Renin Distribution in the Rabbit Renal Microvasculature

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Immunocytochemical studies have shown that renin, which is normally located in the juxtaglomerular afferent arteriole, may also be found farther upstream toward the interlobular artery during chronic stimulation of the renin-angiotensin system. We assessed the renin distribution along the renal microvasculature using both quantitative analysis and immunocytochemistry in rabbits that received a normal sodium diet (0.48% NaCl), a low sodium diet (0.04% NaCl), or enalapril (1 mg/kg/day) for 4 weeks. From the outer cortex we microdissected 1) the proximal portion of the afferent arteriole (p-AF) extending from the interlobular artery to a point 50 μm from the glomerulus, 2) the distal 50 μm including its intact terminus (d-AF), and 3) the glomerulus without the vascular pole (GL) and measured their renin content. In controls, renin was 0.3 ± 0.2, 27.0 ± 5.2, and 2.8 ± 0.5 ng angiotensin I/hr/arteriole (or GL) in the p-AF, d-AF, and GL, respectively. The low sodium diet and enalapril increased renin in the d-AF (53.1 ± 6.9 and 68.4 ± 8.1, respectively) but not in the GL (3.3 ± 1.0 and 3.6 ± 0.7). In the p-AF, both caused a small increase (Δ = 1.5); however, this increase was minuscule compared with the large increase in the d-AF (Δ = 41). Although the average length of positive immunostaining along the afferent arteriole was increased during chronic stimulation, only a small percentage of afferent arterioles showed staining farther than 50 μm from the GL. We conclude that in the rabbit 1) approximately 90% of active renin is located in the d-AF, 1% in the p-AF, and 9% in the glomerulus during a normal sodium diet; and 2) renin content increases almost exclusively in the d-AF during a low sodium diet or enalapril treatment. Thus, the GL and p-AF contribute little to the increased renal renin content seen with these chronic stimuli. (Hypertension 1992;19[ suppl II]:II-36-II-40)

In the normal adult mammalian kidney, renin is located primarily in the distal afferent arteriole within 30 μm of the glomerulus. Most immunocytochemical studies have found that during chronic stimulation of the renin-angiotensin system, such as a low sodium diet, converting enzyme inhibition, and adrenalectomy, renin immunoreactivity extends farther upstream along the afferent arteriole as well as in the efferent arteriole.1-4 Some studies have also suggested that intraglomerular and extraglomerular mesangial cells may also be recruited.5-8 Such changes in renin distribution may be physiologically important in the regional control of renal hemodynamics and sodium excretion. However, immunocytochemical studies are not quantitative and do not differentiate between active and inactive renin. In fact, Gomez et al1 found that although both the percentage of afferent arterioles and the average length of positive immunostaining for renin along the afferent arterioles were increased in kidneys of enalapril-treated rats, there was no increase in active renin content.

The majority of studies of renin distribution have used rats and mice, whereas relatively few have used rabbits. However, it is essential to characterize renin distribution in the rabbit for comparison, because studies of renin release from isolated perfused afferent arterioles, or perfused macula densa with its attached glomerulus, have mainly used rabbits9-12 (because of ease of dissection). Furthermore, the distribution of renin in rabbits may more closely resemble that of humans.13-16

In the present study, the renin distribution in microdissected renal microvessels was assessed quantitatively in rabbits maintained on a normal sodium diet as well as those given either a low sodium diet or enalapril. The distribution of renin immunoreactivity was also assessed for comparison. The results show
that in the rabbit, renin is located mostly in the distal portion of the afferent arteriole and increases almost exclusively in this portion in response to a low sodium diet or enalapril.

