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# Effects of captopril administration on isoelectric heterogeneity of pig renal renin

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Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline, the prototype of a new class of antihypertensive drugs which inhibit angiotensin I converting enzyme (EC 3.4.15.1) (ACE),* is widely used to treat various forms of hypertension [1]. As a consequence of ACE inhibition, plasma concentrations of angiotensin II (AII) are decreased, and increased renin (EC 3.4.99.19) release and plasma renin activity (PRA) occur as a result of loss of AII "feedback" inhibition. It has been reported recently that captopril (Capoten, SQ 14,225) administration can result in juxtaglomerular (JG) cell hyperplasia in rabbits [2], induce intracisternal granule formation in the distended, rough endoplasmic reticulum of the JG cells of mice where renin is produced [3], inactivate oxytocin and vasopressin, and dissociate IgG into two subunits *in vitro* [4], and convert a high molecular weight pig renal renin to a low molecular weight renin *in vitro* [5]. The present study was performed chiefly to assess the potential effects of captopril administration on the molecular weight and isoelectric (pI) forms of renal renin in the pig.

Three 8.5-month-old mini-pig sows, weighing 32–39 kg, from a litter were used. They were individually penned, fed regular pig chow blocks, and provided with water *ad lib*. The food intake was limited to 1.0 kg/pig per day. Captopril (Squibb & Sons, Inc., Princeton, NJ) was administered to two pigs, designated CPT-I and CPT-II, by mixing it with jam to increase palatability, and spreading it onto a chow block at a dose of 1.57 mg/kg body weight per day (equal to a common human dose of about 350 mg/day). Chow was fed to the control pig and the treated pigs twice a day by hand to ensure consumption, and the drug dose was adjusted weekly. Blood samples were taken prior to the administration of the first dose of captopril (week 1), again at 3 weeks (week 3), 4 weeks (week 4), and just prior to necropsy (week 8).

At necropsy one kidney was removed from each pig and the cortex was homogenized for renin extraction. The contralateral kidney was simultaneously perfused *in situ* with 3.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for subsequent light and scanning electron microscopy (SEM).

Routine hematological studies using automated methods were performed on all animals just before captopril administration, and at the four subsequent periods just specified. These included the determination of: hematocrit, hemoglobin, leukocyte number, and differential counts. Measurements of serum ACE activity [6] and PRA [7] were also made.

Renin was extracted from the renal cortex of each pig separately by method II (toluene extraction and purification through step 5) of Levine *et al.* [8]. An important feature of this method is that the pH of the extraction mixture is maintained between 5.0 and 7.5. Renin activity and protein concentration [9] were determined after each extraction stage.

The method used for isoelectric focusing (IEF) of renal renin has been described [10]. Prior to IEF, portions of the purified extract were dialyzed against three changes of 20 mM sodium phosphate buffer (pH 6.9) over a 36-hr period at 5°, followed by two changes of 1% glycine over 24 hr. The amount of protein initially used for each IEF was 130–150 mg. A minimum of three IEF procedures was performed with each kidney extract to verify isoelectric values (pI). Control data, previously obtained in our laboratory from normal pig cortex, were also used for comparison. An equal amount of renin activity from each kidney extract was subjected to IEF for comparison of the various pI forms. Then, relatively low concentrations of renin activity from each extract were used for IEF to ensure that pI forms with lesser activity were not obscured by forms with higher activity; higher concentrations were used to survey for outlying minor constituents.

Renin substrate (angiotensinogen) was extracted from control pig plasma by the method described by Printz *et al.* [11]. Substrate consumption determinations using increasing amounts of substrate, with a constant concentration of control pig renin (capable of generating 1493 ng angiotensin I/hr at substrate saturation), demonstrated the production of an average of 894.3 ng AI equivalents per ml substrate per 4 hr of incubation at 37°, or the equivalent of 690.1 pmoles substrate per ml [12].

Angiotensin I formation was measured in duplicate by radioimmunoassay [7] and was expressed as ng AI generated per ml of sample (plasma, column eluates and IEF fractions) per hr at 37°. For the analysis of either column eluates or IEF fractions, each assay tube contained 3.4 μmoles of 8-hydroxyquinoline, 8 μmoles of dimercaprol, 10 μl of renin, 100 μl of purified homologous substrate (69 pmoles), and sodium phosphate buffer (20 mM $PO_4$–5 mM NaCl) (pH 7.0), to a total volume of 1.0 ml. Adequate substrate concentrations, based upon data from substrate saturation curves, were used throughout the study to ensure that substrate would not be limiting. Angiotensinogen control incubations (no renin added) were used, and net renin activity was calculated accordingly.

