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Distribution of Aminopeptidases in Various Nephron Segments Isolated from Rat Kidney

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The present study was undertaken to investigate the activities of aminopeptidases which were reported to degrade physiologically active peptides including angiotensins, vasopressin, oxytocin and so on, in various nephron segments isolated from the rat kidney. These peptidases include leucine aminopeptidase-, aminopeptidase A-, angiotensinase A- and cystine aminopeptidase-like enzymes. The activities of these peptidases were found to be higher in the proximal tubule than in other segments. In the proximal tubule, these peptidases showed their highest activities in the pars recta. The activities of aminopeptidase A- and angiotensinase A-like enzyme(s) were also high in the glomerulus. In contrast, the activities of these peptidases were hardly detectable in distally located nephron segments. From this distribution of the peptidases, we speculate that the above physiologically active peptides are metabolized in the proximal tubule, particularly in the pars recta, and that angiotensins are metabolized in the glomerulus in addition to the proximal tubule.

Keywords——leucine aminopeptidase; aminopeptidase A; angiotensinase A; cystine aminopeptidase; angiotensin; vasopressin; oxytocin; nephron; microdissection; rat

It is well known that the physiologically active peptides, such as angiotensin, vasopressin, oxytocin, bradykinin, *etc.*, which have vasoactive effects in the general circulatory system and which play a significant role in adjusting the balance of water and electrolytes in the kidney, are metabolized in their target organ.[1] In perfusion studies with these peptides from the renal artery, it was observed that the plasma concentration in the renal vein was lower than that in the renal artery, and that breakdown products appeared in the urine. Accordingly, the kidney is considered to be capable of degrading these peptides in circulating blood.[1]

On the basis of perfusion studies in the renal tubule employing micropuncture techniques, it has been reported that angiotensin and bradykinin are degraded and reabsorbed mainly in the proximal tubule, and not at all in the distal nephron.[2] It has also been observed that the fluid collected by perfusion in the proximal tubule contains breakdown products which are thought to have been formed by various peptidases, and it was suggested that characteristic peptidases for these peptides exist in the proximal tubule.[2,3]

Figure 1 shows a diagrammatic representation of the breakdown of angiotensin, vasopressin and oxytocin by the aminopeptidases.[3] Aminopeptidase A[3b,c] cleaves the N-terminal aspartic acid[1] of angiotensin, and leucine aminopeptidase[3a] cleaves the N-terminal amino acid of angiotensin, and then, in succession, aspartic acid,[1] arginine,[2] valine,[3] tyrosine,[4] and isoleucine.[5] Cystine aminopeptidase[3g,i] cleaves the Cys^1–Tyr^2 bond of both vasopressin and oxytocin.

The localizations of the above-mentioned aminopeptidases, however, remain to be determined, since samples of nephron collected by microdissection have been too small to assay. Thus, we have developed new micromethods to assay the activities of peptidases in each nephron segment, in order to compare the results with the above physiological data obtained by micropuncture and microperfusion techniques.