Methods

Young male New Zealand White rabbits (1.2–2.0 kg) were divided into three groups. Group 1 (n = 14) was maintained on standard rabbit chow (Ralston Purina Co., St. Louis, Mo.), containing 0.48% NaCl; group 2 (n = 12) was maintained the same way but received enalapril maleate (Merck Sharp & Dohme, Rahway, N.J.) by gavage (1 mg/kg body wt in distilled water) daily for 4 weeks; group 3 (n = 19) was maintained for 4 weeks on a low sodium diet (Ralston Purina) containing 0.04% NaCl. On day 1 of the low sodium diet, 20 mg furosemide was administered intravenously to facilitate sodium depletion. All groups received tap water ad libitum. Plasma renin activity was significantly higher in conscious rabbits fed the low sodium diet (26.96 ± 4.98 ng angiotensin I/ml/hr) or given enalapril (45.77 ± 8.31 ng angiotensin I/ml/hr) than in those fed a standard diet (4.67 ± 8.31 ng angiotensin I/ml/hr), confirming stimulation of the renin-angiotensin system.

Isolation of Arterioles and Glomeruli

We microdissected superficial afferent arterioles and glomeruli using methods described previously.17,18 The segments dissected were as follows: 1) the proximal afferent arteriole (p-AF), which extended from the interlobular artery to a point 50 μm from the glomerulus (this segment ranged from 50 to 100 μm in length); 2) the distal 50 μm of the afferent arteriole with its intact terminus (d-AF); and 3) the glomerulus without the vascular pole (GL). Only arterioles from the superficial cortex were used because they are longer (approximately 150 μm) than midcortical and juxtamedullary afferent arterioles (50 μm), allowing us to cut them into smaller segments. Because of the difficulty in dissecting one segment without damaging the others, only one segment from each arteriole was used. The length of the microdissected arterioles was measured using a scale in the eyepiece of the microscope. From each rabbit, three to five d-AF, five to seven p-AF, and five to seven GL were dissected and transferred to a small plastic ladle (Tetko, Elmsford, N.Y.). The ladle was examined microscopically to confirm that all arterioles (or GL) were present, after which it was rinsed and blotted from the bottom three times with 100 μl oxygenated minimum essential medium containing 0.1% bovine serum albumin (MEM–0.1% BSA). The p-AF, d-AF, and GL were placed in 20, 100, and 50 μl distilled water, respectively, and immediately frozen. After the samples had thawed, 180, 900, or 450 μl MEM–0.1% BSA was added to bring the total volume to 0.2, 1, or 0.5 ml, respectively. The sample volume was determined based on a pilot study that showed large differences in renin content among the different segments. Samples were stored frozen until the renin assay. As negative controls, 10–20 proximal tubules (more than 500 μm in length) were dissected from all rabbits, and their renin content was measured. There was no detectable renin activity in the proximal tubules.

Analysis of Renin Activity

Tissue samples were incubated with partially purified rabbit renin substrate equivalent to 600 ng of angiotensin I as described previously.17,18 Generated angiotensin I was measured by radioimmunoassay. Tissue renin content was expressed as nanograms angiotensin I per hour per arteriole (or glomerulus).

Renin Immunocytochemistry

The immunocytochemical methods have been described previously.1-2-19-21 Briefly, renal cortical slices obtained from kidneys perfused with modified Bouins solution in situ were dehydrated and embedded in paraffin. Eighteen slices (7 μm thick) from each animal were processed after rehydration according to the PAP method of Sternberger.22 Epithelioid cells in afferent arterioles containing renin were identified using a sheep antimouse-renin serum (dilution 1:2,500) that cross-reacts with rabbit renin.21 The juxtaglomerular (JG) index was calculated by comparing the number of glomeruli with adjacent immunostained cells to the total number of glomeruli in the section and was expressed as a percentage. In addition, the length of the renin-positive arteriolar segments was measured from vessels visible (in the plane of the section) for more than 100 μm from the glomerulus. These measurements were done on entire cortical sections, as well as on only the superficial cortex of the kidneys (at a depth less than 650 μm), which normally contains two layers of glomeruli (for comparison with renin content in microdissected segments). All evaluations were done in coded preparations by two observers.

