Dynamic Responses of Active and Inactive Renin in Patients with Essential and Renovascular Hypertension

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SUMMARY We studied the dynamic responses of inactive renin and the form of renin released by the kidney in the hypertensive patients. Significant increase of active renin concentration (p < 0.01) and decrease of the percentage of inactive renin concentration (p < 0.01) after sodium depletion was observed in 15 essential hypertensive subjects with normal plasma renin activity. In eight of 15 patients, significant increase of inactive renin concentration (p < 0.01) was observed after sodium depletion. In the remaining seven patients, no significant change of inactive renin concentration was demonstrated. A small increase of active and inactive renin concentration was observed following sodium depletion in six essential hypertensive subjects with low plasma renin activity (PRA). In the unilateral renal hypertension after upright tilting, active renin concentration in the renal vein of the affected kidney was significantly (p < 0.02) higher than that in the renal vein of the non-affected kidney and the inferior vena cava. Inactive renin concentration in the renal vein of the affected kidney was significantly (p < 0.02) lower than that in the renal vein of the non-affected kidney and the inferior vena cava. In four of five cases, the inactive renin concentration in the femoral artery was less than that in the inferior vena cava. Therefore, we might conclude that only active renin was released from the affected kidney, and active renin became inactive by unknown mechanisms; the ischemic kidney might also activate inactive renin. (Hypertension 3: 126-133, 1981)

KEY WORDS • active renin concentration • inactive renin concentration • total renin concentration • essential hypertension • renovascular hypertension

THE presence of acid-activable renin in human amniotic fluid and plasma was suggested by Lumbers1 and Morris and Lumbers.2 An inactive renin that can be activated by acid,3 cold,3,4 and proteolytic enzymes5,6 is present in human amniotic fluid6,5,7 and in plasma from anephric patients,8 pregnant women,8 and patients with nephropathies and renal tumors.8,9 The characterization of an inactive renin is incomplete. Whether an inactive renin represents a true proenzyme8,9,10 or renin bound to an inhibitor11-13 remains unclear. However, it has been suggested that inactive renin may be the intrarenal storage form of the enzyme.14

In addition, Rubin14 reported an increase in renin activity after acidification of pig kidney extracts. After that, Baillie and Derk15 demonstrated that isoproterenol and furosemide stimulated and propranolol or indomethacin suppressed the release of both active and inactive renin in anesthetized pigs, controlled by similar mechanisms. However, the form of renin released by the human kidney and the regulation of its secretion remain controversial.

The present studies were carried out to demonstrate the presence and the dynamic responses of an inactive form of renin in normal subjects and patients with essential or secondary hypertension. We also studied the form of renin released by the kidney in certain hypertensive patients.

Materials and Methods

Buffer

Buffer solutions of pH 3.3 and pH 7.5 were prepared according to Morris and Lumbers. All buffers were made up at 4°C. The following standard buffers were utilized: 1) sodium phosphate buffer, pH 7.5, containing 98.9 mM sodium phosphate, 5 mM Na2EDTA, and 76 mM NaCl; 2) glycine-HCl buffer, pH 3.3, containing 50 mM glycine, 10 mM HCl, 5 mM Na2EDTA, and 95 mM NaCl; and 3) citrate phosphate buffer, pH 4.5, containing 27.8 mM citric acid, 45 mM Na2HPO4 • 2H2O 5 mM Na2EDTA and 82.1 mM NaCl.
Preparation of Renin Substrate

Semi-purified sheep renin substrate was prepared from the plasma of nephrectomized sheep according to the method of Skinner. In brief, a ewe was heparinized and exsanguinated 5 days after bilateral nephrectomy performed under pentobarbital anesthesia. The plasma was immediately separated by centrifugation at 4°C, dialyzed for 24 hours at pH 4.5 against citrate-phosphate buffer. Then, it was heated at 32°C for 45 minutes at pH 4.5, dialyzed to pH 7.5 against the phosphate buffer, and stored at −20°C. In the renin assay, it was used at a final concentration of 1.3 nmo/min, expressed as the maximum quantity of angiotensin I generated with excess human renin. No angiotensinase activity was detectable under assay conditions in the sheep renin substrate.

