THE INACTIVE TO ACTIVE RENIN RATIO IN THE KIDNEYS AND THE PLASMA IN DIABETIC NEPHROPATHY

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We examined the inactive to active renin ratio in the renin granules of the cadaver kidneys and the plasma in patients with diabetic nephropathy. The inactive renin in the break-through fraction when the plasma or the renin from the renin granules was put into a pepstatin column was determined. The inactive renin in this fraction was activated by trypsin. Concerning plasma, the inactive to active renin ratio was 90 in the patients and 9 in the normal subjects. On the other hand, this ratio was 0.29 in the patients' kidneys and 0.28 in the control kidneys. These results suggest that the increase of the inactive to active renin ratio in plasma of diabetic nephropathy does not result from the change of the renin storage in the kidneys.

AN inactive renin develops renin-like activity following limited proteolysis by trypsin. It is supposed that this activation mechanism plays a role in the regulation of plasma renin activity. Some workers¹⁻³ have reported that the plasma in diabetic nephropathy contains large amounts of inactive renin and amounts of active renin are less than normal. These results suggest that a conversion of inactive renin to an active form may be impaired in the kidney or in the plasma. However, there is no report on the renin storage form in the kidneys of patients with diabetic nephropathy, except our report on the inactive to active renin ratio in the renin granules isolated from the non-pathological

human kidneys, which was 0.33⁴

This is a report on the renin storage form in the renin granules isolated from the kidneys of patients with diabetic nephropathy. In addition, the inactive to active renin ratio was examined in the plasma of diabetic nephropathy and of normal subjects.

MATERIALS AND METHODS

Subjects

The kidneys from 6 human cadavers (mean age: 62 ± 2) were obtained during autopsy, within 4 hours after death. All had suffered from diabetes mellitus for over 10 years and had complications including proteinuria.

Plasma was obtained from 7 patients with diabetic nephropathy (mean age: 59 ± 4) and from 10 volunteers (mean age: 54 ± 4). All patients had suffered from diabetes mellitus for over 10 years and had complications including proteinuria. The volunteers had no evidence of

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TABLE I RENIN ACTIVITY AND PROTEIN CONCENTRATION IN EACH FRACTION

Fraction	Renin		Protein (c)
	(a)	(b)	
F 0	2.36 ± 0.58	—	4.96 ± 0.57
1	0.53 ± 0.09	9.0 ± 0.7	1.24 ± 0.11
2	0.86 ± 0.18	6.2 ± 0.3	0.47 ± 0.05
3	1.30 ± 0.43	7.1 ± 0.7	0.34 ± 0.04
4	2.44 ± 0.71	14.3 ± 0.9	0.35 ± 0.03
5	2.50 ± 0.80	21.8 ± 1.8	0.55 ± 0.05
6	5.02 ± 1.26	37.4 ± 2.9	0.30 ± 0.02
7	1.36 ± 0.47	4.5 ± 1.9	0.18 ± 0.04

Fractions were obtained from a sucrose density gradient.

Values are means ± SE of 6 experiments.

a) Specific activity of renin in fractions isolated. Units are μg of angiotensin I/hr/mg protein.

b) Distribution of renin activity (fraction volume $\times$ enzyme activity) expressed as percentage of the total activity recovered from fractions F1 to F7.

c) Protein concentration (mg/ml) in fractions isolated.

any disease. All subjects had the dietary intake of sodium of 100–200 mEq/day for at least 5 days. Venous blood (20 ml) was taken after one hour of recumbency by vacutainers, into a precoded tube containing EDTA and the plasma was separated using a cold centrifuge.

