

Proteinuric Potentials of Angiotensin II, [des-Asp¹]-Angiotensin II, and [des-Asp¹, des-Arg²]-Angiotensin II in Rats

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Received June 16, 1994; accepted August 8, 1994

To compare proteinuric potentials among angiotensin II (ANG II) and its fragments, [des-Asp¹]-angiotensin II (ANG III) and [des-Asp¹, des-Arg²]-angiotensin II (ANG IV), the peptide was intravenously infused for 30 min at doses of 0.015, 0.05, 0.15, 0.45 and 1.45 nmol/kg body weight/min. The infusion of ANG II and ANG III increased the fractional clearance of albumin in a dose-dependent manner: most extensively for ANG II, and moderately for ANG III. In contrast, the infusion of ANG IV hardly showed any proteinuric action, even at the maximal dose of 1.45 nmol/kg body weight/min. These results denoted that the cleaving of the N-terminal aspartic acid¹ from ANG II weakened the proteinuric action in the glomeruli, and the further cleaving of the N-terminal arginine² from ANG III led to a complete loss of proteinuric action in the glomeruli.

Keywords angiotensin II; [des-Asp¹]-angiotensin II; [des-Asp¹, des-Arg²]-angiotensin II; albuminuria; rat

Angiotensin II (ANG II) has been known to increase the urinary excretion of proteins (mainly albuminuria) through increasing the transglomerular passage of macromolecular proteins.^{1–4)} This ANG II is degraded into smaller peptides and amino acids by a variety of peptidases in the body.^{5–8)} Among the fragments of ANG II, [des-Asp¹]-ANG II (ANG III) plays physiological roles in the body^{9–11)}; however, it remains to be investigated whether or not [des-Asp¹, des-Arg²]-ANG II (ANG IV, called conventionally here) is physiologically active.

We hypothesized that ANG III and ANG IV have a similar proteinuric action to ANG II, and tried to estimate the potentials of ANG II, ANG III, and ANG IV in rats.

MATERIALS AND METHODS

Materials [Ile⁵]-Angiotensin II (ANG II) and [des-Asp¹, Ile⁵]-angiotensin II (ANG III) were purchased from Protein Research Foundation (Minoh, Osaka), and [des-Asp¹, des-Arg², Ile⁵]-angiotensin II (ANG IV) from Seikagaku Kogyo (Tokyo). Male Wistar rats (Hokudo; Abuta, Hokkaido) weighing approximately 200 g were used.

General Procedures The animal was anesthetized with ether, and the left jugular vein was catheterized. This route was used for the continuous infusion of saline containing inulin; when necessary, the peptide or the anesthetic was injected through this route. Injections of urethane (500 mg/kg body weight) and α -chloralose (70 mg/kg body weight) were then given for deeper anesthesia. Ten ml/kg body weight of saline containing 1.2% inulin was injected as a prime, followed by the infusion of the same saline at a rate of 0.2 ml/kg body weight/min; an equilibration period of 50 min was allowed. The left ureter was retro-peritoneally approached and catheterized.¹²⁾ Urine was collected every 5 min before, during and after the continuous infusion of the peptide. Blood was taken from the tail vein at 15 min before and at 15 and 45 min after the commencement of the peptide-infusion. Hematuria

that might be caused artificially during the above operation, was checked out by the microscopic observation of sediments which were obtained by centrifugation of the urine. When hematuria was observed in a rat, the data obtained from that rat was excluded from the experiments.

Analytical Procedures Inulin was estimated by the anthrone method.¹³⁾ Albumin was determined by the HPLC method.¹⁴⁾ The sample for the determination of albumin was diluted properly by water, and applied onto the HPLC: column, Superose 12 HR 10/30 (Pharmacia LKB Biotechnology, Uppsala, Sweden); column temperature, ambient temperature; elution, isocratic mode; mobile phase, 20% acetonitrile in 100 mM KH₂PO₄-Na₂HPO₄ buffer (pH 7.4); flow rate, 0.6 ml/min; wave length for detection, 214 nm; injection volume, 100 μ l. The fractional clearance of albumin was calculated as shown below from the observed values, including concentrations of inulin and albumin (mg/ml) in urine (*U*) and plasma (*P*).¹⁵⁾

$$\text{fractional clearance of albumin} = (U_{\text{albumin}}/P_{\text{albumin}})/(U_{\text{inulin}}/P_{\text{inulin}})$$

Statistics Values are shown as the mean \pm S.E.M. The data were subjected to one-way analysis of variance, and subsequently to Bonferroni's method¹⁶⁾; *p* values of less than 0.05 were considered to be significant.