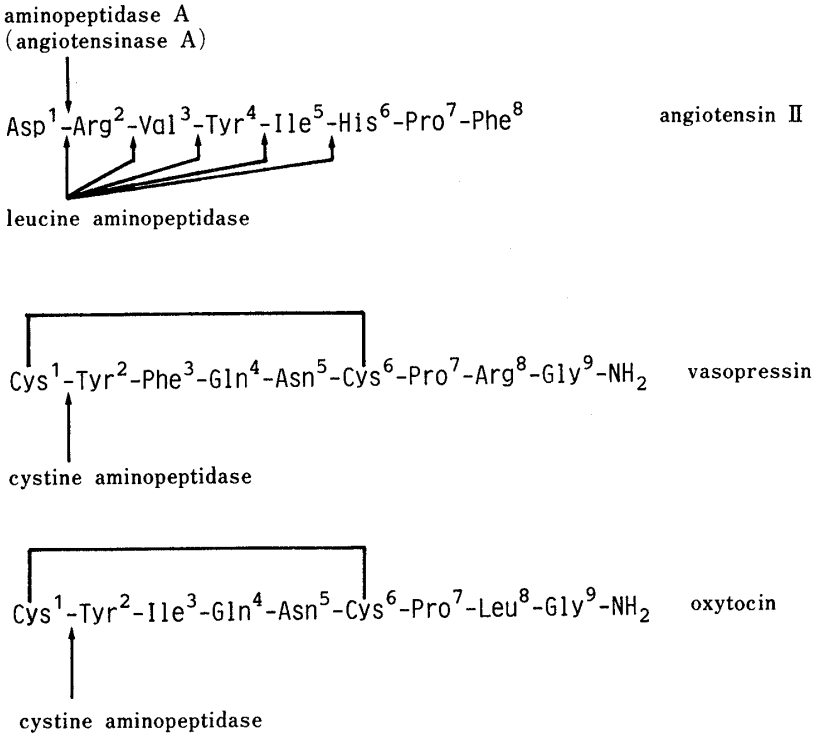

Fig. 1. A Hypothetical Scheme for the Breakdown of Angiotensin II, Vasopressin and Oxytocin by Various Aminopeptidases

Experimental

Chemicals——Hyaluronidase (bovine testes, Type 1-S), L-leucine β-naphtylamide, L-cystine di-β-naphthylamide, β-naphthylamine, Tween-20, Triton X-100, Fast Garnet GBC, oxoglutarate, β-NADH (reduced nicotinamide adenine dinuclotide), bovine serum albumin, and dextran (molecular weight, about 40000), were purchased from Sigma Chemical Co. (U.S.A.), collagenase (type 1, CLS I) from Worthington Biochemical Co. (U.S.A.), α-L-aspartic acid β-naphthylamide from Bachem Fine Chemicals Inc. (U.S.A.), angiotensin II from the Protein Research Foundation (Osaka), Eagle's MEM from Nissui Seiyaku Co. (Tokyo), and malate dehydrogenase and glutamate–oxaloacetate transaminase from Boehringer Mannheim GmbH (West Germany). All other reagents used were commercial products of the highest grade available.

Preparation of Isolated Nephron Segments——Male Wistar rats weighing 200±20 g were used for the experiments, and nephron segments were prepared with collagenase and hyaluronidase by modifying the methods of Imbert-Teboul *et al.*[4a] and Sudo and Morel.[4b] Each rat was fed a standard laboratory diet and had free access to tap water until the day of the experiment. Under pentobarbital anesthesia (50 mg/kg body weight, *i.p.*), 1 ml of a solution containing 10% mannitol and 0.45% NaCl was injected *via* the jugular vein, in order to induce osmotic diuresis and to dilate the tubules. When the polyuria was established, the left kidney was exposed and the aorta was ligated between the branching of the two renal arteries. Then, 4 ml of cold Eagle's solution (lacking kanamycin and phenol red) containing collagenase (10 mg/ml), hyaluronidase (1 mg/ml), bovine serum albumin (0.1%) and dextran (6%), was rapidly perfused *via* the abdominal aorta. After excision, the left kidney was sliced along the corticomedullary axis, and the slices were aerobically incubated for 15 min at 30 °C in the above Eagle's medium containing collagenase (0.7 mg/ml), hyaluronidase (0.5 mg/ml), bovine serum albumin (0.1%) and dextran (6%). After repeated rinsing of the slices in cold collagenase- and hyaluronidase-free modified Hanks' solution, microdissection was performed by hand under stereomicroscopic observation in the modified Hanks' solution at 4 °C. The composition of this modified Hanks' solution was as follows (mM): NaCl, 115; KCl, 5; $MgSO_4$, 0.8; Na_2HPO_4, 0.33; KH_2PO_4, 0.44; $MgCl_2$, 1; $NaHCO_3$, 4; $CaCl_2$, 1; D-glucose, 5; Na acetate, 10; HEPES, 20; the pH was adjusted to 7.4 with 1 N NaOH.

