ANNALS OF CLINICAL AND LABORATORY SCIENCE, Vol. 12, No. 3

Hyperplasia of Juxtaglomerular Cells and Renin Localization in Kidneys of Normotensive Animals Given Captopril

Electron Microscopic and Immunohistochemical Studies

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ABSTRACT

Captopril, a competitive inhibitor of angiotensin I-converting enzyme (ACE), is an orally potent antihypertensive agent. Light and electron microscopic studies of the kidneys of mice, rats, and monkeys given large oral doses of captopril for long duration were conducted. All mice and some rats and monkeys developed hyperplasia of the renin-secreting cells which appeared in several layers surrounding the vascular wall of the afferent arterioles. In the electron microscope, these epithelioid cells appeared heavily loaded with aggregates of homogeneous, electron dense, osmiophilic amorphous granules filling distended spaces of the endoplasmic reticulum. The Golgi cisterns often included small, sharply outlined triangular or rhomboid osmiophilic granules. The use of specific renin antibodies and the application of the "three-layer bridge technique" for peroxidase-antiperoxidase defined and verified the accumulation of renin in the juxtaglomerular cells. After cessation of dosing, hyperplasia of the juxtaglomerular cells markedly regressed, and there was a significant reduction in the number and size of the renin granules in such cells.

Introduction

The anatomical and histochemical features of the renal juxtaglomerular apparatus (JGA) have been studied extensively in experimental animals[3,4,11–16,24,25,27–29,36,37,42,43] and in man.[5–7,10,14,16]. Basically, The JGA is formed of four essential com-

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ponents: (1) terminal portion of the afferent glomerular arteriole near the hilum of the glomerular tuft; (2) efferent arteriole of the glomerulus; (3) a specialized segment of the distal tubule, the macula densa; and (4) extraglomerular mesangial region formed of two basic cell types: (a) agranular or lacis cells which control electrolyte balance, and (b) granular or juxtaglomerular cells (JGC) which synthesize, store and release renin.[9,17,28] These cells are usually confined within

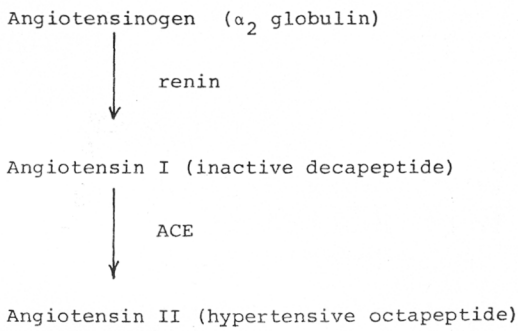

FIGURE 1. The renin-angiotensin system.

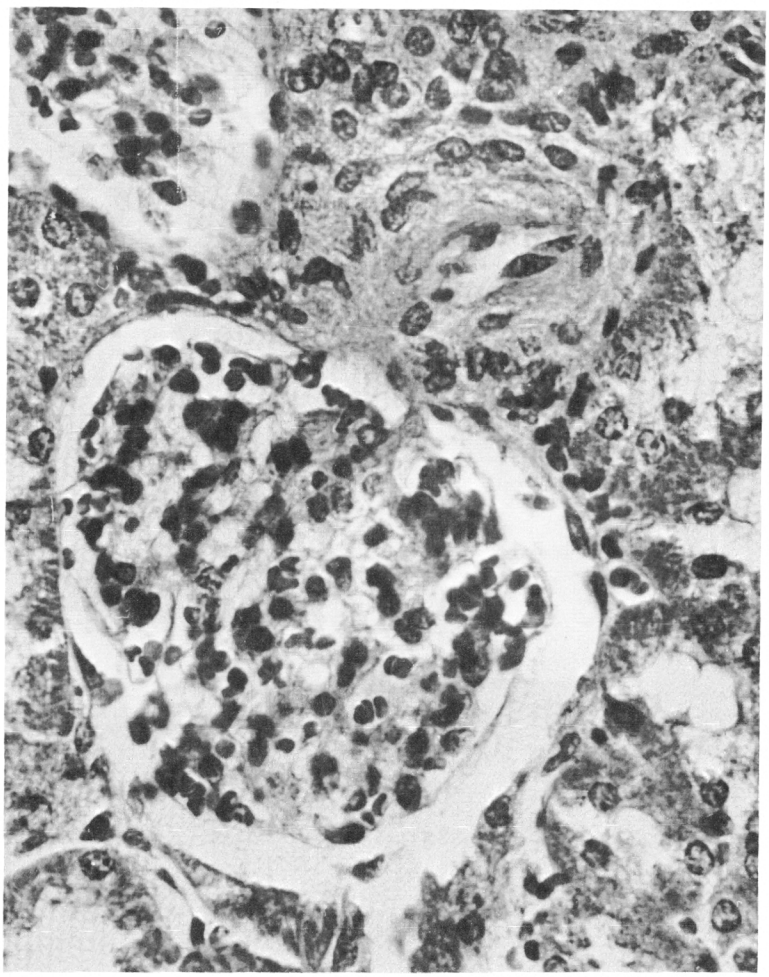

FIGURE 2. Hyperplasia of juxtaglomerular cells around two afferent arterioles in a mouse given captopril (150 mg per kg daily) for one year. Hematoxylin and eosin stain, × 640.