Activation of Inactive Renin by Acidification

Activation of inactive renin was done according to the method reported by Lumbers. In brief, to study the activation of inactive renin, 0.5 ml of plasma was dialyzed for 24 hours at 4°C in 8/32 inch Visking cellophane tubing against the Glycine-HCl buffer at pH 3.3. Each sample was then dialyzed against the sodium phosphate buffer at pH 7.5 for 24 hours at 4°C.

Renin Assay

Blood samples (5 ml) were collected into chilled Vacutainers (Neotube 5 ml, Nipro) containing Na₂EDTA (7.5 mg, granules) as anticoagulant, and placed immediately in ice water at 0°C. Blood samples were centrifuged at 4°C. Plasma for renin determination was stored at −20°C until assayed.

Plasma renin activity (PRA) was measured by radioimmunoassay by Haber et al’s method. Nonacified and acidified plasma was added to sheep renin substrate, respectively, with angiotensinase and converting enzyme inhibitors and incubated for 1.5 hours at 37°C in a shaking incubator. The amount of angiotensin I thus generated was quantified by the radioimmunoassay of the Haber’s method, 1' corrected for angiotensinase activity. The plasma renin activity (PRA) was stored at −20°C until assayed.

Experimental Procedures

Normal Subjects

Twenty-one healthy subjects aged 7 to 46 years were studied as controls. To estimate active, inactive, and total renin concentration, blood samples were obtained from an arm vein, between 8:00 and 9:00 a.m., after overnight fasting and 60 minutes of recumbency under normal diet.

Hypertensive Subjects

Twenty-one patients with essential hypertension aged 15 to 64 years and seven patients with secondary hypertension (three with renal hypertension due to chronic glomerulonephritis, two with renovascular hypertension, one with primary aldosteronism, and one with Cushing’s syndrome) were included in this study. For at least one week during hospitalization, they took a normal sodium diet (NaCl 12 g/day). Plasma for the renin assay was obtained from an arm vein in the early morning after the patient had lain flat overnight (supine), then after 4 hours of ambulation, and then after 4 hours of ambulation following 3 days of restricted sodium intake below 3 g of NaCl. The 21 patients with essential hypertension were divided into two groups, the normal renin group (n = 15), and the low renin group (n = 6), on the basis of plasma renin activity after 3 days of restricted sodium intake below 3 g of NaCl.

Upright Tilting Study

The eight hypertensive patients with renal artery stenosis and two patients with hypertension probably due to unilateral pyelonephritis were included, to investigate the form of renin released from the human kidney. After 1 hour of recumbency, a catheter was introduced into the saphenous vein under local anesthesia. Samples of plasma were obtained simultaneously from the renal veins of affected and nonaffected kidneys, hepatic vein, and inferior vena cava below the kidney, with the patients supine. After that, plasma was also obtained from the same sites and the femoral artery after 60° tilt for 20 minutes, which stimulated renin secretion.

Results

Active and Inactive Renin Concentration in the Normal Subjects

Figure 1 shows plasma renin activity, active renin concentration, total renin concentration, and inactive renin concentration under normal diet in the 21 normal subjects aged 7 to 46 years. Plasma renin activity was 1.3 ± 1.1 ng/ml/hr (mean ± SD), active renin concentration 4.9 ± 3.9, total renin concentration 20.1 ± 9.2, and inactive renin concentration 15.2 ± 7.1 ng/ml/hr. The percentage of inactive renin concentration was 77% ± 14.6%.