In this study, the activities of the aminopeptidases were measured using artificial substrates, and the activities were represented as U per length as well as U per protein content. In the assay of enzyme activities per protein content, a large quantity of identical nephron segments was gathered and homogenized, and then the protein content and enzyme activity were measured. In the assay of enzyme activities per length, photographs were taken to measure the length of the nephron segment(s), the segment(s) was aspirated into a capillary pipet, and then a constant volume of 2% Triton X-100 detergent, was aspirated. Next, the detergent solution was passed into a fixed volume of the

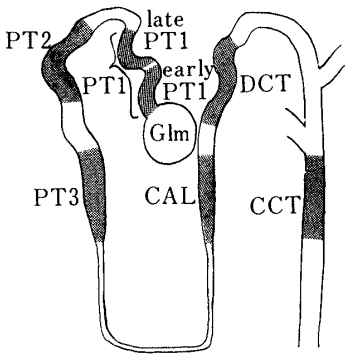

Fig. 2. Nephron Segments Employed for This Study

Abbreviations of nephron segments are as follows. Glm, glomerulus; PT1 (early PT1, late PT1), segment 1 of proximal tubule; PT2, segment 2 of proximal tubule; PT3, segment 3 of proximal tubule; CAL, cortical thick ascending limb of Henle's loop; DCT, distal convoluted tubule; CCT, cortical collecting tubule. The details are given in Experimental.

incubation medium without the substrate, and the medium was divided equally into two parts for measurement of the enzyme activity: one as a sample, and the other as a blank.

In the measurement of the protein content per length, photographs were taken, the nephron segment(s) was aspirated into the capillary pipet, and then a constant volume of alkaline copper reagent[5] was aspirated. The solution was then passed into a fixed volume of alkaline copper reagent.

Each nephron segment used for this experiment consisted of the following 7 (or 8) portions[4a,6] (Fig. 2). (1) Glomerulus (Glm). (2) Proximal convoluted tubule (PT1): early PT1 was within 2 mm of the proximal tubule from the glomerulus, late PT1 was a further 2 mm from the early PT1, and thus PT1 was within 4 mm from the glomerulus. (3) The transitional portion (PT2) from the pars convoluta to the pars recta of the proximal tubule, which contained mainly the upper portion of the pars recta, about 1 mm. (4) The terminal portion of the pars recta of the proximal tubule (PT3), about 1 mm. (5) Cortical thick ascending limb of Henle's loop (CAL). (6) The distal convoluted tubule (DCT), which has been defined as the portion extending from the macula densa up to the first branching (it includes several distinct segments).[4a] In this study, the assay was done in the DCT as a whole, about 1 mm from the macula densa, without distinction between the component parts. (7) Cortical collecting tubule (CCT), about 1 mm.

Measurements of Activities of Leucine Aminopeptidase-, Aminopeptidase A- and Cystine Aminopeptidase-Like Enzymes——These three enzyme activities were measured by micromodifications of the methods of Oya *et al.*,[7] Nagatsu *et al.*[3c] and Appel,[3i] respectively, involving diazotization of β-naphthylamine.

Leucine Aminopeptidase-Like Enzyme(s)——L-Leucine β-naphthylamide was used as the substrate; the volume of the incubation medium[7] was 45 μl; protein content, about 200 ng; incubation was carried out at 37 °C for 30 min.

Aminopeptidase A-Like Enzyme(s)——α-L-Aspartic acid β-naphthylamide was used as the substrate; the volume of the incubation medium[3c] was 30 μl; protein content, about 300 ng; incubation was carried out at 37 °C for 60 min.

Cystine Aminopeptidase-Like Enzyme(s)——L-Cystine di-β-naphthylamide was used as the substrate; the volume of the incubation medium[3i] was 30 μl; protein content, about 1500 ng; incubation was carried out at 37 °C for 3 h.

In the measurement of each enzyme, the incubation was stopped by adding a solution of 10% Tween-20 and 0.015% Fast Garnet GBC in 1 M acetate buffer (pH 4.2): 15 μl for leucine aminopeptidase-like enzyme(s), 10 μl for aminopeptidase A- and cystine aminopeptidase-like enzyme(s). After 30 min, the absorbance was measured at 525 nm, and activity was calculated from a standard curve made using β-naphthylamine.