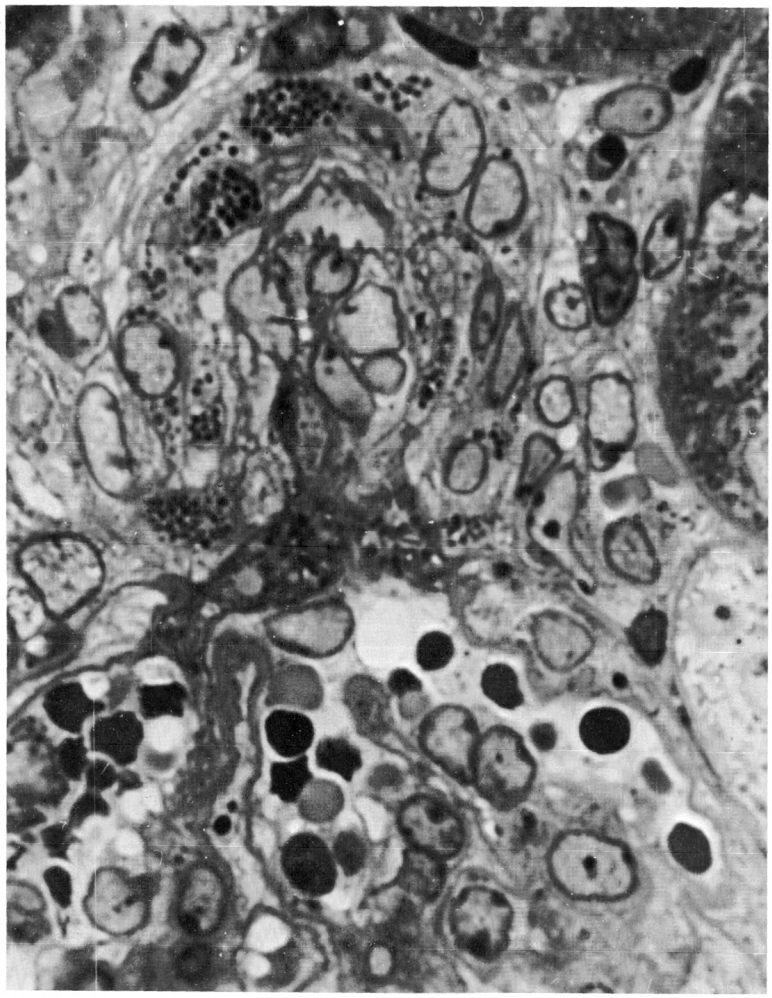

FIGURE 3. Renin granules in juxtaglomerular cells of a mouse treated with captopril (150 mg per kg daily) for one year. Toluidine blue stained plastic-embedded 0.5 μ-thick section, × 1,000.

the sleeve of the preglomerular portion of the afferent arterioles.

Renin, a highly specific proteolytic enzyme, is released into the blood from the JGC in response to lowered blood pressure or decreased sodium ion concentration. Through its proteolytic action, renin cleaves angiotensinogen, plasma protein (α_2 globulin formed by the liver), to generate a relatively inactive decapeptide known as angiotensin I. In turn, angiotensin I-converting enzyme (ACE), a dipeptidylcarboxypeptidase which is mainly found in the lung but also in circulating plasma, kidneys and a variety of other organs,[33] converts the inactive angiotensin I to a vasoactive octapeptide or angiotensin II, a potent vasoconstrictor responsible for the elevation of blood pressure (figure 1).

Captopril* is a novel, potent, and specific orally-active inhibitor of ACE,[1,18,38,39]

* Capoten®; SQ 14,225; *D*-3-mercapto-2-methyl propanoyl-*L*-proline.

one of the enzymatic components of the renin-angiotensin-aldosterone system that regulates blood pressure and electrolyte balance.

Prolonged oral administration of captopril to mice, rats, and monkeys induced hyperplasia of the renal juxtaglomerular cells (JGC) with varied dose-related response.[26] Such hyperplasia was self-limiting and regressed upon cessation of dosing.

This investigation has been carried out to study the renin granules and their localization in such hyperplastic JGC using electron microscopic, morphometric and immunohistochemical approaches.

Materials and Methods

Animals

During chronic oral toxicologic testing of captopril, mice and rats † were given 0, 50, 150, and 1350 mg per kg per day for two years; and rhesus monkeys were given 0, 50, 150, and 450 mg per kg per day

† Charles-River CD outbred albino.

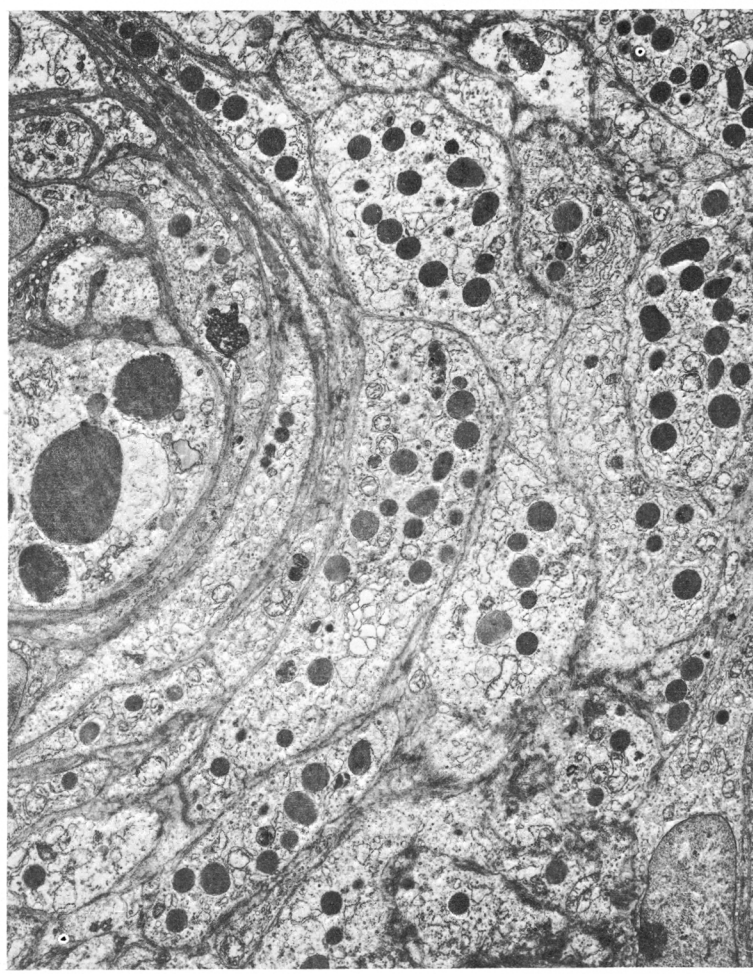

FIGURE 4. Same mouse as in figure 2 showing renin granules. Uranyl acetate-lead citrate stain, × 3,600.