Effects of Ambulation and Sodium Depletion

Figure 2 demonstrates the dynamic responses of active renin concentration, inactive renin concentration, and percentage of inactive renin concentration in the supine position, and after ambulation and sodium depletion in 15 essential hypertensive patients with normal plasma renin activity after restricted sodium
intake. A significant increase of active renin concentration after ambulation and sodium depletion was observed, as compared with that at rest (p < 0.01). On the other hand, no significant change of inactive renin concentration was consistently demonstrated following ambulation and sodium depletion.

However, in eight of fifteen patients with normal renin essential hypertension, marked increase of inactive renin concentration after sodium depletion was observed; they were classified as a high response group. In the remaining seven patients with normal renin essential hypertension, no significant change of inactive renin concentration was demonstrated; they were classified as an unresponsive group. The percentage of inactive renin concentration was consistently decreased after ambulation and sodium depletion, compared with that at rest.

Figure 3 compares the inactive renin concentration after sodium depletion in the high response group mentioned above and the unresponsive group. No significant difference in active renin concentration in the supine position and after sodium depletion was observed in the two groups. However, the inactive renin concentration in the supine position, and after ambulation or sodium depletion was significantly lower in the unresponsive group than in the high response group (p < 0.01).

As shown in figure 4, six essential hypertensive patients with low plasma renin activity showed a small
increase of active and inactive renin concentration following sodium depletion.

Figure 5 demonstrates the dynamic responses of active and inactive renin concentration following ambulation and sodium depletion in the patients with secondary hypertension. In patients with low plasma renin activity, including renal hypertension, primary aldosteronism, and Cushing's syndrome, a small increase of active renin concentration was observed with sodium depletion; but no significant change of inactive renin concentration was demonstrated. In two patients with renovascular hypertension, significant high levels of active renin concentration in all three situations were observed. On the other hand, in one case, high levels of inactive renin concentration were found while the other patient showed normal levels of inactive renin concentration.

Upright Tilting Study

Figure 6 demonstrates active and inactive renin concentrations in the plasma samples of both the renal veins and the inferior vena cava in eight patients with renovascular hypertension and two patients with hypertension probably due to unilateral pyelonephritis. As shown in figure 6, the plasma in the renal vein of the affected kidney had significantly higher values of active renin concentration, compared with that in the inferior vena cava and in the renal vein of the nonaffected kidney (respectively, \( p < 0.02 \)). On the other hand, the inactive renin concentration in the renal vein of the affected kidney was significantly lower (\( p < 0.05 \)) than that in the renal vein of the nonaffected kidney and in the inferior vena cava.

Figure 7 shows active and inactive renin concentrations in the plasma samples of both the renal veins and the inferior vena cava in six patients with renovascular hypertension and two patients with hypertension probably due to unilateral pyelonephritis after 60° tilt for 20 minutes. As shown in figure 7, the plasma in the renal vein of the affected kidney had significantly higher values of active renin concentration compared with that in the inferior vena cava and in the renal vein of the nonaffected kidney after upright tilting (respectively, \( p < 0.02 \)). On the other hand, the inactive renin concentration in the renal vein of the affected kidney was significantly lower (\( p < 0.05 \)) than that in the renal vein of the nonaffected kidney.

Figure 8 demonstrates that active renin concentration in the femoral artery was significantly higher than that in the hepatic vein (\( p < 0.05 \)). On the other hand, no significant difference in inactive renin concentration was observed between the femoral artery and hepatic vein.

As demonstrated in figure 9, the active renin concentration in the femoral artery was higher than in the inferior vena cava in four of five cases. However, the inactive renin concentration in the femoral artery was less than that in the inferior vena cava in four of five cases.

**Figure 3.** Comparison between the high response group and the unresponsive group of inactive renin concentration after sodium depletion in the essential hypertensive patients with normal plasma renin activity. Solid lines depict the high response group of inactive renin concentration. Dotted lines indicate the unresponsive group of inactive renin concentration. Vertical bars represent mean ± 1 SD. See text for details on rest, ambulation, and Na depletion.
FIGURE 4. Dynamic responses of active renin concentration, inactive renin concentration, and percentage of inactive renin concentration in the six essential hypertensive patients with low plasma renin activity. See text for details on rest, ambulation, and Na depletion.