Measurement of Activities of Angiotensinase A-Like Enzyme(s)——The term "aminopeptidase A" has been sometimes used with the same meaning as "angiotensinase A."[3b,c] In this study, however, for the sake of convenience, aminopeptidase A-like enzyme(s) was defined as the peptidase whose substrate is α-L-aspartic acid β-naphtylamide, and angiotensinase A-like enzyme(s) as that whose substrate is angiotensin II. The activity of angiotensinase A-like enzyme(s) was measured by a micromodification of the method of Oelkers and Goldacker,[8] with increased sensitivity. With angiotensin II as the substrate, the amount of aspartic acid cleaved from angiotensin II was measured. The volume of the incubation medium[8] was 40 μl, the protein content was about 400 ng, and the incubation was carried out at 37 °C for 4 h. After the incubation, 10 μl of 63 mM acetic acid was added, and the mixture was boiled for 5 min, then centrifuged. Next, 30 μl of the supernatant was transferred into another microtube, and 35 μl of 0.1 M phosphate buffer (pH 7.2) containing 5 mM oxoglutarate, 0.32 mM β-NADH, and 0.16 mg/ml of malate dehydrogenase was added. The mixture was allowed to stand for 10 min, and the absorbance (E1) was read at 340 nm. Then, 10 min later, 1 μl of 2 mg/ml glutamate–oxaloacetate transaminase was added, and the absorbance (E2) was read at 340 nm. The activity of angiotensinase A-like enzyme(s) was calculated by subtracting E2 from E1.

Measurement of Protein Contents——The protein content of each sample was measured with a micromodification of the method of Lowry *et al.*[5] The final volume was 26 μl. Bovine serum albumin was used as a standard. In the above measurements, absorbance was measured using a microcuvette adapted for the Hitachi-320 spectrophotometer (Hitachi Ltd., Tokyo).

Statistics——Results are given as means ± S.E. Statistical significance was assessed by means of Student's *t* test; *p* values of less than 0.05 were considered significant.

Results

Table I shows the protein content per length of various tubular segments and per glomerulus. The protein content of one glomerulus was about 76 ng. While the proximal tubule showed an increase from the pars convoluta up to the upper portion of the pars recta [early PT1 ≒ late PT1 (N.S.), late PT1 < PT2 ($p<0.01$)], it showed a decrease from the upper portion to the terminal portion of the pars recta [PT2 > PT3 ($p<0.001$)]; the upper portion of the pars recta showed the highest protein content. In the distally located nephron segments (CAL, DCT, CCT), the distal convoluted tubule showed the highest protein content

TABLE I. Protein Content per Length or per Glomerulus in Various Nephron Segments

Nephron segment	Protein content per mm of tubular length or per glomerulus (ng/mm or glomerulus) mean ± S.E.
Glm	76 ± 3.8
Early PT1	233 ± 8.9
Late PT1	232 ± 6.3
PT2	282 ± 11.6
PT3	179 ± 15.3
CAL	77 ± 5.3
DCT	129 ± 9.2
CCT	100 ± 8.8

Values represent the means ± S.E. The number of experiments for each nephron segment was 10. Abbreviations of nephron segments are as in Fig. 2. Statistics (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$): Glm < early PT1***, Glm < late PT1***, Glm < PT2***, Glm < PT3***, Glm < DCT***, Glm < CCT*, early PT1 < PT2**, early PT1 > PT3**, early PT1 > CAL***, early PT1 > DCT***, early PT1 > CCT***, late PT1 < PT2**, late PT1 > PT3**, late PT1 > CAL***, late PT1 > DCT***, late PT1 > CCT***, PT2 > PT3***, PT2 > CAL***, PT2 > DCT***, PT2 > CCT***, PT3 > CAL***, PT3 > DCT***, PT3 > CCT***, CAL < DCT***, CAL < CCT*, DCT > CCT*.

TABLE II. Activities per Protein Content of Leucine Aminopeptidase-, Aminopeptidase A-, Angiotensinase A- and Cystine Aminopeptidase-like Enzyme(s) in Each Nephron Segment

Nephron Segment	Activity per protein content (U/g·protein)			
	Leucine aminopeptidase-like enzyme(s)	Cystine aminopeptidase-like enzyme(s)	Aminopeptidase A-like enzyme(s)	Angiotensinase A-like enzyme(s)
Glm	9 ± 3.1	0.25 ± 0.090	16.7 ± 2.50	23.8 ± 4.94
PT1	44 ± 3.4	0.39 ± 0.082	7.4 ± 1.37	17.6 ± 2.66
PT2	172 ± 14.5	0.74 ± 0.095	24.6 ± 3.00	33.1 ± 6.42
PT3	261 ± 7.2	1.13 ± 0.068	10.2 ± 2.08	23.3 ± 5.34
CCT	9 ± 3.8	0.25 ± 0.053	1.0 ± 1.09	1.8 ± 1.31