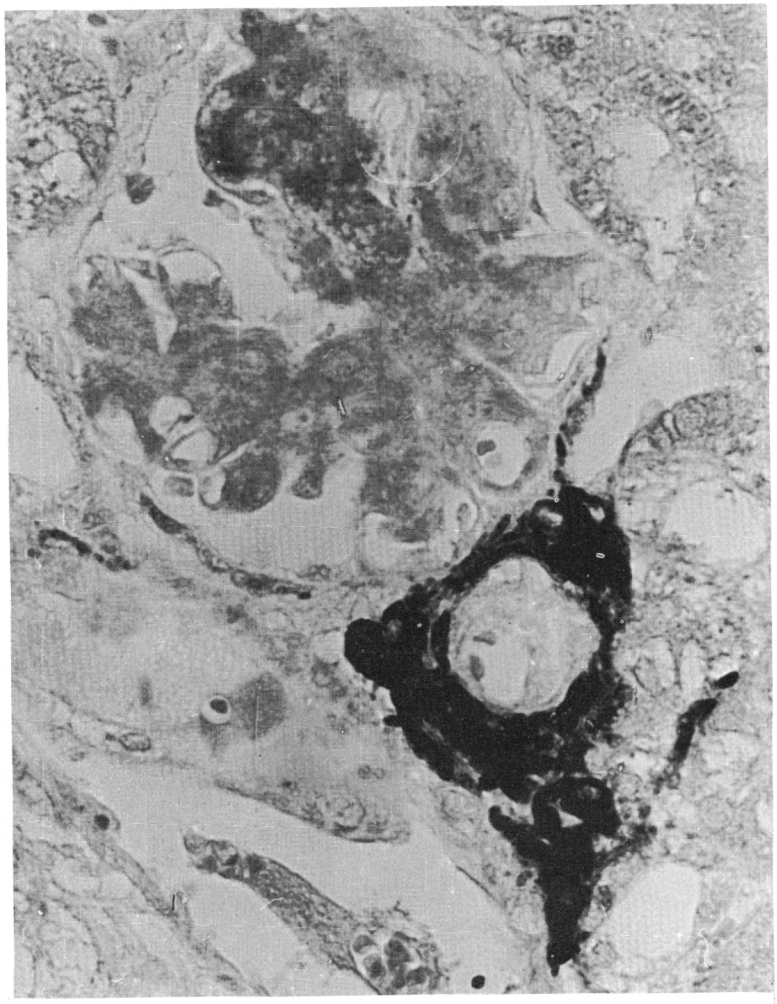

FIGURE 5. Indirect peroxidase-antiperoxidase staining around an afferent arteriole representing a dense accumulation of renin in a mouse treated with captopril (150 mg per kg daily) for one year, × 640.

for one year. Some of the monkeys were kept for a post-recovery period of six months.

LIGHT MICROSCOPY

The incidence of JGC hyperplasia was studied in buffered formalin-fixed and paraffin-embedded 6 μ-thick sections stained with hematoxylin and eosin (HE). Special stains[17,22,41,48] were also used in the detection of the JGC granules.

ELECTRON MICROSCOPY

Small pieces were randomly taken from the renal cortex, then immediately fixed in ice-cold s-collidine-buffered osmium tetroxide at pH 7.4. After dehydration in methanol followed by propylene oxide, the specimens were embedded in Epon 812.[30] Plastic-embedded, 0.5 μ-thick sections, stained with toluidine blue, were then examined in the light microscope to localize the areas to be studied at the ultrastructual level. Ultrathin sections,

usually stained with uranyl acetate and lead citrate,[35] were then examined with Zeiss 9S electron microscope.

Morphometric Analysis

A morphometric study was carried out on the monkey kidneys in an attempt to evaluate, at least arbitrarily, the degree of granulation and the frequency of the JGC in the hyperplastic areas using different parameters, namely: (1) the juxtaglomerular granulation index (JGI) according to the method of Hartroft and Hartroft[23]; (2) the JGC cell count per glomerulus, and (3) the percentage of the glomeruli affected. Toluidine blue-stained 0.5 μ-thick plastic-embedded sections were examined under the oil immersion power of the light microscope and then randomly selected hyperplastic areas were photographed. The number of renin granules per cell was counted from at least 50 cells examined from different sections. The mean number of the granules per cell was then determined. In well oriented semi-thin sections, epithelioid cells loaded with renin

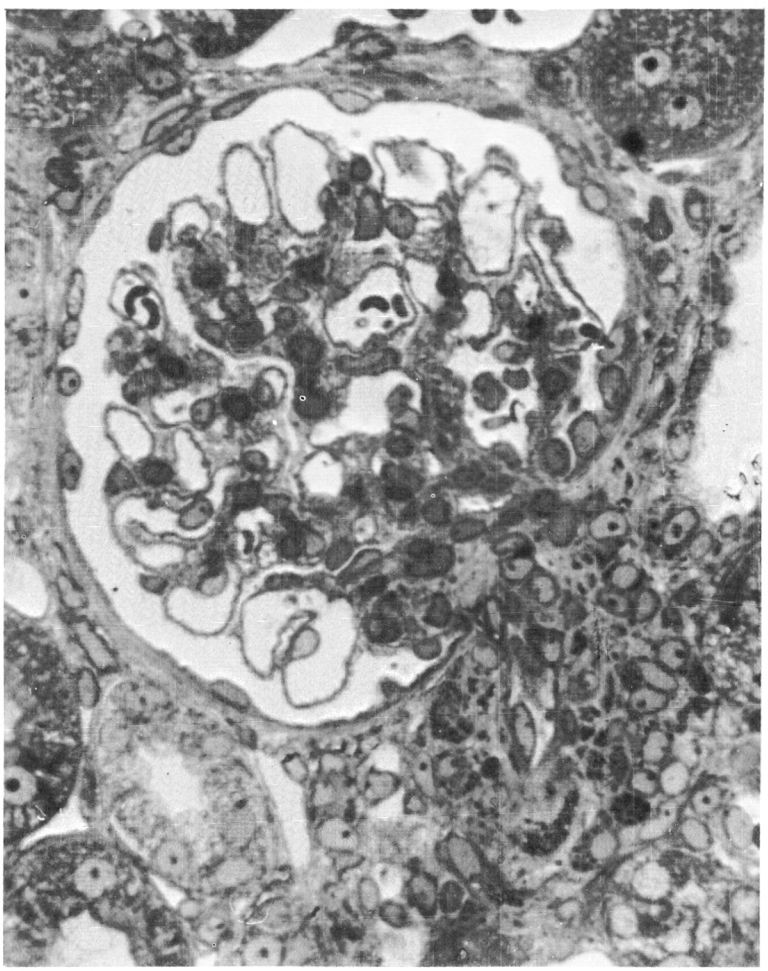

FIGURE 6. Hyperplasia of juxtaglomerular cells in a rat given captopril (150 mg per kg daily) for one year showing mild accumulation of renin granules, × 640.

