Evidence for an Extrarenal Source of Inactive Renin in Rats

Yutaka Doi, Roberto Franco-Saenz, and Patrick J. Mulrow

SUMMARY We studied the source of inactive renin in plasma by investigating the changes of active and inactive renin after bilateral nephrectomy in the rat. Active renin rapidly decreased after bilateral nephrectomy, with a half-life of approximately 15 minutes. Inactive renin, on the other hand, was 20.96 ± 1.63 ng/ml/hr before nephrectomy and gradually increased to reach a peak at 20 hours after nephrectomy (193 ± 62 ng/ml/hr). The molecular weight of active renin was approximately 40,000 and that of inactive renin was approximately 60,000 on a Sephacryl S-200 column. Inactive renin was separated from active renin by a Cibacron blue column, and the 0 time inactive renin eluted in the same fractions as the inactive renin from 20 hours after nephrectomy. The pH optimum of inactive renin in rat renin substrate was between 5.5 and 7.5, which differs from the optimal value of pepsin or cathepsin D. The increase of inactive renin in nephrectomized rats was not prevented by removal of the salivary glands, uterus, spleen, pancreas, stomach, intestines, adrenal glands, or pituitary. In summary, inactive renin is present in the anephric rat and does not appear to be converted to active renin in the peripheral blood. The source and control of this extrarenal inactive renin are still unclear, but this renin is secreted in the rat within hours after nephrectomy.

(Hypertension 6: 627-632, 1984)

KEY WORDS • inactive renin • prorenin • nephrectomy • angiotensin • rat

SINCE Lumbers first reported the existence of inactive renin in amniotic fluid by acid activation, several other investigators have demonstrated the presence of an inactive form of renin in plasma that can be activated by either acid activation, cryoactivation, trypsin, plasmin, or kallikrein. The source of plasma inactive renin is still controversial. Although Takii and Inagami isolated inactive renin from hog kidneys and Atlas et al. found inactive renin in the perfusate of human kidney, others have found inactive renin in anephric patients. In humans, active and inactive renin falls rapidly after nephrectomy. The study of the source of inactive renin has been difficult because of the lack of a good animal model.

Recently Barrett et al. showed trypsin activation of rat inactive renin. Because of the increased concentration of trypsin inhibitors in rat plasma, a much higher concentration of trypsin is needed to demonstrate inactive renin in rat plasma. The same group of investigators demonstrated a parallel disappearance of active and inactive renin after nephrectomy in the rat. However, complete occlusion of the renal veins and arteries resulted in a significant increase of inactive renin. In the present study, we investigated the changes of rat active and inactive renin after bilateral nephrectomy and studied some of the characteristics of rat inactive renin. Also, we studied the effects of anesthesia, sodium loading, and infusion of angiotensin II (ANG II) on the levels of active and inactive renin after nephrectomy.

