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INFLUENCE OF THE TUBULAR FLOW RATES ON THE ENDOCYTIC UPTAKE AND THE EXCRETION OF HORSERADISH PEROXIDASE BY RAT KIDNEY

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SUMMARY

The renal clearances of horseradish peroxidase (HRP) in diuretic and anti-diuretic rats were compared with the concentrations of HRP in the renal cortex of the same rats. The injection of mannitol, hypertonic saline, or antagonists to histamine and serotonin, together with HRP, caused diuresis. The renal clearance of HRP was 7-15% of the inulin clearance, relatively small amounts of HRP were reabsorbed into the renal cortex, and relatively large amounts were excreted in the urine. However, when HRP was injected alone causing vascular leakage and anti-diuresis, 6-10 times higher concentrations of HRP were found in the renal cortex, and only a few percent of the protein were excreted in the urine during the first 20 minutes after injection. It is suggested that the low or high endocytic uptake of HRP by the renal cortex was related to the high or low tubular flow rates during diuresis or anti-diuresis.

INTRODUCTION

It was reported previously (1-3), that the endocytic reabsorption of horseradish peroxidase (HRP) by the renal tubule cells was several times higher when a relatively large dose of HRP was injected alone into Sprague-Dawley rats than when the same dose of HRP was injected together with antagonists to histamine and serotonin, with mannitol or with hypertonic saline. HRP, at relatively high dose levels, is known to cause vascular leakage due to the release of histamine and serotonin from mast cells (4). Other changes observed during the vascular leakage caused by HRP were a rise in the hematocrit; a decrease in the serum protein concentration; a decrease in the urinary excretion of sodium; an accumulation of HRP in the peritubular spaces; and a temporary drop in the blood pressure (3,5).

A preliminary report on this work was presented at the meetings of the American Physiological Society in St. Louis, Missouri, October 1978.

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The drastic changes in the endocytic uptake of HRP under the experimental conditions mentioned were difficult to understand. In the present work, the renal clearance of HRP alone, at dose levels causing vascular leakage, was compared with the clearances of HRP after treatment with mannitol, hypertonic saline, or with antagonists to histamine and serotonin. In the same animals, the uptake of HRP by the renal cortex was measured. The observations suggest that the tubular flow rate or contact time has a strong influence on the reabsorption of protein by the renal tubule cells, the flow rates being low during vascular leakage (anti-diuresis) and high during diuresis induced by mannitol, hypertonic saline or the antagonists.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 210-270 g in weight were used. In early experiments, a high dose of HRP was infused continuously, following a priming injection of the protein. The blood pressure, glomerular filtration rate (GFR), and urine flow decreased, and the hematocrit increased considerably. Some of the animals died. In subsequent experiments, it was preferred, therefore, to measure the renal clearance of a single, medium to high dose of HRP which had been well tolerated by the animals in previous experiments (1-3).

The animals were anesthesized by intraperitoneal injection of Inactin (100 mg/kg body weight), and a 4% solution of polyfructosan (Laevosan Gesellschaft, Linz, Austria) in physiological saline was infused at a rate of 1.8 ml/hr through the jugular vein. The urine was collected by a bladder catheter into polyethylene tubings (0.86 mm inner diameter). The volume of the urine was calculated from the length of the fluid columns. The blood pressure was monitored continuously with the aid of a transducer and polygraph through a catheter placed into the carotid artery.

After a period of equilibrization, the clearance of polyfructosan alone was measured in order to determine the GFR under control conditions. A single dose of HRP (Sigma type II) was then injected into the jugular vein over a period of 1.0-1.5 minutes. The dose of HRP was 60,000 units (7 mg approximately) per 100 g body weight. Six to nine urine collections and 4-5 plasma samples were taken during the following 60-90 minutes. The animals were killed 90 minutes after injection of HRP. The cortex of one kidney was homogenized and the total particulate fraction was prepared as previously (1-3). The activities of HRP and protein in the total particulate fraction were determined by the methods mentioned below.

For the experiments summarized in Table II, the proportion of the injected HRP which was excreted in the urine 20 and 90 minutes after injection of the standard dose and the concentrations of HRP in the total particulate fractions, 90 minutes after injection, were measured in the same animals used for the clearance experiments (Table I). However, the concentration of HRP in the total particulate fraction and in the urine, 20 minutes after injection of HRP alone, was obtained from separate animals so that the urine from the bladder catheter could be included. The same commercial preparation of HRP was used for all experiments since the contaminants may differ in different batches and may influence the extent of renal reabsorption and excretion.