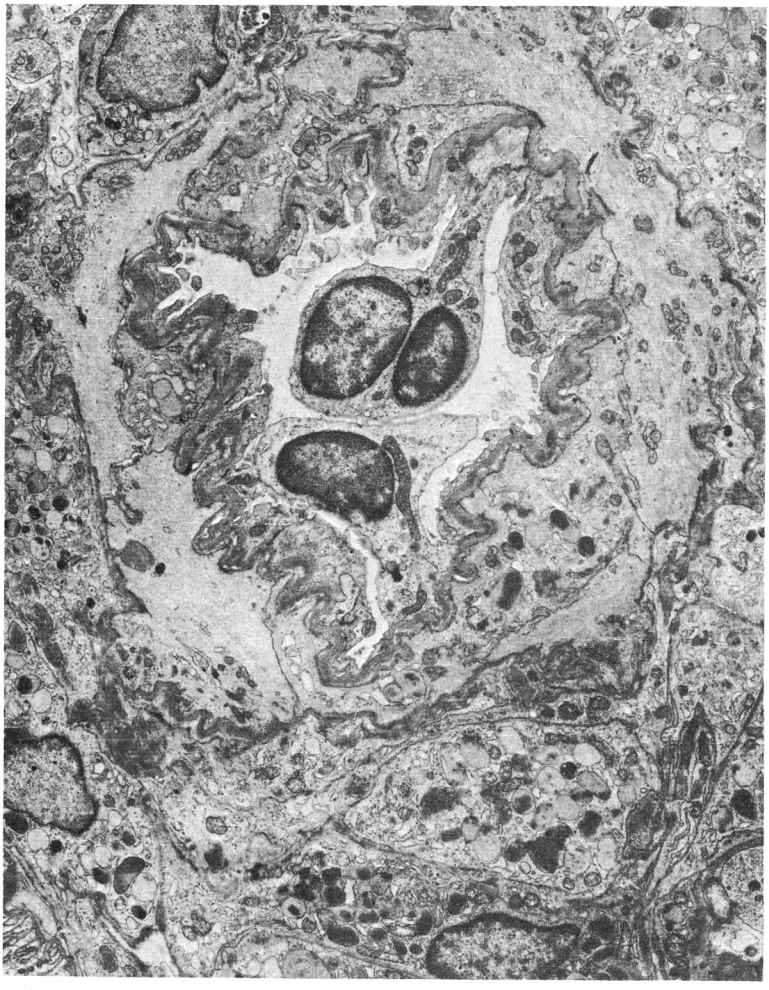

FIGURE 7. Same rat as in figure 6 showing an afferent arteriole surrounded by juxtaglomerular cells containing renin granules. Uranyl acetate-lead citrate stain, × 3,600.

granules were usually found for counting in an eccentric position with respect to the axis of the afferent arteriole and were often located close to the macula densa.

The incidence of the juxtaglomerular hyperplasia was expressed as percent of affected glomeruli that were calculated from 200 glomeruli randomly examined in HE-stained sections.

IMMUNOHISTOCHEMICAL TECHNIQUES

Preparation of Renin Antibodies: Antibodies to rat and human renin were produced using rat and human renin purified to homogeneity by published methods.[31,49] These preparations satisfied multiple criteria of purity which include single bands by polyacrylamide gel electrophoresis, SDS-electrophoresis, isoelectric focusing, and immuno-double diffusion.

Human renin insolubilized with glutaraldehyde was used first to immunize Dutch-belted rabbits according to Yokosawa et al.[50] Antisera with higher titers were produced by boosting the same rabbits with a renin-tetanus toxoid conjugate which was prepared by allowing 150 μg of

renin and 75 μg of tetanus toxoid to react with 50 μg of 1-ethyl-3-(3-dimethylaminopropyl) carbodimide. The conjugate, containing approximately 10 μg of renin, was used for each booster administered every three weeks in Freund's incomplete adjuvant. Plasma collected after 16 weeks of immunization had a titer of 60,000 as defined by the dilution required for 50 percent inhibition of renin (90 g) activity. This antiserum showed species specificity directed to primate renin. Renins from hog or rat kidney, or mouse submaxillary gland did not react with this antibody. Cathepsins from human kidney were not inhibited by this antibody.[5]

Anti-rat renin antibodies were produced in a similar manner using a rat renin-tetanus toxoid conjugate as immunogen. Dutch-belted rabbits were injected initially with 80 μg of renin equivalents of the conjugates at multiple intradermal sites followed by 10 μg of biweekly boosters. Antisera with a titer of 41,000 for 50 percent inhibition of renin were used for this study. These antisera