Material and Methods

Sprague-Dawley female rats weighing 190 to 220 g were maintained on regular Purina Chow diet and underwent bilateral nephrectomy under pentobarbital anesthesia (30 mg/kg). To study the effect of anesthesia on the levels of active and inactive renin, a group of rats underwent pentobarbital anesthesia without nephrectomy. Twenty hours after pentobarbital anesthesia, active renin was lower in intact rats (5–8 ng/ml/hr) whereas inactive renin was the same as zero time. The sodium loading was performed by the addition of 1% sodium chloride to the drinking water 5 days before nephrectomy. The ANG II (20 mg/kg/min) (Sigma Chemical Company, St. Louis, Missouri) was infused continuously into the jugular vein by osmotic mini pump (Alzet 2001) starting 2 days before nephrectomy. Blood samples were obtained immediately after
the rats were decapitated at frequent intervals from 0 to 48 hours after nephrectomy. Active renin was measured by radioimmunooassay (RIA) of angiotensin I (ANG I) by a modification of the method of Haber et al. Fifty µl of plasma was incubated with 5 µl of 8-hydroxyquinoline solution (0.34 M), 5 µl of dimercaprol (2%), 25 µl of EDTA (4%), 90 µl of Tris acetate buffer (0.1 M, pH 7.4), and 75 µl of rat renin substrate. The renin substrate was obtained from rat at 48 hours after nephrectomy. The incubation was carried out for 1 hour at 37° C at pH 7.4. Aliquots (50 µl) were used for the RIA of ANG I. Total renin was measured by the trypsin activation method. Trypsin (Sigma Type III) was prepared immediately before use in Tris acetate buffer (0.1 M, pH 7.4). Plasma was incubated with trypsin (5 mg/ml plasma) for 1 hour at 4° C. The reaction was stopped by the addition of lima-bean trypsin inhibitor (Sigma Type II-L) (5 mg/ml of plasma). Inactive renin was calculated as the difference between total renin and active renin. To make certain that the trypsin and lima-bean trypsin inhibitor were not interfering in the RIA of ANG I, trypsin plus trypsin inhibitor were first mixed and then incubated with or without substrate, and an aliquot was tested on the RIA. No angiotensin was measured. Also, trypsin inhibitor plus substrate did not generate ANG I. Moreover, increasing the concentrations of substrate had no effect on the levels of inactive renin.

The molecular weight of renin was estimated by Sephacryl S-200 column (Pharmacia) 1.5 × 99 cm with the use of bovine serum albumin, ovalbumin, chymotrypsin A, and ribonuclease A as the molecular weight standards (Pharmacia). Rat plasma (1 ml) was placed on the column and eluted with 0.05 M Tris chloride buffer pH 7.4 containing 0.1 M sodium chloride and 0.02% sodium azide. Then 1 ml fractions were collected, and in each fraction the concentration of trypsin inhibitor present in each fraction. Cibacron blue F 3G-A affinity column size (1.5 × 25 cm) (Pharmacia Sepharose Blue CL-6B) was used for separation of active and inactive renin according to the method of Yokosowa et al. The column was eluted with 0.02 M sodium phosphate buffer pH 7.1. The concentration of sodium chloride was changed in step-wise increments from 0.2 to 1.4 M. Inactive renin in the samples was activated by the addition of trypsin, 2.5 mg/ml of sample, and the samples were incubated as before. The recovery of active renin was approximately 90%, whereas the recovery of inactive renin was 50%. The results of the experiments are expressed as the means ± the standard error (SEM). Statistical analysis was performed by Student's t test. Significance was defined as a p value less than 0.05.

Results

The time course of active and inactive renin after bilateral nephrectomy is shown in Figures 1 and 2. At 0 time, active renin was 13.52 ± 2.3 ng/ml/hr, and inactive renin was 20.96 ± 1.64 ng/ml/hr. Active renin decreased rapidly after bilateral nephrectomy, with a half-life of approximately 15 minutes. At 16 hours after nephrectomy, active renin was close to zero. On the other hand, inactive renin gradually increased after nephrectomy and reached a peak at 20 hours (193 ± 62 ng/ml/hr). It then slowly decreased. At 48 hours after nephrectomy, inactive renin was still significantly higher than at 0 time (33.69 ± 1.31 ng/ml/hr vs 20.96 ± 1.63 ng/ml/hr).

The molecular weight of active and inactive renin before and after nephrectomy is shown in Figures 3 and 4. The molecular weight of active renin was approximately 40,000 and that of inactive renin was approximately 60,000 at 0 time. However, at 20 hours after nephrectomy (Figure 4), the peak of active renin completely disappeared, and an extremely high peak of inactive renin was apparent. The molecular weight is approximately 60,000 at 0 time, and it completely disappeared at 20 hours after nephrectomy. The molecular weight of inactive renin is approximately 40,000 at 0 time and it increased after nephrectomy and reached a peak at 20 hours. It then slowly decreased. At 48 hours after nephrectomy, inactive renin was still significantly higher than at 0 time (33.69 ± 1.31 ng/ml/hr vs 20.96 ± 1.63 ng/ml/hr).
Molecular weight of active and inactive renin in 0 time plasma calculated from their elution volume on a Sephacryl S-200 column. Inactive renin was located by trypsin activation of the eluates from the column. The trypsin concentration used was adjusted for the amount of trypsin inhibitor present in each fraction. Fractions were collected in 1 ml aliquots.