FIGURE 5. Dynamic responses of active renin concentration, inactive renin concentration, and percentage of inactive renin concentration in the patients with secondary hypertension. Black circle indicates primary aldosteronism, open triangle indicates renal hypertension due to chronic glomerulonephritis, open circle indicates renovascular hypertension, and cross indicates Cushing's syndrome.
Figure 6. Active and inactive renin concentration in plasma from the renal veins of nonaffected and affected kidneys and the inferior vena cava (IVC) in eight patients with renovascular hypertension (solid line) and two patients with hypertension probably due to unilateral pyelonephritis (broken line) after 1 hour of recumbency.

Figure 8. Active and inactive renin concentration in the plasma from the femoral artery and the hepatic vein in four patients with renovascular hypertension (solid lines) and one patient with hypertension probably due to unilateral pyelonephritis (dotted lines) after 60° tilt for 20 minutes.

Figure 7. Active and inactive renin concentration in plasma from the renal veins of nonaffected and affected kidneys and the inferior vena cava (IVC) in six patients with renovascular hypertension (solid line) and two patients with hypertension probably due to unilateral pyelonephritis after 60° tilt for 20 minutes.

Figure 9. Active and inactive renin concentration in the plasma from the femoral artery and the inferior vena cava (IVC) in four patients with renovascular hypertension (solid lines) and one patient with hypertension probably due to unilateral pyelonephritis (dotted lines) after 60° tilt for 20 minutes.
Discussion

An inactive form of renin may be activated in vitro by exposure to acid, cold, or by action of enzymes like peptin, trypsin, or kallikrein. Although an inactive renin has been demonstrated in the plasma of the human subjects by several investigators, its characterization and physiological role in hypertension remain obscure. The definition of "inactive renin" is entirely descriptive and in this paper refers to the increment in renin concentration obtained during dialysis against acid, as described.

Skinner et al. reported that most of the renin in liquor amnii and plasma existed in an inactive form which could be activated by acidification to pH 3.3 and that the inactive renin ratio in first trimester plasma was greater than in term plasma or in normal male and female control plasma. More recently, Boyd and Weinberger et al. reported the presence of an inactive renin in the normal human plasma, which was consistent with our findings in the present studies.

The plasma concentration of active and inactive renin has been demonstrated under varying conditions in man. Derkx et al. and Weinberger et al. found that acute stimulation or suppression of renin release could result in opposite response in the active and inactive renin concentrations. Derkx et al. demonstrated that isoproterenol increased active but not inactive renin concentration in the human plasma and propranolol suppressed active but elevated inactive renin concentration. Weinberger et al. reported that in normal subjects, saline infusion suppressed active but not inactive renin, whereas 10 mEq sodium diet and furosemide caused both to increase, although the relative increase in active renin concentration was greater. Furthermore, Atlas et al. demonstrated that sodium depletion stimulated total renin release, while propranolol and clonidine produced divergent response of active renin and inactive renin (activated by cold) in plasma from hypertensive patients, the changes in inactive renin (activated by cold) depending on the changes induced in blood pressure.

In the present studies, ambulation and sodium depletion caused active renin concentration in the plasma to increase in the patients with normal and low renin essential hypertension. Millar et al. reported that inactive renin concentration showed no significant change during either sodium depletion or upright tilting in the normal subjects, but rose significantly 30 minutes after intravenous furosemide. However, Aoi et al. demonstrated that inactive renin concentration did not change significantly 30 and 60 minutes after intravenous furosemide in the normal and hypertensive subjects.