Ascending gel filtration column chromatography (Sephadex G-100) at 5° was used to estimate the molecular weight of renin extracted from each pig kidney [12]. A 1.0-ml (<20 mg protein) aliquot of each renin sample was applied to the column and eluted with phosphate–saline buffer. Assay of these fractions was performed using 20 μl of each fraction and 100 μl of the homologous substrate preparation.

Blocks of cortex (1 × 2 × 4 mm) were fixed in glutaraldehyde, dehydrated through freon, and critical point dried for SEM. After coating with gold-palladium, the specimens were examined in a JEOL 100c electron microscope with a scanning attachment. Additional sections were embedded in paraffin for light microscopy. The sections for light microscopy were stained with hematoxylin and eosin, and with alcian blue-periodic acid-Schiff (PAS) stain for the demonstration of juxtaglomerular granules.

During the 8-week experimental period, there was little difference in the rate of weight gain between the control pig and the two captopril-treated pigs, with the net weight gains being 13.6, 16.5 and 16.2 kg respectively. During the first 7 days that the pigs were receiving captopril, they appeared to be hyperactive; however, this was not observed after the seventh or eighth day.

Both pigs treated with captopril developed large, 3.5 to 5.0 cm diameter, ulcerous skin lesions on their snouts and several much smaller lesions on their labia within 2 weeks of drug administration. These lesions were not treated and persisted for the duration of the experimental period. No lesion was seen on the control pig.

* Abbreviations: ACE, angiotensin converting enzyme; AII, angiotensin II; PRA, plasma renin activity; pI, isoelectric point; JG, juxtaglomerular; SEM, scanning electron microscopy; and IEF, isoelectric focusing.

The routine hematological studies performed on blood samples taken at five intervals over the course of the study showed no difference in hematocrits, hemoglobin values, leukocyte numbers, or differential counts between the two groups.

As anticipated, serum ACE levels were lower in the CPT-I and CPT-II pigs, when compared to either pretreatment levels or to control pig ACE levels at each time interval. At week 8, the ACE activity for the control pig was 372 nmoles [$^3$H]-hippuric acid generated per min per ml of serum compared to a mean ACE value of 198 for the CPT pigs. Concurrently, PRA was increased in the treated animals; however, the increase in PRA of one treated pig (CPT-II) was less than that observed in the other. At week 8, PRA of the control pig was 1.00 ng AI generated per ml plasma per hr of incubation compared to a mean PRA value of 13.92 for the CPT pigs.

Although there were slight differences in the fresh weight of the kidneys and the cortical tissue from the three pigs, these differences were not attributed to drug administration since the heaviest, as well as the lightest, values were observed from CPT-I and CPT-II pigs, respectively.

The molecular weights, isoelectric forms and kinetics of substrate consumption for renin extracted from the three kidneys were compared using either equal amounts of renal protein or equal renin activities. In contrast to our earlier *in vitro* study which demonstrated that captopril could convert high molecular weight pig renin to a low molecular weight form [5], the results of G-100 column chromatography demonstrated that the molecular weight of renin extracted from all three animals was approximately 60,000 ± 1500 daltons. The mean specific activity of renin eluted from the column of the control pig was 4,960.1 ng AI generated per mg protein per hr, whereas the mean