Figure 4. Elution profile of active and inactive renin on a Sephacryl S-200 column from plasma collected 20 hours after nephrectomy. Trypsin activation was done as in Figure 3.

Figure 7 shows the pH optimum of active (0 time) and inactive renin (at 20 hours after nephrectomy) following sequential chromatography on Sephacryl S-200 and Cibacron blue affinity columns. The pH optimum of inactive renin was from 5.5 to 7.5, the same as that of inactive renin 20 hours after nephrectomy was approximately 60,000.

To further characterize and purify inactive renin, we used a Cibacron blue affinity column. Figure 5 shows the elution pattern of active and inactive renin at 0 time on a Cibacron blue affinity column. Active renin eluted in the first peak between Fractions 12/25, and inactive renin eluted in the last peak between Fractions 92/105. Figure 6 shows the elution pattern of inactive renin 20 hours after nephrectomy. There was no active renin peak, and the inactive renin peak eluted in the same fractions as that of the zero time inactive renin.

Figure 5. Elution profile of active and inactive renin of 0 time plasma chromatographed on Cibacron Blue affinity column. Protein concentration in each fraction was measured by spectrophotometry, and inactive renin was measured by trypsin activation of the eluates.
of active renin. To investigate the possible source of inactive renin, various organs were removed at the time of nephrectomy. Figure 8 shows that removal of the salivary glands, uterus, adrenals, and pituitary at the time of nephrectomy did not prevent the increase in inactive renin 20 hours later. Similarly, removal of the pancreas, stomach, and spleen in one rat and removal of the intestines in another did not blunt the rise in inactive renin 20 hours later.

To determine if inactive renin levels are influenced by physiological mechanisms that inhibit the secretion of active renin, we examined the effect of sodium loading and ANG II on inactive renin after nephrectomy. Figure 9 shows that 5 days of sodium loading and ANG II infusion could not prevent the increase of inactive renin in nephrectomized plasma.
EXTRARENAL INACTIVE RENIN/Doi et al.

Discussion

The presence of inactive renin in human plasma after bilateral nephrectomy has been reported by several investigators. In humans, active and inactive renin decrease rapidly after nephrectomy, but the half-life of inactive renin is significantly longer than that of active renin. In the rat, Barrett et al. reported a parallel decrease in active and inactive renin after bilateral nephrectomy. However, their results are difficult to interpret since, in contrast to normal rats in which inactive renin accounts for approximately 80% of the total renin, their nephrectomy experiments showed that inactive renin was lower than active renin and accounted for only 33% of the total renin. It is possible that differences in the experimental design may account for the discrepancy in the results, since they reported that stress caused significant stimulation of active renin with a reciprocal decrease in inactive renin. It is conceivable that stress may have influenced the levels of active renin in their nephrectomy rats, and therefore the experimental conditions were different from ours. Furthermore, they measured the levels of active and inactive renin during the first hour after nephrectomy and 24 hours later, whereas in our experiments we followed the levels at frequent intervals for 48 hours.

There is considerable variation in the literature regarding the normal levels of inactive renin in the rat. This variability is probably the result of different experimental conditions as well as differences in the methods employed to activate inactive renin, which include the amount of trypsin used for activation as well as the time and temperature of incubation. In the dog, an inactive renin-like enzyme has been reported to increase after nephrectomy. In the experiments of Gallagher et al. and Wilczynski and Osmond, the increase in inactive renin was demonstrated by using a pH in the range of 5.7 to 6.0 for the ANG I generation. Gallagher et al. suggested that the increase in activity did not appear to result from an increase in the concentration of renin, since the optimum pH of angiotensin formation in trypsin-treated plasma from anephric dogs was pH 5 or less. They concluded that the trypsin activatable renin-like enzyme was cathepsin D.