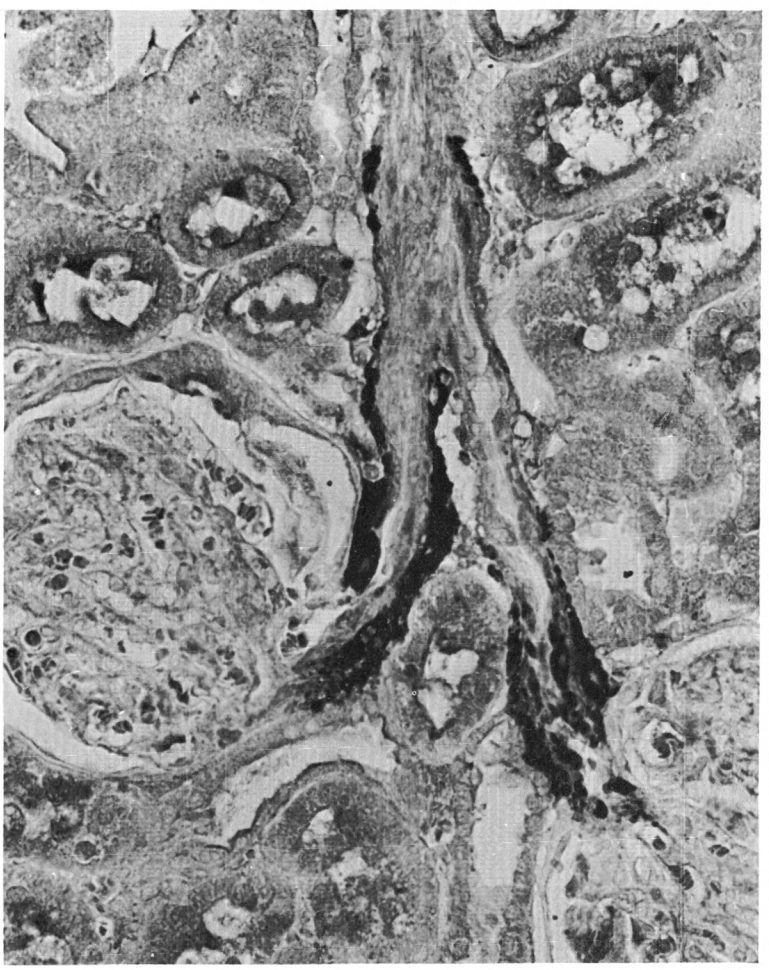

FIGURE 8. Two adjacent glomeruli in a rat given captopril (150 mg per kg daily) for one year showing the afferent arterioles surrounded by JGC loaded with renin granules densely stained with the immunoperoxidase-antiperoxidase technique, × 640.

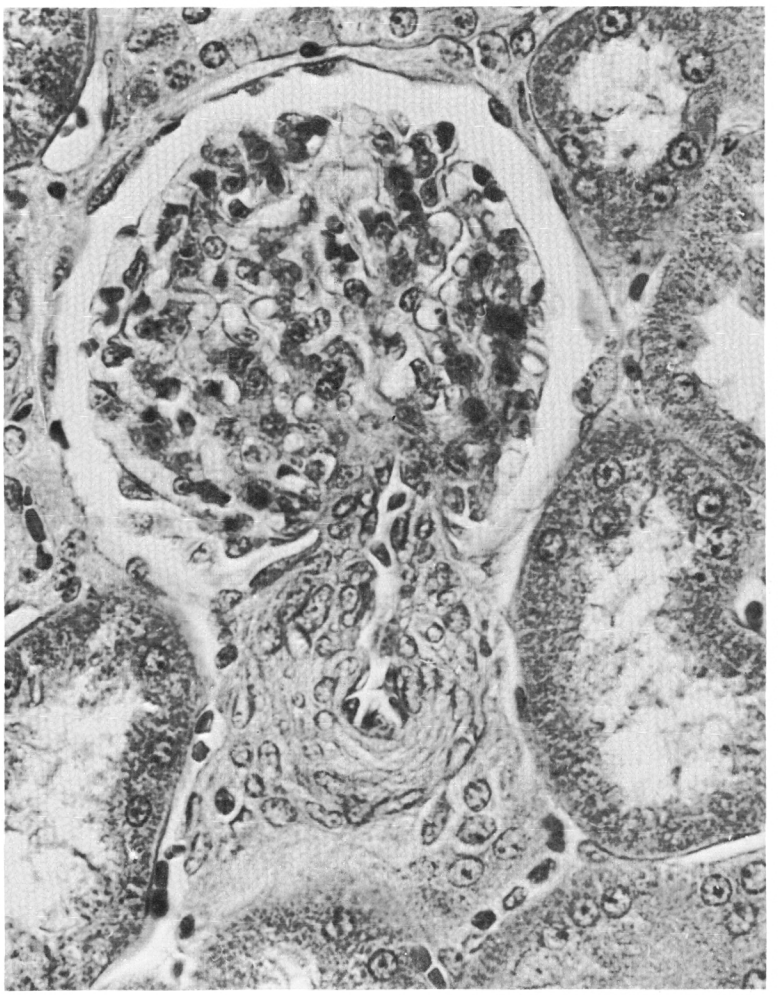

FIGURE 9. Hyperplasia of juxtaglomerular cells around an afferent arteriole of a monkey treated with captopril (450 mg per kg daily) for one year. Hematoxylin and eosin stain, × 640.

did not cross react with human renin or rat cathepsin examined at 1:500 dilutions.

Peroxidase-Antiperoxidase Procedure: The "three-layer bridge technique" for peroxidase-antiperoxidase (PAP) originated by Sternberger et al[44] and modified by others[8, 19, 21, 32, 45, 47] is a highly sensitive and specific immunohistochemical procedure widely used at present for the identification and localization of immunoglobulins, complement deposits, cell products, etc. The PAP procedure adopted by Taugner et al[46] for the localization of renin was followed in this study with slight modification. The deparaffinized formalin-fixed sections were incubated with the specific renin rabbit-antisera diluted $1:10^6$ with normal swine serum (NSS) for 24 hours at 4°C instead of 48 hours. Optimal results were obtained when the incubation period with swine anti-rabbit IgG and with soluble PAP-complex* were extended to one hour instead of 10 minutes.

* Both purchased from DAKO, Accurate Chemical & Scientific Corp., Westbury, NY.