The number of experiments in each nephron segment was 5. Other details are given in Table I. Statistics (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). Leucine aminopeptidase-like enzyme(s): Glm < PT1***, Glm < PT2***, Glm < PT3***, PT1 < PT2***, PT1 < PT3***, PT1 > CCT***, PT2 < PT3***, PT2 > CCT***, PT3 > CCT***. Cystine aminopeptidase-like enzyme(s): Glm < PT2**, Glm < PT3***, PT1 < PT2*, PT1 < PT3***, PT2 < PT3*, PT2 > CCT**, PT3 > CCT***. Aminopeptidase A-like enzyme(s): Glm > PT1*, Glm > CCT*, PT1 < PT2***, PT1 > CCT**, PT2 > PT3**, PT2 > CCT***, PT3 > CCT**. Angiotensinase A-like enzyme(s): Glm > CCT**, PT1 > CCT***, PT2 > CCT**, PT3 > CCT**.

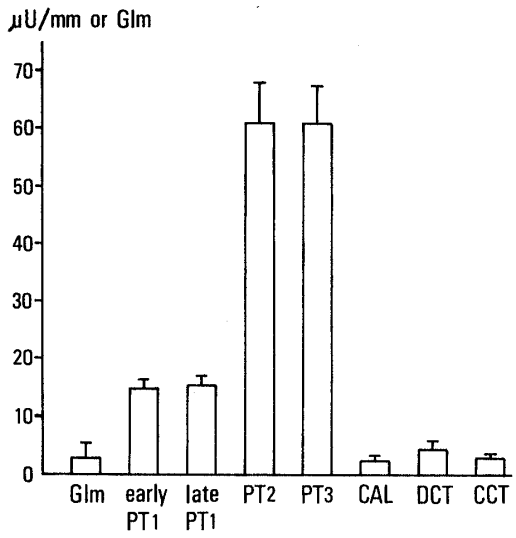

Fig. 3. Activity of Leucine Aminopeptidase-Like Enzyme(s) in Each Nephron Segment

Vertical bars represent the means ± S.E. The number of experiments for each nephron segment was 10. Abbreviations of nephron segments are as in Fig. 2. Statistical analyses were carried out only among the tubular segments excluding the glomerulus (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$): early PT1 < PT2***, early PT1 < PT3***, early PT1 > CAL***, early PT1 > DCT***, early PT1 > CCT***, late PT1 < PT2***, late PT1 < PT3***, late PT1 > CAL***, late PT1 > DCT***, late PT1 > CCT***, PT2 > CAL***, PT2 > DCT***, PT2 > CCT***, PT3 > CAL***, PT3 > DCT***, PT3 > CCT***.

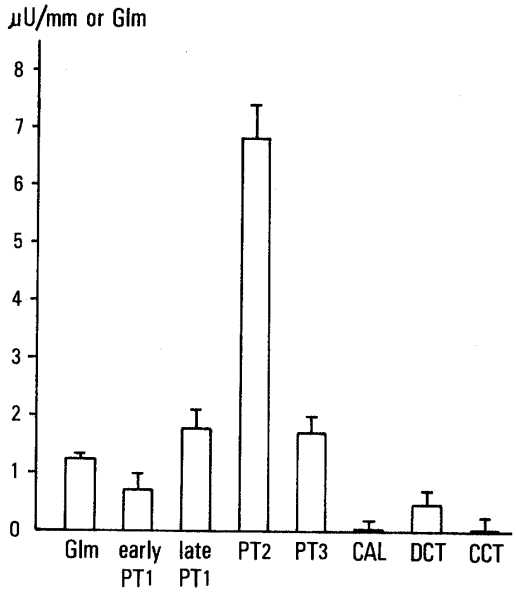

Fig. 4. Activity per Length or per Glomerulus of Aminopeptidase A-like Enzyme(s) in Each Nephron Segment

The number of experiments in each nephron segment was 10. Other details are as in Fig. 3. Statistics are as in Fig. 3: early PT1 > late PT1*, early PT1 < PT2***, early PT1 < PT3**, early PT1 > CAL**, early PT1 > CCT*, late PT1 < PT2***, late PT1 > CAL***, late PT1 > DCT**, late PT1 > CCT***, PT2 > PT3***, PT2 > CAL***, PT2 > DCT***, PT2 > CCT***, PT3 > CAL***, PT3 > DCT**, PT3 > CCT***.