In our present study, we have shown a remarkable increase of rat inactive renin after bilateral nephrectomy. In our study, inactive renin has a molecular weight of approximately 60,000, and its pH optimum is between 5.5 and 7.5, which is different from that of cathepsin D and pepsin. Also, inactive renin was capable of generating ANG I when natural renin substrate was used at pH 7.4. Cathepsin D usually cannot act on natural renin substrate, but trypsin has been reported to have generated tetradecapeptide from natural renin substrate in horse plasma. Therefore, cathepsin D could possibly act upon the tetradecapeptide cleaved by trypsin from the natural substrate. However, the pH optimum should be lower and no ANG I should be generated at pH 7.4. Also, if this were the case, one might expect that the rate of ANG I generation after nephrectomy would be proportional to the renin substrate concentration. Our data did not show a direct relationship with substrate concentration, because the maximum value of inactive renin was at 20 hours and then gradually decreased, while substrate concentration after nephrectomy peaked at between 8 and 24 hours and remains elevated for at least 48 hours.

Furthermore, after elution from Sephacryl S-200 and Cibacron blue, activated inactive renin generates ANG I from natural substrate that had never been exposed to trypsin. Finally, under our experimental conditions, trypsin did not produce any substance that interfered in the RIA for ANG I. If tetradecapeptide were generated, one might expect cross-reactivity in the RIA. These observations suggest that in the rat inactive renin present after nephrectomy is not cathepsin D.

The molecular weight of inactive renin after nephrectomy in the rat has not been reported previously. In this study, we showed that inactive renin has a molecular weight of approximately 60,000. It does not change after nephrectomy, and it does not change after trypsin activation.

The source of plasma inactive renin is still controversial. Although inactive renin has been found in human and hog kidneys, the ratio of inactive-to-active renin is lower in the kidney than in the peripheral plasma. These data suggest that, in addition to the kidney, there is an extrarenal source of inactive renin. Furthermore, inactive renin has been found in humans after bilateral nephrectomy, which indicates that the kidney is not the only source of inactive renin.

Weinberger et al. suggested that the source of inactive renin after nephrectomy was the salivary gland. In our study, however, the salivary gland did not appear to be the only source of inactive renin in the rat, since the rise of inactive renin 20 hours after nephrectomy was not prevented by the simultaneous removal of the salivary glands. Furthermore, removal of the adrenals, pituitary, uterus, pancreas, stomach, and intestines did not prevent the rise of inactive renin. It is possible that other organs such as the brain, liver, lungs, or the vascular endothelium may be the source of this enzyme. The physiological role and mechanism of the secretion of inactive renin are still unknown. In our experiment, inactive renin increased markedly after nephrectomy. Sodium loading and ANG II did not prevent the increase of inactive renin after nephrectomy. In contrast to active renin, these data suggest that the secretion of inactive renin is not controlled by volume or negative feedback by ANG II.

In summary, we confirmed an enzyme in the plasma of nephrectomized rats with the characteristics of inactive renin. We were unable to determine the tissue of origin nor its physiological role, but this enzyme is not converted to active renin in peripheral blood. Furthermore, any changes in the concentration of inactive renin in peripheral blood of rats cannot be ascribed to changes in renal secretion unless arteriovenous differences across the kidney are measured.
References

Evidence for an extrarenal source of inactive renin in rats.
Y Doi, R Franco-Saenz and P J Mulrow

Hypertension. 1984;6:627-632
doi: 10.1161/01.HYP.6.5.627

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/6/5/627

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/