glomeruli of the control groups were morphologically normal, presenting random, sparse and pale ferritin reactivity (Fig 6).

The proximal convoluted tubules of rats with both renal and DOCA-induced hypertension appeared dilated with degenerate cytoplasm and luminal casts (Fig 7 and 8). Ferritin was deposited extensively within the tubular epithelium. The tubules from both control groups were morphologically unremarkable with an apparent total absence of uptake of ferritin by the intact tubular epithelial cells (Fig 9).

The renomedullary interstitial cells of control as well as hypertensive groups retained ferritin. The amount of stainable iron was relatively limited in the control groups (Fig 10) while it was very intense and extensive in both forms of experimental hypertension (Fig 11 and 12). Increased uptake of ferritin appeared to be related to the degree of proliferation of these interstitial cells.

Electron Microscopic Observations

The renomedullary interstitial cells of all groups contained ferritin, which was exclusively concentrated in their osmiophilic granules. Frequently the deposits in the control groups assumed a dense convex pattern extending inward from the periphery of the single limiting membrane (Fig 13). These inclusions were rarely filled with electron-dense ferritin particles whereas the osmiophilic granules of the group made hypertensive with DOCA always appeared to be completely occupied with aggregates of ferritin (Fig 14). Granules from both the control
and DOCA-treated rats were uniformly round to oval (Fig 13 and 14) while those from the renal hypertensive animals were usually irregularly shaped (Fig 15). The ferritin was extensively condensed within the renal hypertensive granules and sometimes appeared as intense round foci (Fig 15). The size of the ferritin-containing cytoplasmic bodies of these cells appeared to parallel the sustained elevation of blood pressure. Normal rats had granules measuring 400–570 μ in diameter (Fig 13) while those of DOCA-treated animals were considerably larger—i.e., 630–1,000 μ (Fig 14). In the rats with renal hypertension, these structures were enlarged grossly, measuring 1010–2160 μ (Fig 15).

Discussion

This study indicates that the pattern of ferritin uptake in the normal kidney is site selective. Similar abnormalities in distribution of the tracer were seen in the two forms of experimental hypertension investigated.

Increased renovascular permeability, arteriolar lesions, and glomerulosclerosis associated with the development of hypertension have been described previously. These well-documented morphologic changes also have been observed in this study. With ferritin as a light microscopic marker, increased iron deposition was found in the hypertension-changed renal arteries, arterioles and glomeruli. This observation is further evidence for the pathologic involvement of these structures. Enhanced deposition of ferritin occurs in the glomeruli of the amino-nucleoside nephrotic rat, due to a defect in the basement membrane. Defects in the basement membrane and/or endothelium may be involved in the abnormal incorporation of ferritin that was observed.

Dilatation and atrophy of the proximal convoluted tubules of hypertensive rats have been described. The authors of the present study concur with these previous observations and further point out that extensive deposits of exogenous ferritin are now found in the cytoplasm of the damaged tubular epithelium. It is not clear why these cells from both control groups remained devoid of ferritin but this may be related to the permeability characteristics of their intact plasma membranes. From this study, one cannot conclude with surety whether the hypertension-mediated differences in uptake were due to alteration of the cell membrane or were a consequence of stimulated vesicular transport processes in the membrane.

The ultrastructure of the renomedullary interstitial cells has been characterized; however, their precise role remains speculative. These cells enclose numerous lipid-rich cytoplasmic granules that are thought to contain either a secretory product or its precursor.
interstitial cells from glomeruli, arteries, and arterioles and the deposition of ferritin has been correlated with the presence of the osmiophilic granules. An inverse relationship between DOCA as well as renal hypertension and the abundance of osmiophilic granules has been established. In the present study, the renal medullary interstitium generally was observed to proliferate in rat kidneys with either DOCA-induced or renal hypertension. In the light microscope, these proliferated cells present intensified reactivity to iron, 10 hours after the intravenous injection of horse spleen ferritin. The renomedullary interstitial cells of the normal rat apparently are capable of accumulating ferritin, the process being accelerated by potassium deficiency. The present authors are in basic agreement that these cells normally take up ferritin, but now report that they do so at an apparently slower rate compared to that of hypertensive tissue. Evidence that a potassium deficiency mechanism may not be involved in explaining our observations is given in the report that the renal medulla of normal and hypertensive rats contain similar concentrations of potassium. Employing electron microscopy, the present authors have observed that the ferritin taken up by the renomedullary interstitial cells of the rat accumulates exclusively in their osmiophilic granules. Ferritin appeared to be stored more readily in the distended osmiophilic granules of renomedullary interstitial cells in both forms of hypertension. The exact nature of the uptake process and the reason for the exclusive infiltration of the ferritin into these specific sites is unknown. It would appear that a relationship exists between the sustained hypertensive state—i.e., the notable hypercellularity of the renomedullary interstitial cells—and the attendant intensified deposition of ferritin within their enlarged granules. Hypertrophy of these inclusions may represent an attempt to incorporate increased quantities of nutrients or precursor substances for the formation of their hypothetic secretory product. The irregular shape of the osmiophilic granules observed in the renal hypertensive animals suggests that ferritin may be engulfed by a process similar to phagocytosis. It is possible that the heightened deposition of ferritin in the osmiophilic granules may be merely the replacement of particulate material in a depleted storage site.