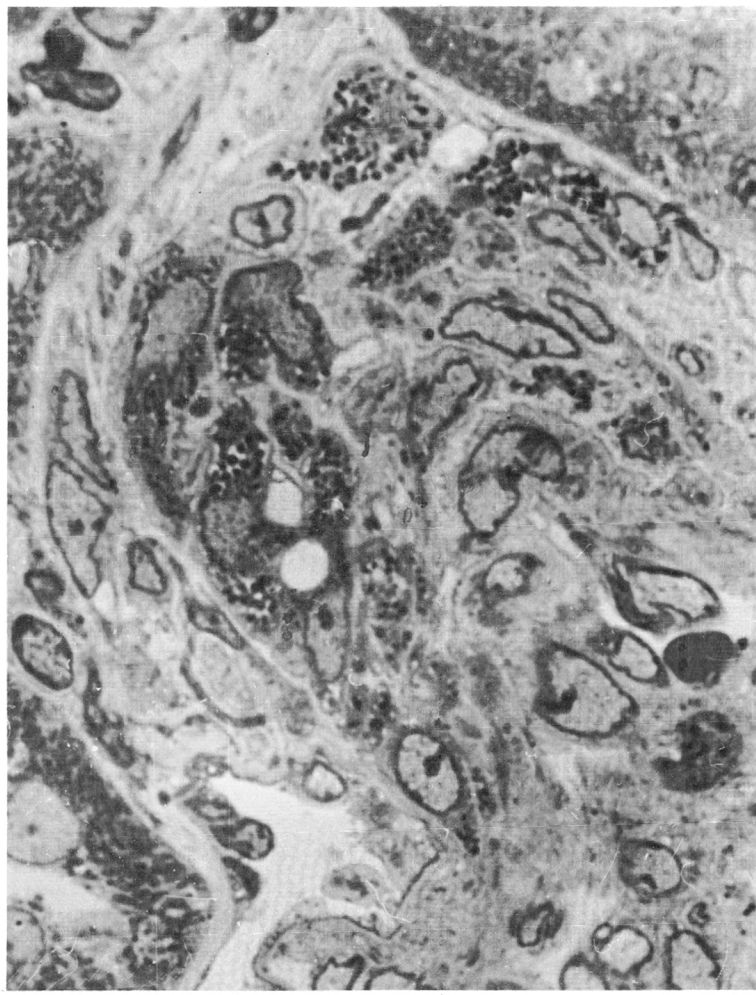

FIGURE 10. Accumulation of renin granules in JGC of a monkey treated with captopril (450 mg per kg daily) for six months. Toluidine blue stained plastic embedded section, × 1,000.

Results

MICE

Marked hyperplasia of the JGC was noted in all treated mice (figure 2), regardless of the dose level of captopril. The female mice appeared to be more susceptible than the males as they manifested a higher incidence of JGC hyperplasia (45 percent) than their male counterparts (28 percent). Although there was a noticeable increase in the number of renin granules in the JGC (figures 3 and 4), it was not possible to obtain a precise JGI because of the extreme deposition of the naturally occurring amyloid that is commonly seen in aged mice.

Following exposure to renin-antisera and PAP treatment, an intensely staining reaction product was found around the wall of the afferent arterioles (figure 5) and in some instances also around the wall of the efferent arterioles. This staining product indicated the site of renin accumulation.

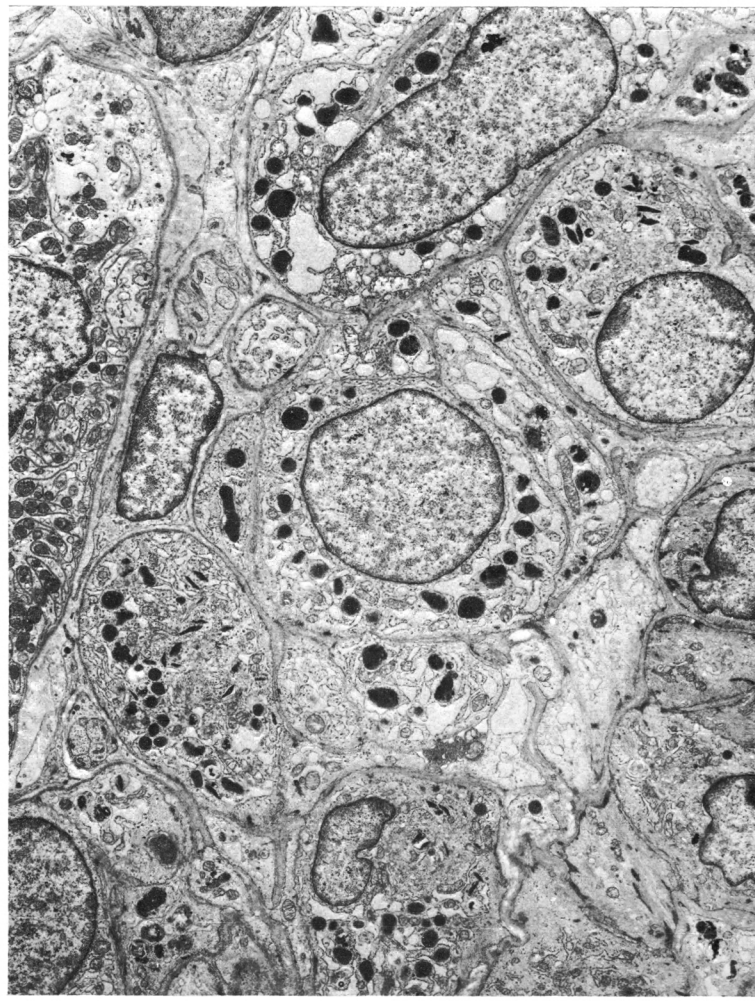

FIGURE 11. Juxtaglomerular cells loaded with renin granules (same monkey as in figure 10). Uranyl acetate-lead citrate stain, × 3,600.

RATS

A small number (two percent to seven percent) of the glomeruli in the kidneys of some rats developed mild hyperplasia of the JGC. Incidences of animals showing hyperplasia of JGC were greater in the higher dose-group and in males compared to females. In affected rats, the JGC appeared moderately increased in number and retained renin granules of normal size and count (figures 6 and 7). The PAP-staining product, corresponding to the presence of renin, appeared prominent in the walls of the afferent arterioles (figure 8).

MONKEYS

Microscopic examination of the kidneys of dosed monkeys revealed well preserved, normal structure of the nephron components, i.e., glomeruli, proximal and distal tubules, as well as collecting tubules. They appeared identical to their controls.