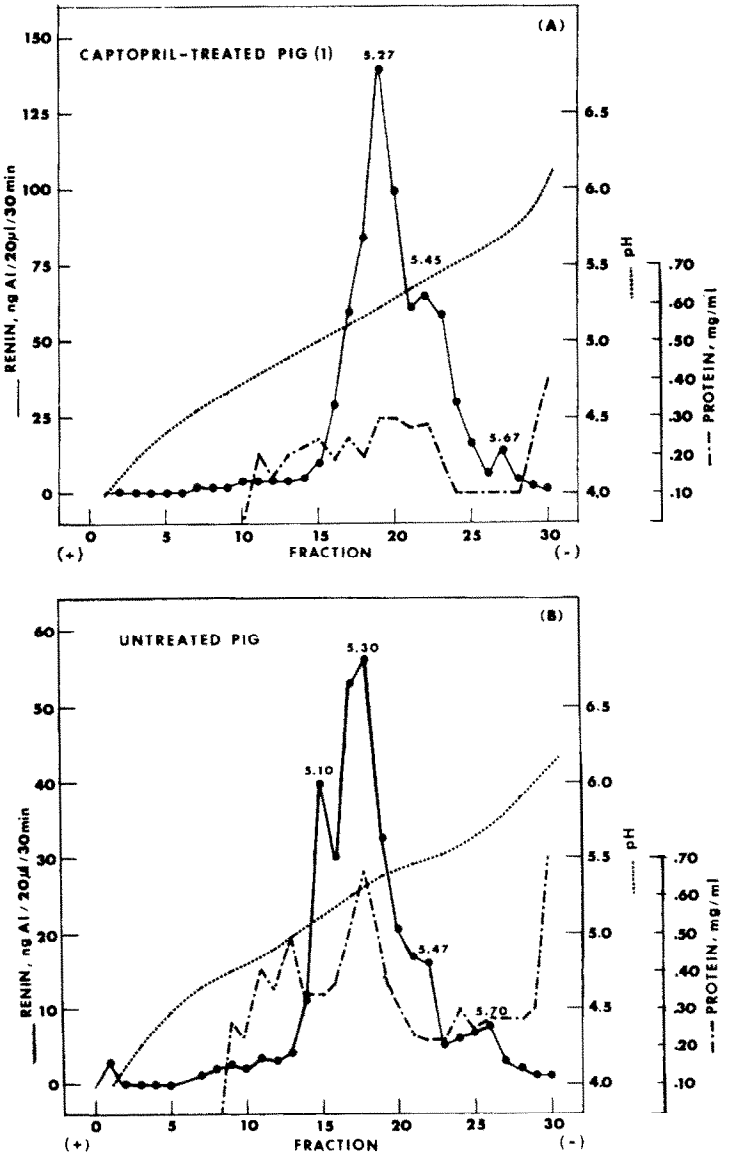


Fig. 1. Isoelectric focusing profile. The absence of the pI 5.10 renal renin form from the captopril-treated pig (A), when compared to the untreated pig (B), is emphasized by the IEF profile obtained by using twice the amount of total renin activity of the treated pig preparation. The higher specific activity of the renin activity from the treated pig is also demonstrated.

specific activities of renin from the two captopril-treated pigs (CPT-I and CPT-II) were 11,002 and 12,457 ng AI generated per mg protein per hr respectively.

The results of the IEF studies demonstrated a distinct difference in the renin profiles of the control pig vs the captopril-treated pigs. The renal forms from the control pig consistently focused into four peaks with isoelectric points (pI) of 5.10, 5.30, 5.47 and 5.70 and represented 25.02, 57.48, 10.99 and 6.49% of the total renin activity recovered from the gels. The form with the lowest pI value (5.10) was undetectable in the treated pigs. Even the application of twice the amount of renin activity to the IEF media failed to demonstrate any pI 5.10 (Fig. 1). It is noteworthy that this form was not only consistently isolated from our normal pig preparations, including those from four pigs not included in this study, but it has also been reported by others [13]. With the absence of the most acidic pI form, the relative percentages of pI forms 5.27, 5.45 and 5.67 were 63.32, 31.53 and 6.49 respectively. This observed alteration in the pI profile further demonstrates that renal renin can be affected qualitatively, as well as quantitatively, by agents which increase renin release. Previous studies have shown that: (a) numerous renin granules appear in the distended, rough endoplasmic reticulum in the JG cells of captopril-treated mice [3]; (b) a sharp increase in plasma and renal renin activity occurs in captopril-treated rabbits [2] and mini-pigs; and (c) isoproterenol, a sympathomimetic drug, which causes increases in renin secretion, preferentially increases the secretion of some of the multiple pI forms of rat renin by renal cortical slices [14].

The histologic appearance of the kidney from the control animal was normal. Sections through the glomeruli which revealed the JG apparatus did not display hyperplasia. The histopathology of the kidneys from the treated animals was normal except for a mild to moderate degree of JG hyperplasia in several of the glomeruli examined. This contrasts sharply with our earlier observation of captopril-treated rabbits, receiving the same dosage, which showed not only a much greater increase in PRA and renal renin activity but also profound JG hyperplasia [2]. Scanning electron microscopy of the control and treated animals revealed normal appearing podocytes with their foot processes surrounding the capillaries in the glomerular tuft. The JG apparatus was not visualized by this technique.

In summary, captopril-treated pigs exhibited morphological and biochemical responses to the drug. The early development of cutaneous lesions on the snout and labia, and a mild to moderate degree of JG hyperplasia were observed. The treated animals became hyperreninimic, and the specific activity of renal renin was increased sharply. An alteration in the isoelectric profile of the renal renin of the treated pigs was manifested by the absence of the most acidic isoelectric form (pI 5.10) which otherwise has been observed consistently in untreated animals.

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