Summary

The uptake of ferritin by kidney tissue of rats with both DOCA-induced and renal hypertension has been studied. In the renal arterioles, arteries, glomeruli, proximal convoluted tubules and renomedullary interstitial cells from the experimental groups, increased deposition of
ferritin was observed. Electron microscopy demonstrated exclusive accumulation of ferritin particles in the osmiophilic granules of the interstitial cells. These granules appeared hypertrophic and ameboid in the hypertensive state. Possible mechanisms and the pathophysiologic significance of these changes are discussed.

References


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[ Illustrations follow ]
Figures 1–12 stained with Prussian blue-fast red (× 330).

**Fig 1.**—Arteriole from rat with DOCA-induced hypertension with hyalinized thickened walls and narrowed lumen. Amorphous ferritin deposition in intima (arrow).

**Fig 2.**—Subendothelial aggregates of ferritin (arrow) in small renal artery of rat with unilateral renal hypertension. Reactive proliferation of intimal and medial layers with occlusion of lumen.

**Fig 3.**—Perivascular deposits of ferritin (arrows) observed in small renal artery of vehicle-injected control.

**Fig 4.**—Hypertrophic glomerulus from rat with renal hypertension. Note thickened capsule and iron-containing exudate in Bowman’s space (arrow). Heavy ferritin aggregation in hilar region (arrow) and mesangial cells (arrows).

**Fig 5.**—Hypertrophic glomerulus from rat with DOCA-induced hypertension. Intense iron-reactive exudate in Bowman’s space (arrow). Large amounts of ferritin deposited throughout hypercellular tuft (arrows).

**Fig 6.**—Normal-appearing glomerulus from vehicle-injected control. Sparse pale and random ferritin granulation (arrow).
Fig 7.—Proximal convoluted tubule from rat with DOCA hypertension. Degenerative cytoplasm contains extensive fine foci of ferritin (arrow) and iron-reactive luminal casts.

Fig 8.—Dilated proximal convoluted tubule from rat with renal hypertension displaying prominent cytoplasmic ferritin reactivity (arrow) and large quantities of iron-staining luminal casts.

Fig 9.—Proximal tubule from sham-operated control devoid of ferritin deposits.

Fig 10.—Renomedullary interstitial cells of vehicle-injected control containing relatively few areas of ferritin involvement (arrow).

Fig 11.—DOCA-mediated proliferation of interstitial cells with increased ferritin deposition (arrow).

Fig 12.—Intense collection of ferritin in hypertrophic interstitial cells observed in rat with renal hypertension. (arrow).
Fig 13.—Deposits of ferritin in osmiophilic granules (G) of renomedullary interstitial cell of vehicle-injected control. Note dense convex distribution pattern extending from periphery and incomplete filling of granules (arrow) (uranyl acetate and lead citrate. X 34,000).

Fig 14.—Enlarged round-to-ovoid ferritin-filled osmiophilic granules (G) from rat with DOCA hypertension. Observe electron density and exclusive involvement of ferritin sedimentation (uranyl acetate and lead citrate, X 27,200).

Fig 15.—Intense aggregation of ferritin (arrow) in enlarged, irregularly shaped osmiophilic granules (G) of rat with renal hypertension (uranyl acetate and lead citrate, X 26,400).