[CAL < DCT ($p<0.001$), DCT > CCT ($p<0.05$)]. Although the cortical collecting tubule was thick in appearance, its protein content was low.

Figures 3—6 and Table II show the activity of each enzyme in various nephron segments. In these figures excluding Fig. 6, the enzyme activity values per length of each tubular segment and per glomerulus were investigated in the whole portions as in Fig. 2, while the enzyme activity values per protein content were investigated only in five portions (Glm, PT1, PT2, PT3, and CCT), because relatively more nephron pieces were necessary to measure both protein amount and enzyme activity. In addition to this, it was very difficult to collect large amounts of DCT and CAL in the rat, and also to measure the exact length of large amounts of tubular segments.

Figure 3 and Table II show the activity of leucine aminopeptidase-like enzyme(s) in each nephron segment. The activity was higher in the proximal tubule than in other segments. In terms of activity per protein content, the proximal tubule showed an increase from the pars convoluta to the terminal portion of the pars recta [PT1 < PT2 ($p<0.001$), PT2 < PT3 ($p<0.001$)]. In terms of activity per length, the proximal tubule showed an increase from the pars convoluta to the pars recta [early PT1 ≒ late PT1 (N.S.), late PT1 < PT2 ($p<0.001$)]. In the pars recta, both the upper portion and the terminal portion showed equally high activity. The glomerulus and the distally located nephron segments (CAL, DCT, CCT) showed very low activities, and there were no significant differences among the distally located nephron segments.

Figure 4 and Table II show the activity of aminopeptidase A-like enzyme(s) in each

nephron segment. In terms of the activity per protein content, the glomerulus showed relatively high activity [Glm > PT1 ($p < 0.05$)]. The proximal tubule showed an increase from the pars convoluta up to the upper portion of the pars recta [PT1 < PT2 ($p < 0.001$)], but showed a decrease from the upper portion to the terminal portion of the pars recta [PT2 > PT3 ($p < 0.01$)]; the upper portion of the pars recta showed the highest activity. In terms of activity per length, the same tendency was recognized in the proximal tubule [early PT1 < late PT1 ($p < 0.05$), late PT1 < PT2 ($p < 0.001$), PT2 > PT3 ($p < 0.001$)]; the upper portion of the pars recta showed the highest activity. The activities in the distally located nephron segments (CAL, DCT, CCT) were very low, and no significant differences were found.

Leucine aminopeptidase and aminopeptidase A possess the ability to degrade the N-terminal aspartic acid[1] of angiotensin, as mentioned in the introduction. However, leucine aminopeptidase- and aminopeptidase A-like enzymes showed different distributions from each other, particularly in the glomerulus. In order to clarify the distribution of ability to degrade the N-terminal aspartic acid[1] of angiotensin II, we investigated the distribution of angiotensinase A-like enzyme(s) using angiotensin II as the substrate, in each nephron segment, particularly in the glomerulus.

Figure 5 and Table II show the activity of angiotensinase A-like enzyme(s) in each nephron segment. In terms of activity per protein content, the glomerulus and the whole proximal tubule showed high activities, while no significant differences were recognized among Glm, PT1, PT2 and PT3. In terms of activity per length, the proximal tubule showed an increase from the pars convoluta up to the upper portion of the pars recta [early PT1 ≒ late PT1 (N.S.), late PT1 < PT2 ($p < 0.01$)], and showed a decrease from the upper portion to the terminal portion of the pars recta [PT2 > PT3 ($p < 0.001$)]. The distally located nephron segments (CAL, DCT, CCT) showed very low activities, and no significant differences were found among them.