In experiments in which the effects of antagonists to histamine and serotonin (methapyrilene hydrochloride; Histadyl, Eli Lilly Company, Indianapolis, Ind., and methysergide maleate, Sansert, Sandoc, Inc., East Hanover, N.J.) were tested, 0.3 ml of a mixture containing 1.0 mg of each antagonist per kg body weight was slowly injected into the jugular vein, 2-3 minutes before injecting HRP (3). The clearance of HRP during mannitol diuresis was measured after infusing 0.5 ml of a 5% solution of mannitol into the jugular vein immediately before injecting HRP. Three to four additional i.v. injections of mannitol were given during the next 90 minutes in order to maintain a continuous diuresis. Diuresis by hypertonic saline was induced by injecting 0.5 ml of a 10% solution of NaCl repeatedly. The first injection was given immediately before injecting HRP, and 3-4 injections were made during the following 90 minutes. In some clearance experiments, physiological saline containing 1.5 mg HRP, Sigma type VI, per ml was infused continuously at a rate of 1.8 ml/hr, following a priming injection with 0.5 mg HRP per 100 g body weight.

HRP was assayed with o-dianisidine and H_2O_2 according to Steinman and Cohn (6). The concentrations of HRP in the plasma usually decreased in a logarithmic fashion so that the concentrations at the midpoints of the 10-minute clearance periods could be extrapolated. Polyfructosan in plasma and urine were assayed by the method of Führ et al. (7). Protein was analyzed by the method of Lowry et al. (8). Sodium in plasma and urine was determined by flame photometry.

RESULTS

When highly purified HRP, Sigma, type VI, was infused continuously at a low dose level as indicated above, the clearance of the protein was 7-9% of the inulin clearance. No appreciable changes in the glomerular filtration rate, blood pressure or hematocrit occurred. When a relatively high dose of 7 mg HRP, Sigma, type II, per 100 g body weight was injected during 1-2 minutes into rats in which diuresis had been induced by mannitol or hypertonic saline, the renal clearance of HRP amounted to 10-15% of the inulin clearance (Table I). The injection of the antagonists to histamine and serotonin, together with HRP, resulted in similar clearance values as those obtained during mannitol- and hypertonic saline- induced diuresis (Table I). The antagonists to histamine and serotonin also caused diuresis. After the injection of HRP together with mannitol, the GFR and blood pressure sometimes showed a moderate and transient decrease and the hematocrit a moderate increase.

The uptake of HRP into the renal cortex was 6-10 times lower when HRP was injected together with the agents causing diuresis than when it was injected alone (Table II). After the injection of HRP combined with mannitol, hypertonic saline or the antagonists, 21-25% of the injected protein was excreted

in the urine during the first 20 minutes (Table II). However, when HRP was injected alone, only 6% of the injected protein was recovered in the urine during the first 20 minutes. The renal cortex contained relatively high concentrations of HRP at this time (Table II). During the period of high uptake and low excretion of HRP, the following other changes were observed: a 30-50 mm Hg decrease in the blood pressure, a 50-80% decrease in the GFR, a 50-80% decrease in the urine flow, and a 15-30% rise in the hematocrit.

TABLE I

Comparison of urine flow, hematocrit, GFR, and renal clearances of HRP and sodium in diuretic and anti-diuretic rats[a]

	Urine flow µl/min	Hemato-crit %	GFR ml/min	C_{HRP}/C_{In}	C_{Na}/C_{In} Δ %
		HRP alone			
Controls	8.3 ± 1.8	43 ± 1	2.5 ± 0.1	--	
+ HRP (0-30')	1.4 ± 0.4	56 ± 5	0.6 ± 0.4	<0.001	-83[c]
+ HRP (30-60')	4.3 ± 1.2	54 ± 5	1.1 ± 0.3	0.1[c]	
		HRP + antagonists[b]			
Controls	5.4 ± 2.4	42 ± 2	1.6 ± 0.4	--	
+ HRP	12.7 ± 8.1	42 ± 3	1.5 ± 0.3	0.12 ± 0.02	+64 ± 13
		HRP + hypertonic saline			
Controls	4.7 ± 2.0	42 ± 1	2.2 ± 0.6	--	
+ HRP	33.4 ± 22.6	46 ± 2	2.2 ± 0.6	0.10 ± 0.03	+97 ± 2
		HRP + mannitol			
Controls	4.7 ± 1.1	44 ± 2	2.1 ± 0.4	--	
+ HRP	14.3 ± 5.8	50 ± 7	1.3 ± 0.4	0.14 ± 0.04	+64 ± 18

The values observed during the first 30 minutes after injection of HRP alone, when the urine flow was very slow, are shown separately from the values observed from 30-60 minutes, when the urine flow approached normal values again.