Isolation of Renin Granules from the Cadaver Kidneys with Diabetic Nephropathy

Renin granules were prepared from the cadaver kidneys as reported previously⁴. Cortical slices of the kidneys were homogenized with 0.45M sucrose (1:4 w/v). After centrifugation of the homogenate at $500 \times g$ for 10 min, the supernatant was placed on a discontinuous sucrose-density-gradient solution (1.2 to 1.7M with 0.1M intervals). After centrifugation at $60,000 \times g$ for 90 min the gradient was fractionated into 7 fractions: Fraction 0 corresponded to the supernatant, Fraction 1 to 1.2M sucrose, Fraction 2 to 1.3M sucrose, Fraction 3 to 1.4M sucrose, Fraction 4 to 1.5M sucrose, Fraction 5 to 1.6M sucrose and Fraction 6 to 1.7M sucrose. The sediment was resuspended in 7 ml of 0.45M sucrose and used as Fraction 7. Five milliliters of each fraction was added to 5 ml of 0.1M phosphate buffer containing 0.2% Triton X-100 (pH 7.2) and then centrifuged at $100,000 \times g$ for 30 min. The supernatant was analysed for protein and renin. The supernatant in Fraction 6 was used as renin from renin granules.

Affinity Chromatography

Separation of the inactive renin from the active one was carried out as reported previously⁴. Five milliliters of the samples were put into a pepstatin column (1.6 $\times$ 30 cm) and the column was washed thoroughly with 0.05M phosphate buffer containing 0.1M NaCl (pH 7.2). The break-through fraction was concentrated up to 5 ml using a Toyo UK-10 membrane filter.

Activation of Inactive Renin

Inactive renin was activated by trypsin. Trypsin treatment was carried out as reported previously⁴. The samples were incubated for 1, 5, 15, 30, 60 or 180 min at 0°C or 37°C with trypsin at a concentration of 0, 1, 10, 100 or 1000 $\mu\text{g}/\text{ml}$ and the reaction was stopped by the addition of a two-fold molar excess of soy-bean trypsin inhibitor.

Measurement of Renin Activity

Tubes containing 50 μl portions of dilution of the sample, 20 μl of sheep renin substrate prepared by the method of Skinners et al. (maximum amount of angiotensin I that could be liberated was 100 ng), 10 μl of phenylmethylsulfonylfluoride (250 mM) and 170 μl of phosphate buffer (0.1M, pH 7.0) with EDTA (15 mM), dimercaprol (4.8 mM) and 8-hydroxyquinoline sulfate (2.4 mM), were incubated at 37°C for one hour. If a concentration of plasma active renin in diabetic nephropathy was undetectable after this incubation period, incubation was carried out for 3 hours. The reaction was

Fig.1. Effects of trypsin concentration, incubation temperature and incubation time in break-through fractions obtained from plasma of the controls (a) and of patients with diabetic nephropathy (b). Incubation was carried out with three trypsin concentrations (0 $\mu\text{g/ml}$ ($\blacktriangle$), 100 $\mu\text{g/ml}$ ($\circ$) and 1000 $\mu\text{g/ml}$ ($\bullet$)) at 0°C (—) or at 37°C (---). The reaction was stopped by soy-bean trypsin inhibitor and then renin activity was measured after incubation with renin substrate.

stopped by immediate cooling. The angiotensin I generated was determined by radioimmunoassay (CEA-IRE-SOLIN, Italy), according to the method of Haber et al.⁵ Angiotensin I in the unincubated sample was measured before incubation and this value was subtracted.

Protein

The protein content of the fraction was determined by the method of Lowry et al.⁶ using bovine serum albumin as the standard.

Statistics

Statistical analyses were performed using Student's *t*-test. The mean value and standard errors are indicated.

RESULTS

Subcellular Distribution of Renin

Table I shows the renin activity and protein concentration in each fraction obtained in 6 experiments. The renin activity was mainly

recovered from the supernatant fraction (Fraction 0), showing $71 \pm 4\%$ of the total activity recovered from Fractions 0 to 7, while it was $44 \pm 5\%$ in the 10 non-pathological kidneys as reported previously.⁴ Among Fractions 1 to 7, Fraction 6 showed the highest renin activity, being about 37% of the total granular activity.