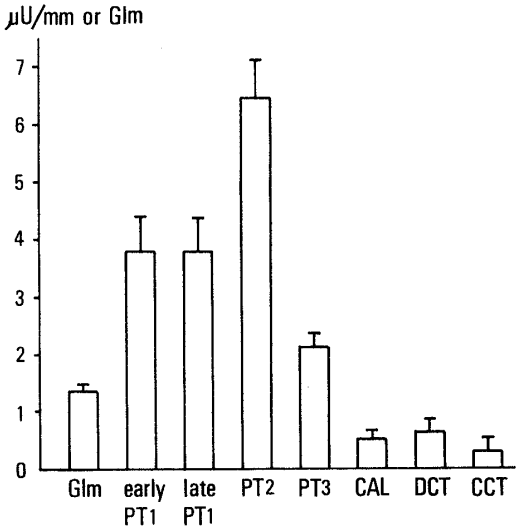

Fig. 5. Activity per Length or per Glomerulus of Angiotensinase A-Like Enzyme(s) in Each Nephron Segment

The number of experiments in each nephron segment was 10. Other details are as in Fig. 3. Statistics are as in Fig. 3: early PT1 < PT2**, early PT1 > PT3*, early PT1 > CAL***, early PT1 > DCT***, early PT1 > CCT***, late PT1 < PT2**, late PT1 > PT3*, late PT1 > CAL***, late PT1 > DCT***, late PT1 > CCT***, PT2 > PT3***, PT2 > CAL***, PT2 > DCT***, PT2 > CCT***, PT3 > CAL***, PT3 > DCT***, PT3 > CCT***.

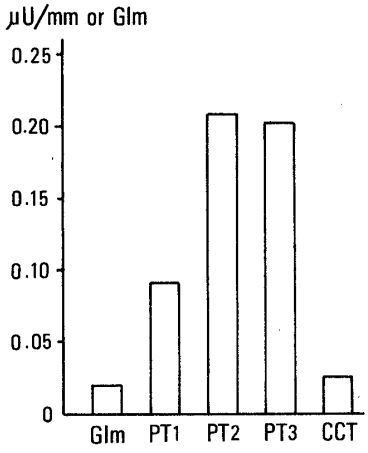

Fig. 6. Activity per Length or per Glomerulus of Cystine Aminopeptidase-Like Enzyme(s) in Each Nephron Segment

The values of activity per length or per glomerulus were calculated from the mean values of the activity per protein content (Table II) and from those of the protein content per length or per glomerulus (Table I). Vertical bars represent the means. Other details are as in Fig. 3.

In the collection of nephron segments in the rat, it was very difficult to collect a large amount of the distal convoluted tubule (DCT) and the cortical thick ascending limb of Henle's loop (CAL). Accordingly, in the measurement of cystine aminopeptidase-like enzyme(s), which required a large amount of nephron segments for assay, the activity per protein content was assayed in the following five portions (Glm, PT1, PT2, PT3, CCT) which could be collected relatively easily, and the activities per length and per glomerulus (Fig. 6) were calculated from the protein content per length and per glomerulus (Table I) and from the activity per protein content (Table II).

Figure 6 and Table II show the activity of cystine aminopeptidase-like enzyme(s) in each nephron segment. In terms of activity per protein content, the proximal tubule showed an increase from the pars convoluta to the terminal portion of the pars recta [PT1 < PT2, ($p < 0.05$), PT2 < PT3 ($p < 0.05$)], with the activity of the glomerulus and the cortical collecting tubule being very low. In terms of activity per length, the proximal tubule showed an increase from the pars convoluta to the pars recta; cystine aminopeptidase-like enzyme(s) showed a distribution pattern similar to that of leucine aminopeptidase-like enzyme(s).

Discussion

In obtaining various nephron segments from the rat kidney, the method of Imbert-Teboul *et al.*[4a] has usually been used, though an improved method was recently established by Sudo and Morel.[4b] This new method was established for measuring intracellular Na and K in the nephron segments, and is considered to be the best among the methods available at present, because the intracellular electrolytes are reported to be well maintained in spite of the collagenase treatment. Accordingly, with this new method, the enzymes in the cells are considered to be retained without significant loss; minimization of the leakage of enzymes from the cells of isolated nephron segments during maceration and microdissection processes is clearly of crucial importance for studies on the enzyme distributions along the nephron. We have further modified this method, and the enzymatic distributions along the rat nephron were investigated by using this new method.

In this study, the activities of various aminopeptidases were investigated in isolated nephron segments from the rat kidney, though purification of individual enzymes from the nephron homogenate was not carried out. In addition, the specificities for the substrates employed were not characteristic of each aminopeptidase.[3,8,11] Accordingly, we have used the term "-like enzyme(s)" in naming the enzymes studied in this study.