Statistical Analysis

Data from the renin content studies are expressed as mean ± SEM. Student's unpaired t tests were used for the statistical evaluation; a value of p < 0.05 was considered significant. Statistical evaluation of the immunocytochemical studies was performed using a rank test (U test of Wilcoxon, Mann, and Whitney).

Results

Renin Content Studies

In rabbits maintained on standard chow, renin content of the d-AF was 27.0 ± 5.2 ng angiotensin I/hr/d-AF. The p-AF and GL contained only 0.33 ± 0.16 and 2.8 ± 0.46 ng angiotensin 1/hr/p-AF (or GL), respectively, indicating that approximately 90% of renin was located in the d-AF, 1% in the p-AF, and 9% in the GL. As shown in Figure 1, renin content in the d-AF was significantly higher in rabbits given either the low sodium diet or enalapril (53.11 ± 6.89 and 68.35 ± 8.1 ng angiotensin I/hr/d-
FIGURE 1. Bar graph shows renin content in microdissected proximal (p-AF) and distal (d-AF) afferent arterioles and glomeruli (GL) from rabbits that received either a normal sodium diet, a low sodium diet, or enalapril. Values are mean±SEM. Unpaired t tests were used to test for significant differences between controls and experimental groups for each segment. Ang I, angiotensin I.

AF, respectively), whereas the renin content in the GL was not altered significantly (3.3±1.0 and 3.6±0.7 ng angiotensin I/hr/GL, respectively). In the p-AF, there was a small increase in both low sodium and enalapril-treated groups (1.80±0.64 and 1.81±0.56 ng angiotensin I/hr/p-AF, respectively). Although statistically significant in the enalapril-treated group, the absolute increase was 30 times less in the p-AF than in the d-AF group (A=1.5 versus 41 ng angiotensin I/hr/p-AF or d-AF, respectively).

Renin Immunocytochemistry

Rabbits on the low sodium diet or enalapril had a significantly higher JG index (22.4±4.9% and 29.9±6.3%, respectively) than controls (12.9±2.8%). When only the superficial cortex was analyzed, this effect was less pronounced because of the higher percentage of renin-immunoreactive JGs in rabbits on the normal sodium diet. Histograms of the length of the renin-positive arteriolar segments in control, sodium-depleted, and enalapril-treated kidneys are illustrated in Figure 2, which shows that both the low sodium diet and enalapril increased the fraction of renin-immunoreactive cells near the glomerulus. Although both chronic stimuli increased renin immunoreactivity somewhat farther upstream along the arteriole, immunostaining was rarely seen farther than 50 μm from the glomeruli (Figures 2 and 3).

Discussion

The bulk of renin in normal adult mammals is found in the media of the afferent arterioles within 30 μm of the glomeruli. In previous studies, chronic stimulation of the renin-angiotensin system (converting enzyme inhibition, sodium depletion, adrenalectomy, or renal artery stenosis) caused renin immunoreactivity to extend farther upstream along the afferent arteriole toward the interlobular artery in rats and mice, suggesting that these newly recruited segments may contribute significantly to arteriolar renin content. To study this possibility, we determined quantitatively renin content in d-AF, p-AF, and GL in control, sodium-depleted, and enalapril-treated rats. In control animals, more than 90% of renin content was located in the d-AF, which is consistent with the previous immunocytochemical studies; however, in contrast to those studies, chronic stimulation caused renin to increase almost exclusively in the d-AF. The low renin levels in the p-AF are not due to renin being lost by this segment during dissection, because renin content in the d-AF in animals on the low sodium diet was similar to that in the whole afferent arterioles in our previous studies.