All animals of the high-dose (450 mg per kg daily) group and some of the inter-

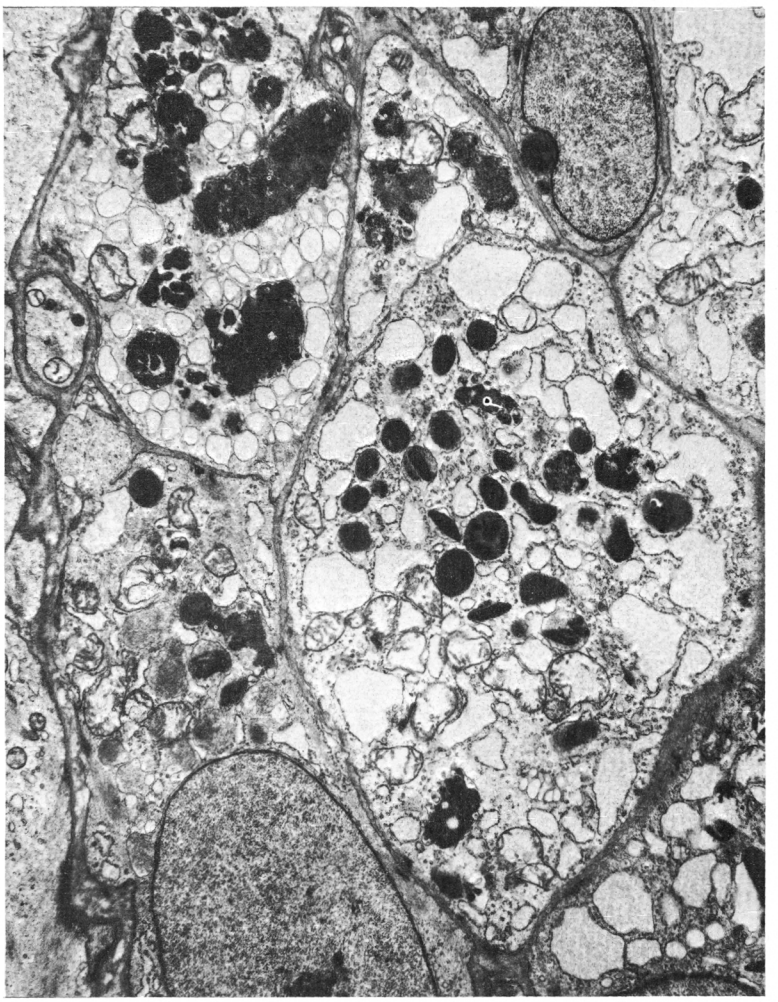

FIGURE 12. After one year of treatment with captopril (450 mg per kg daily), renin granules appeared to be disintegrating and taken up by lysosomes (left side of photo). Uranyl acetate-lead citrate stain, × 4,400.

mediate-dose (150 mg per kg daily) group developed hyperplasia of the JGC surrounding the vascular wall of the afferent arterioles (figure 9). These cells appeared heavily loaded with renin granules (figures 10 and 11).

After three months of dosing with captopril, the animals of the high-dose group showed hyperplasia of JGC involving about 57 percent of the glomeruli examined. In the intermediate-dose group, about 25 percent of the glomeruli showed mild hyperplasia of the JGC. After six and 12 months of dosing, marked hyperplasia of the JGC involved 80 percent of the glomeruli. Attempts to evaluate the JGI failed because most of the "stagnant" renin granules appeared disintegrated and engulfed by lysosomes (figure 12).

Since there were no monkey renin-antisera at the time of this study, it was suggested to use human renin antibodies instead, at least to check the possibility of cross-reaction between human and monkey antibodies. Surprisingly enough, when the PAP-technique was applied to formalin-fixed, paraffin-embedded sections of monkey kidneys, a cross-reaction took

place and an intensely staining product representing renin was detected at the site of the renin-loaded JGC around the afferent arterioles (figure 13).

After a period of six months post-dose recovery, the JGC hyperplasia markedly regressed (table I) with a significant reduction in the number and size of renin granules (figure 14).

Discussion

Captopril, a highly specific and potent inhibitor of ACE of various animal species (mouse, rat, guinea pig, rabbit, cat, dog, and monkey) and man, is active in lowering the blood pressure in several experimental models of hypertension like the Goldblatt model[20,33,34,38] and in the two types of hypertension caused by hereditary factors in the rat, namely: (1) a normal renin form of hypertension resembling essential hypertension in man that occurs in the Okamoto-Aoki Wistar-Kyoto strain of spontaneously hypertensive rats (SHR); and (2) a high renin form of hypertension that occurs in stroke-prone rats.[1,2,40]