The sodium clearances differed widely in different animals. Therefore, only the percentage differences of sodium clearances in the same animals, before and after injection of HRP, are shown in the last column. The differences for 3-4 rats were averaged.

[a]The values are the means of 3-4 experiments, with standard deviations.

[b]Histadyl and Sansert, 1 mg each per kg body weight.

[c]Estimated.

TABLE II

Concentration of HRP in total particulate fractions of the renal cortex and percentage urinary excretion of HRP after injection of HRP alone or together with agents causing diuresis

Injected agents	Concentration of HRP in total particulate fraction (units/mg protein)		Percentage urinary excretion of HRP	
	after 20 min	after 90 min	after 20 min	after 90 min
HRP alone	26.4 ± 0.8[a]	25.5 ± 3.5	6.0 ± 1.0	27.8 ± 0.8
HRP + antagonists	--[b]	3.6 ± 0.9	25.2 ± 2.2	44.9 ± 3.5
HRP + mannitol	--[b]	4.1 ± 0.6	21.1 ± 2.6	38.6 ± 1.7
HRP + hypertonic saline	--[b]	2.7 ± 0.3	25.5 ± 4.9	44.9 ± 6.5

[a]The values are the means of 3-4 experiments, with standard deviations.
[b]Not tested.

Fifteen to 20 minutes after the injection of HRP, high concentrations of the protein suddenly appeared in the urine and about 25% of the injected HRP was excreted during the following 60 minutes (Tables I, II). The blood pressure, GFR, and urine flow gradually returned towards normal values. The clearance of sodium was also much decreased.

DISCUSSION

The slow urine flow occurring during vascular leakage after the injection of a high dose of HRP made it difficult to relate the concentrations of HRP and sodium in the urine accurately to those in the blood, and corrections had to be made for the delayed transit of the urine through the catheter. The clearance values for HRP and sodium in anti-diuretic rats shown in Table I, should be considered, therefore to be estimations. However, the clearances could be measured accurately in diuretic rats when the urine flow was much increased. They also could be measured accurately when low doses of HRP which did not cause vascular leakage, were infused continuously.

As was also observed previously (1-3), considerable amounts of HRP are taken up by the renal cortex 15-20 minutes after its i.v. injection at dose

levels high enough to cause vascular leakage. However, it could only be seen from renal clearance experiments that relatively little HRP was excreted in the urine during this time. It can be estimated that the amounts of HRP reabsorbed into the renal cortex (total particulate fractions) 20 minutes after the injection into diuretic rats represented only 2% of the excreted HRP whereas it represented over 20% in the anti-diuretic rats. Therefore, the glomerular sieving coefficient for HRP (molecular weight 40,000 daltons) in the diuretic rats differed only slightly from the fractional clearance values shown in Table I. These values were similar to those reported by Rennke et al. (9) for HRP and by Galaske et al. (10) for serum proteins of comparable molecular weights. An excellent review on the glomerular filtration, reabsorption and excretion of proteins by the kidney was presented recently by Maack et al. (11).

It may be suggested that early after the injection of a relatively high dose of HRP, the protein becomes highly concentrated in the glomerular filtrate and is reabsorbed by the renal cortex in relatively high concentrations. Such a correlation would be in accordance with previous observations showing the concentration of HRP in the total particulate fractions of the renal cortex to vary in proportion to that in the glomerular filtrate (1). The flow rate or contact time may be related to the differences in protein uptake by the renal cortex. During diuresis, the flow rate is increased. The contact of HRP to the tubular cells is then relatively short and, therefore, relatively small amounts of protein may be reabsorbed (Table II). During vascular leakage (anti-diuresis), the flow rate of the proximal tubular fluid is decreased. The contact of HRP to the tubular cells is longer and thus the reabsorption of HRP into these cells may be relatively high. Although the concentration of HRP in the tubular fluid may be lower during diuresis, the load of HRP is increased and larger amounts are excreted during diuresis than during anti-diuresis (Table II). The latter effect may be related to the decreased plasma disappearance rates of HRP in the diuretic rats (2,3).

The antagonists to histamine and serotonin also caused diuresis (Table I). It is not known whether the effects of the antagonists on the reabsorption and excretion of HRP were related only to their diuretic properties or also to their antagonistic properties.

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