The distributions of the peptidases studied in this paper can be summarized as follows. (1) The peptidases which showed high activities in the glomerulus were aminopeptidase A- and angiotensinase A-like enzyme(s). (2) The peptidases which showed the highest activity in the pars recta of the proximal tubule were leucine aminopeptidase-, aminopeptidase A-, angiotensinase A- and cystine aminopeptidase-like enzyme(s). (3) The activities of the above-mentioned peptidases were all very low in the distally located nephron segments (CAL, DCT, CCT).

With respect to the distributions of the above-mentioned peptidases, when further detailed investigations were done in 3 (or 4) portions of PT1 (early PT1, late PT1), PT2, and PT3 of the proximal tubule, differences in the patterns of the distribution were recognized. Aminopeptidase A[9a] and leucine aminopeptidase,[9a] which were reported to be peptidases of the brush border membrane of the proximal tubule, showed particular differences in the patterns of distribution.

Maunsbach[6] reported that differences exist in the cell structure, particularly concerning the morphological structure of the brush border membrane, among the three portions of the proximal tubule (PT1, PT2, PT3) in his histological study of the nephron. Accordingly, the

differences in the distributions of the above-mentioned peptidases of the brush border membrane of the proximal tubule, might be due to both quantitative and qualitative heterogeneity ("intra-nephron heterogeneity"[10]) of the brush border membrane of the proximal tubule. Such differences might well result in heterogeneity in the metabolism of physiologically active peptides.

Smith *et al.*[11] reported in their study on the hydrolysis of various amino acid amides by leucine aminopeptidase that the degree of hydrolysis of L-aspartic acid amide was only 2.9% based on L-leucinamide as 100%; the N-terminal aspartic acid[1] of angiotensin II is resistant to hydrolysis by leucine aminopeptidase relative to other constituent amino acid amides of angiotensin II. Accordingly, leucine aminopeptidase may further hydrolyze fragment peptides formed by various peptidases to the constituent amino acids.[11]

In the glomerulus, the activity of leucine aminopeptidase-like enzyme(s) was extremely low. Accordingly, the hydrolysis of N-terminal aspartic acid[1] of angiotensin II might be due to aminopeptidase A- and angiotensinase A-like enzyme(s) localized markedly in the glomerulus.

In the distally located nephron segments (CAL, DCT, CCT), the activities of the above-mentioned peptidases were very low. The activities of leucine aminopeptidase-, aminopeptidase A- and angiotensinase A-like enzyme(s) in the distal convoluted tubule, however, were all higher than in the other segments, although the differences were not significant. The distal convoluted tubule can be subdivided into several segments,[3a] but was assayed as a whole in this study. Accordingly, a more detailed analysis might provide interesting results.

Microinjection and microperfusion studies of the renal tubule of the rat have been mainly done *in vivo* (*in situ*) using micropuncture techniques, and it was reported that angiotensin II was degraded and reabsorbed exclusively in the proximal tubule, and not at all in the distally located tubule.[2] Angiotensin II has been suggested to be degraded by peptidases, including leucine aminopeptidase, aminopeptidase A, angiotensinase A and other peptidases, which were reported to be present in the brush border membrane.[2a,b,9]

In these studies of the rat *in vivo* (*in situ*) using micropuncture techniques, however, information was obtained only from parts of the superficial proximal tubule or distal tubule, while little information was obtained regarding the pars recta of the proximal tubule and other nephron segments. Nevertheless, we suggest that both the glomerulus and the proximal tubule play a significant role in degrading angiotensin II, and that the pars recta of the proximal tubule possesses the highest degrading ability for angiotensin II.

In contrast, it has been reported that the degradation of vasopressin and oxytocin in the nephron is very low as compared with that of angiotensin II and bradykinin.[1d,e,2b] The difference in degradation rates between these two groups of peptides is considered to be due to their molecular structures, since angiotensin II and bradykinin are linear molecules, whereas vasopressin and oxytocin contain a disulfide bond (Fig. 1). In the present investigation on the hydrolysis of amino acid β-naphthylamides (Figs. 3, 4 and 6 and Table II), cystine di-β-naphthylamide showed the strongest resistance to hydrolysis by aminopeptidases located in the nephron segment.

In conclusion, we speculate that the physiologically active peptides are metabolized in the proximal tubule, particularly in the pars recta of the proximal tubule, and that angiotensins are metabolized in the glomerulus in addition to the proximal tubule.

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