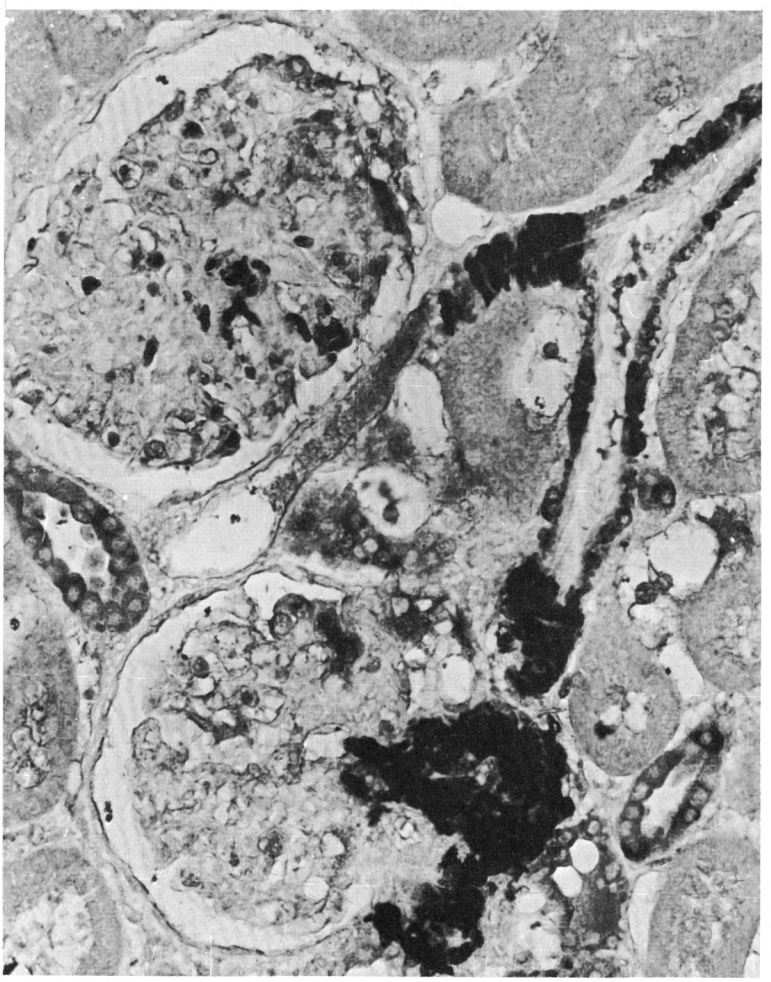

FIGURE 13. Human renin antiserum cross-reacting with monkey renin as seen by the intense peroxidase-antiperoxidase staining for renin in two adjacent glomeruli, × 640.

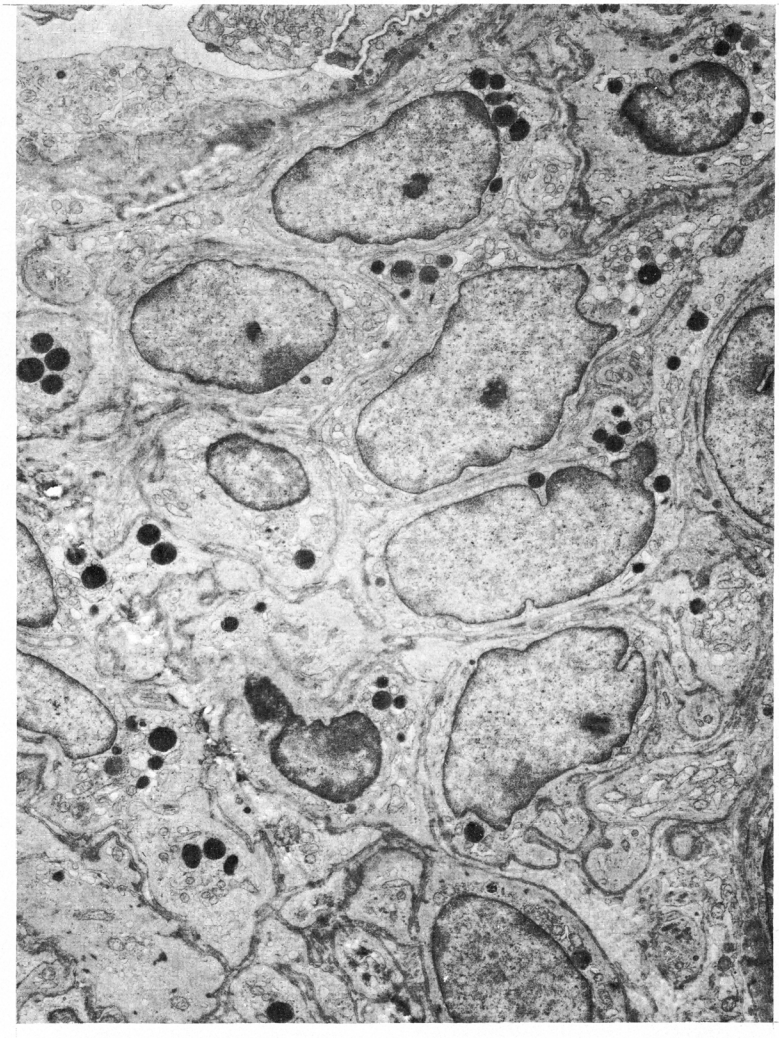

FIGURE 14. Monkey receiving captopril (450 mg per kg daily) for one year, showing marked regression of the hyperplasia of juxtaglomerular cells with significant reduction in the number of renin granules after a post-dose recovery period of six months. Uranyl acetate-lead citrate stain, × 3,600.

Several feedback control mechanisms have been postulated for the release of renin, usually synthesized and stored in the JGC, which is regarded as the rate-limiting step in the production of angiotensin. Such feedback mechanisms are directly and indirectly controlled by angiotensin II through its effect on blood volume, blood pressure and sodium balance.[33] In one of these control mechanisms, renin secretion is mediated through physiologic change in which renin increases renal perfusion pressure by increasing the blood pressure and the extracellular fluid volume. Another feedback control process is mediated through the suppression of renin secretion by angiotensin II without any alteration in the perfusion pressure or the distal tubular sodium. Still another possibility for control by feedback may be the release of renin resulting from alteration of the concentration of sodium ions in the distal tubules which in turn stimulates the macula densa to trigger the secretion of renin by JGC.

TABLE I

Incidence of Juxtaglomerular Hyperplasia in Monkeys Treated with Captropril for One Year

Dose (mg/kg)	Twelve Months on Captopril		Twelve Months on Captopril and Six Months Recovery	
	Animal	Percent Hyperplasia*	Animal	Percent Hyperplasia
450	706-M	87	704-M	29
	708-F	71	710-F	57
150	738-M	32	715-M	14
	718-F	53	717-F	5
	719-F	58		
50	723-M	0	722-M	0
	725-F	0	724-F	0
	726-F	0		
0	732-M	0	728-M	0
	735-M	0	730-M	0

*Percent hyperplasia was estimated as percentage of glomeruli showing hyperplasia of juxtaglomerular cells and calculated from at least 200 glomeruli randomly examined in different hemotoxylin and eosin-stained sections.

Administration of captopril to experimental animals induced hyperplasia of the JGC. This hyperplasia was self-limiting and regressed after cessation of dosing. Because there were no major changes in blood pressure, blood volume, sodium balance, or renal function, hyperplasia of the JGC is considered to reflect stimulation of the renin-secreting cells owing to a loss of feedback inhibition by angiotensin II.

The use of specific renin antibodies defined and verified the accumulation of renin in the hyperplastic JGC of all three species studies.

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