RESULTS

Figure 1 shows changes in the fractional clearance of albumin when ANG II, ANG III, or ANG IV was continuously infused at 0.45 nmol/kg body weight/min. For ANG II, the fractional clearance of albumin increased after the infusion commenced, peaked at the 10–15 min period, and tended to be reverted thereafter. For ANG III, the fractional clearance of albumin increased according to the infusion time elapsed, and the peak was observed at the 25–30 min period. Thereafter, it reverted to the control level. The peak values were higher in ANG II than in ANG III. For ANG IV, the fractional clearance of albumin remained within the control level.

Figure 2 shows interrelations between doses and peak

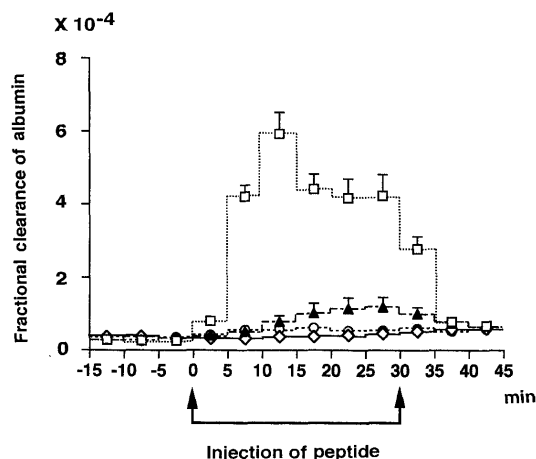


Fig. 1. Changes in the Fractional Clearance of Albumin Induced by a 30-min Period of Continuous Intravenous Infusion (0.45 nmol/kg Body Weight/min) of Angiotensin Peptide

---□---, ANG II; ---▲---, ANG III; ---◇---, ANG IV; ---○---, control. Control: saline without angiotensin peptide was infused. Points and bars, mean \pm S.E.M. Number of rats were used in an identical dose of an identical peptide was 6.

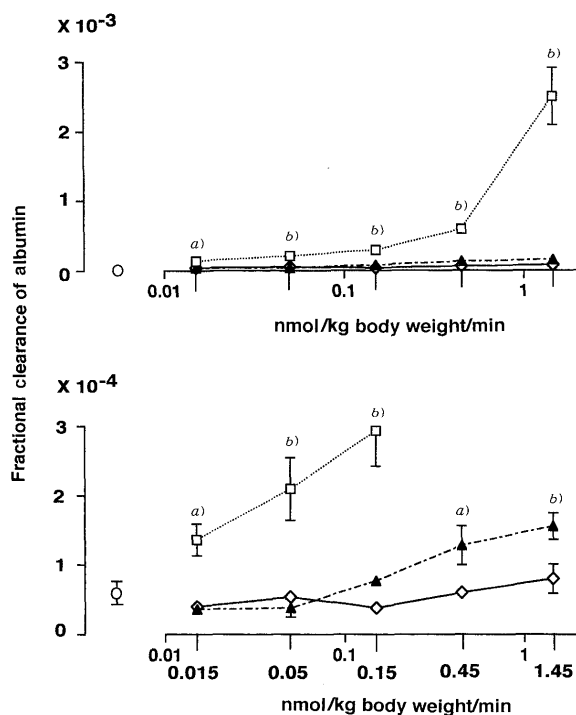


Fig. 2. Interrelation between Doses and Peak Values of the Fractional Clearance of Albumin Induced by a 30-min Period of Continuous Intravenous Infusion of Angiotensin Peptide