Inactive and Active Renin in Renin Granules from Kidneys with Diabetic Nephropathy

The renin activity (active renin) in the renin granules was $1.38 \pm 0.29 \mu\text{g/ml/hr}$. The renin isolated from the renin granules was put into a pepstatin column to remove the active renin. The break-through fraction was treated by trypsin at a concentration of one or 10 $\mu\text{g/ml}$ for 0, 1, 5, 15 or 30 min at 0°C. The maximum amount of renin activity (inactive renin) was obtained at a concentration of 10 $\mu\text{g/ml}$ for 5 or 15 min at 0°C. The inactive renin obtained from 6 experiments was $0.41 \pm 0.17 \mu\text{g/ml/hr}$. The inactive to active renin ratio in the renin granules from the kidneys with diabetic nephropathy is

0.29 ± 0.08 . In our previous study, this ratio in the renin granules from the 10 non-pathological kidneys was 0.28 ± 0.04 . Thus, the ratio was not different in these kidneys.

Inactive and Active Renin in Plasma

The plasma concentration of active renin was 2.49 ± 0.52 ng/ml/hr in the controls and 0.94 ± 0.44 ng/ml/hr in the diabetic patients. The difference was statistically significant ($p < 0.05$).

Inactive renin was examined using the break-through fraction from the affinity column. Figure 1a shows the renin activity in the break-through fraction of the control plasma with 3 concentrations of trypsin for 7 periods at 0°C or 37°C . An initial velocity of an increase of renin activity was more rapid at 0°C than at 37°C . The maximum renin activity was obtained at 1 mg/ml of trypsin concentration with an incubation for 5 min at 0°C . As shown in Fig. 1b, the maximum trypsin-activated renin activity in the plasma of a diabetic patient was obtained in a similar manner to that described above, although the incubation time was 15 min. Inactive renin in the controls was 17.97 ± 1.27 ng/ml/hr and that in diabetic patient was 42.08 ± 3.41 ng/ml/hr. The difference was statistically significant ($p < 0.01$). The inactive to active renin ratio was 9.47 ± 1.33 in the controls and 90.60 ± 20.95 in diabetic patient, and the difference was statistically significant ($p < 0.01$).

DISCUSSION

Renin granules from the cadaver kidneys with diabetic nephropathy, were mainly recovered from the fraction corresponding to 1.7M sucrose, similar to that obtained by us from the non-pathological kidneys.⁴ In the present study the inactive to active renin ratio in the renin granules was about 0.3 which was similar to that obtained from the non-pathological kidneys. This indicates that the renin granules obtained from both the diabetic kidneys and the non-pathological kidneys mainly consist of the active form of renin. On the other hand, the inactive to active renin ratio was about 9 in normal plasma, indicating a predominance of the inactive form of renin. This ratio in the plasma of diabetic nephropathy was about 10 times higher than that in the normal plasma. This result is similar to that reported previously.¹⁻³ Hsueh et al.¹ suggested that the intrarenal conversion mechanism of inactive renin to an active one was

impaired in diabetic nephropathy. However, the present result indicates a defect in this mechanism existing in the plasma rather than in the kidneys. Three possible mechanisms may be considered to explain the difference of the inactive to active renin ratio between the plasma and the kidneys. Firstly, active renin may be inactivated in the plasma. Hsueh et al.⁷, Leckie et al.⁸ and Matsunaga et al.⁹ recently found a reversible conversion between active and inactive renin in human plasma. Secondly, the half-life of inactive renin may differ from that of active renin. The half-life of inactive renin in the plasma has been reported to be longer than that of active renin by Derkx et al.¹⁰ Thirdly, inactive renin may mainly originate from extrarenal organs. Weinberger et al.¹¹ found the existence of inactive renin in anephric patients, suggesting an extrarenal source of inactive renin.

An initial velocity of an increase of the renin activity by trypsin was more rapid at 0°C than at 37°C in the break-through fraction of plasma. This result corresponds to that found by Sealy et al.¹² who used whole plasma. They suggested that the discrepancy between the activation rate of inactive renin and the hydrolytic activity of trypsin was due to the existence of endogenous trypsin inhibitors in human plasma. The endogenous trypsin inhibitors seem to exist in the break-through fraction, because the relation of the activation rate of inactive renin to incubation temperature was similar between the whole plasma and the break-through fraction. On the other hand, inactive renin from human renin granules was observed to be activated by incubation at a higher temperature in our previous study.⁴ These results suggest that the amount of trypsin inhibitors in the renin granules is scanty, as compared to that in the plasma.

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