The lack of increased renin content in the p-AF during chronic stimulation was unexpected, because previous immunocytochemical studies have found
FIGURE 3. Photomicrographs show immunocytochemical staining for renin in kidneys from control (top), sodium-depleted (middle), and enalapril-treated (bottom) rabbits. Afferent arterioles (AA) showed immunoreactivity only in the vicinity of the glomerulus in controls. Both the low sodium diet and enalapril treatment caused variable amounts of immunostaining farther upstream along the afferent arteriole.

abundant renin immunoreactivity in the proximal afferent arteriole (for instance, Gomez et al found that more than 70% of afferent arterioles from enalapril-treated rats stained positive for length greater than 100 μm). We speculated that this discrepancy could be due to 1) increased renin immunostaining upstream from the glomerulus (as found in previous studies) due to inactive renin (as suggested by Gomez et al), or 2) species differences. The first possibility cannot be tested directly, as there is no reliable method of measuring inactive renin in microdissected arterioles. To test the second possibility, we performed immunocytochemical studies, which would also give some insight into the first possibility because both active and inactive renin are stained. The results were consistent with our renin content studies. Renin immunoreactivity was predominantly located in the d-AF; and although chronic stimulation did cause extension of positive staining upstream along the afferent arteriole, it rarely extended more than 50 μm from the glomeruli. Unfortunately, because it is technically not feasible to cut segments shorter than 50 μm, we cannot determine whether these newly appeared immunoreactive segments produce active renin nor how much of the increased renin content is due to these segments.

Whether renin is present in the glomeruli is controversial at best. Most immunocytochemical studies report that renin immunoreactivity is rarely found in the glomerular tuft under control or stimulated conditions. However, there have been reports of renin being found in mesangial cells during various chronic stimuli, such as adrenalec-tomy or renal artery stenosis; and in a recent preliminary report, Hostetter et al found increased renin immunoreactivity in glomerular tufts from remnant kidneys in rats. Furthermore, mesangial cells in culture have been reported to contain renin. In the present study, renin was always present in the glomerulus. The amount of glomerular and arteriolar renin did not correlate, nor did glomerular renin increase with chronic stimuli as did arteriolar renin. These two findings suggest that glomerular renin is not likely due to residual arteriolar segments in the dissected glomeruli. Although our findings support earlier reports of renin being found in the glomeruli, it was not altered by chronic stimuli, whereas in previous studies, glomerular renin was found only during chronic stimulation. We have no explanation for these discrepancies, although they could be due to species differences, the different stimuli used, or the duration of the stimulation.

The findings of the present study are important for the following reasons: 1) There is only limited information on the rabbit renin-angiotensin system, yet rabbits are being used for most studies involving microdissection and microperfusion techniques. 2) In the very few human studies, the pattern of renin immunoreactivity seen under control situations and conditions such as renal artery stenosis, Barter’s syndrome, and adult polycystic kidney disease seems to resemble the pattern seen in rabbits. 3) The distribution of renin along the arteriole described above may be indicative of the way its release is regulated. JG renin-containing cells may be regulated primarily by the macula densa, whereas renin-containing cells farther upstream may be primarily baroreceptor or β-adrenoreceptor regulated. Thus, it is possible that in the rabbit, a larger percentage of the renin-containing cells is influenced predominantly by the macula densa. Indeed, studies using the microperfused macula densa with its glomerulus attached have shown that renin release can increase by more than 10-fold when the sodium chloride concentration at the macula densa is lowered, whereas studies using perfused afferent arterioles have shown only a twofold increase or no increase at all in response to the lowering of intraluminal pressure. 4) It has been speculated that renin in the JG afferent arteriole may be predominantly a regulator of glomerular hemodynamics.
through “short-loop feedback,” whereas renin farther upstream would have predominantly a systemic endocrine function.

In summary, the present study determined the distribution of renin in the rabbit renal microvasculature using two distinct but complementary techniques. Both renin content and immunoreactivity were located almost exclusively in the d-AF within 50 μm of the glomeruli, with small amounts being detected in the p-AF and GL. Four weeks of a low sodium diet or enalapril increased renin almost exclusively in the distal segment of the afferent arteriole within 50 μm of the glomerulus.

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