---□---, ANG II; ---▲---, ANG III; ---◇---, ANG IV; ---○---, control. Upper figure, $0-10^{-3}$ in Y-scale; lower figure, $0-10^{-4}$ in Y-scale. Values of the control were the maximal values during the 30-min infusion of saline instead of the peptide solution. Doses: 0.015, 0.05, 0.15, 0.45, and 1.45 nmol/kg body weight/min. Points and bars, mean \pm S.E.M. Number of rats that were used in an identical dose of an identical peptide was 6. Statistics, compared with control: a) $p < 0.05$; b) $p < 0.01$.

values in the fractional clearance of albumin when the peptide was continuously infused at 0.015 to 1.45 nmol/kg body weight/min. The values in ANG II and ANG III increased in a dose-dependent manner. Compared with the control, the value for ANG II was higher at all the doses examined, while the value for ANG III was higher at more than 0.45 nmol/kg body weight/min. The values

of ANG IV, at all the doses examined, remained within the control level.

Regarding the periods at which the fractional clearance of albumin peaked, ANG II revealed the peak-periods to be 15–30 min at doses of 0.015, 0.05, and 0.15 nmol/kg body weight/min, 10–15 min at 0.45 nmol/kg body weight/min, and 5–15 min periods at 1.45 nmol/kg body weight/min. Also, the peak-periods for ANG III were 25–30 min at doses of 0.45 nmol/kg body weight/min, and 15–25 min at 1.45 nmol/kg body weight/min.

DISCUSSION

The mechanism(s) for ANG II-induced proteinuria (albuminuria) has been explained as follows. Alterations in glomerular capillary ultrastructure, as well as in glomerular hemodynamics, are caused by the ANG II stimulation.^{1–4,17} As a result, the permeability of the glomerular basement membranes to macromolecules increased, and high-molecular proteins could pass through the glomerular basement membranes.^{1–4,17}

Our results denote that, when ANG II, ANG III or ANG IV is intravenously infused in the rat, the proteinuric potential is the highest for ANG II, moderate for ANG III, and the least (nearly at the control level) for ANG IV. The ANG III-induced proteinuria is speculated to result from the same mechanisms as the proteinuria induced by ANG II, since the receptor is considered to be identical for both ANG II and ANG III. This speculation is partially endorsed by the following facts: that the glomeruli have receptors for ANG II,^{18–21} and that ANG III competitively occupies ANG II-receptors in the glomeruli.^{18,19}

Furthermore, our study denoted that the peak-periods of the albuminuria differed by the type and dose of the peptides employed: peaks were observed earlier when the peptide administered was more potent and in a larger amount. These phenomena were surmised to result from the proteinuric ability of the peptide itself, and by the extent it occupied the glomerular receptors.

Regarding the difference in the albuminuric effects among the three peptides, our study evidently proves that the presence or the absence of Asp¹ and Arg² in the amino acid sequence of ANG II decides the potentials. Namely, the cleaving of the Asp¹ of ANG II lowers the albuminuric effect of ANG II, and the further cleaving of Asp¹ and Arg² causes a complete loss of the effect.

The glomeruli have a variety of peptidase-like activities.^{22,23} In particular, aminopeptidases including aminopeptidase A and leucine aminopeptidase cleave the N-terminal Asp¹, and, in turn, the Arg² of ANG II^{4,24,25}; these enzymes convert ANG II to ANG III, and ANG III to ANG IV. In addition, trypsin cleaves the Arg²-Val³ peptide bond^{25,26}; this enzyme directly converts ANG II to ANG IV. Accordingly, we speculate it to be possible that ANG II would be degraded to ANG III and further to ANG IV in the glomeruli, and that the transglomerular passage of albumin is regulated in combination with ANG II and its degraded fragments of ANG III, ANG IV and others. These speculations of ours remain to be investigated in the future.

Acknowledgement This research was supported by grants from the special research foundation of the Health Sciences University of Hokkaido. The authors wish to thank professor emeritus Dr. Tsuneyoshi Tanabe of the Health Sciences University of Hokkaido, who gave us many important and useful suggestions regarding this work.

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