The present studies demonstrated that essential hypertensive patients with normal plasma renin activity were classified into two groups, namely, unresponsive and high response groups, on the basis of the response of inactive renin concentration by sodium depletion. In the former, inactive renin concentration did not increase after sodium depletion, whereas in the latter it markedly increased following sodium depletion. No significant difference in the age, sex, or level of blood pressure were observed between the groups. Furthermore, no significant difference in the active renin concentration was demonstrated between the groups. Three hypotheses would be possible for the differences in the response of inactive renin concentration between the two groups. First, only active renin might be released from the kidney in the unresponsive group, whereas in the high response group, both active and inactive renin was thought to be released from the kidney. Second, only active renin was released from the kidney and an active renin could be rapidly converted to an inactive renin by unknown mechanisms in the high response group. Third, an inactive renin could be rapidly converted to an active renin by unknown mechanisms after it was released by the kidney. Further studies will be required to justify the hypotheses mentioned above and to clarify the physiological and biological characteristics of an inactive renin.

Although a high molecular weight of renin has been extracted from human and animal kidneys, the form of renin released by the human kidney remains controversial. In the present studies, we demonstrated that active renin concentration in the renal vein of the affected kidney showed significantly marked increase by upright tilting, whereas inactive renin concentration in the renal vein of the nonaffected kidney and in the inferior vena cava showed significantly high values, compared with that in the renal vein of the affected kidney. The present observations are supported by the similar findings of Weinberger et al. that renin might have been secreted by the affected kidney in the active form or might have activated an inactive renin delivered to it in patients with renal artery stenosis and "Page" kidney. Furthermore, Vandongen et al. reported that an inactive renin was present in the peripheral plasma whereas obviously no inactive renin was secreted by the perfused kidney of the rat and concluded that an inactive renin was produced outside the kidney of the rat. On the other hand, Derkx et al. and Boyd demonstrated increased quantities of inactive renin in renal venous plasma on the affected side in the patients with renal artery stenosis.

However, Derkx et al. and Boyd investigated only the venoarterial difference in active and inactive renin concentration in the affected kidney and did not evaluate release of active and inactive renin from the affected kidney, especially by stimulation such as tilting, so that direct comparison is difficult.

Increase of sympathetic nerve activity and catecholamines by stimulation such as standing have been reported to increase renin secretion. Derkx et al. demonstrated that infusion of isoproterenol resulted in an increase in active renin but no change in inactive renin, while tilting and diazoxide infusion resulted in a rise in active renin and a fall in inactive renin in the normotensive and essential hypertensive subjects. Furthermore, Vandongen et al. reported that...
stimulation of renin secretion with isoprenaline produced only active renin in the rats. Therefore, an acute stimulus to renin release by tilting might result in a rise in active renin.

Where does an inactive renin come from, because only active renin is released from the affected kidney? The present studies demonstrate that an inactive renin concentration in the renal vein of the nonaffected kidney and in the inferior vena cava showed significantly high values, compared with a value shown in the renal vein of the affected kidney, and that an inactive renin concentration in the femoral artery was lower than that in the inferior vena cava in four of five cases. These findings suggest that an active renin is converted to inactive form by unknown mechanisms during peripheral circulation.

Although the kidney contains activating factors such as cathepsin D and kallikrein, which activate an inactive renin in vitro, there is a possibility of differences in the production of activating or inactivating factors by the affected and nonaffected kidneys. Because an inactive renin concentration in the renal vein of the nonaffected kidney and in the inferior vena cava showed a significantly high value, compared with that in the renal vein of the affected kidney, an inactive form of renin might be activated through the affected kidney by the activating factors such as cathepsin D, kallikrein or other unknown enzymes.

We conclude from these studies that an active form of renin was released from the affected kidney in patients with unilateral renal hypertension and that active renin became inactive by unknown mechanisms during peripheral circulation, and that the ischemic kidney also activated an inactive form of renin. The present studies suggest the conversion of active and inactive renin to each other.

The physiological significance of inactive renin remains obscure, and additional studies will be required to clarify its importance in the regulation of the renin-angiotensin system in normal and hypertensive subjects.

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