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A Method To Evaluate the Renin−Angiotensin System in Rat Renal Cortex Using a Microdialysis Technique Combined with HPLC-Fluorescence Detection

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A microdialysis (MD) technique, combined with HPLC-fluorescence (FL) detection, was developed for the evaluation of the tissue-specific renin−angiotensin system (RAS) in the rat renal cortex. An MD probe constructed with a hydrophilic hollow fiber dialysis tubing, AN69, showed high recovery (more than 50%) in vitro for all four angiotensins: angiotensin I (Ang I), Ang II, Ang III, and Ang (1−7). Angiotensins, successfully derivatized with *m*-BS-ABD-F, a water-soluble fluorogenic reagent that has a 2,1,3-benzoxadiazole (benzofurazan) structure, could be simultaneously determined by coupled-column HPLC. The detection limit for Ang I, Ang II, Ang III, and Ang (1−7) were 94, 44, 47, and 83 fmol, respectively. All these peptides were determined with good linearity (0.0125−3.1 μM, equivalent to 0.25−62 pmol, correlation coefficient >0.99) and good precision (recovery >91%). In the MD studies, generation of Ang (1−7) and Ang II was observed when Ang I was perfused, and Ang (1−7) was the major biologically active angiotensin found in the dialysate samples. The concentration of Ang (1−7) and Ang II in the dialysate samples showed good correlation to that of Ang I in a MD perfusate (20−100 μM). Cleavage of Ang I to Ang (1−7) was drastically suppressed by the co-perfusion of phoshoramidon (0.5−5 mM), an inhibitor of neprilysin, which generates Ang (1−7) from Ang I. These results are consistent with the previously reported characteristics of tissue-specific renal RAS, suggesting that our MD/HPLC-FL system may have the potential to be employed to evaluate tissue-specific RAS in the rat renal cortex.

The kidney plays a central role in the long-term control of arterial pressure by regulating the sodium level and extracellular fluid volume. It is well known that the tissue-specific renal kinin−kallikrein system and renin−angiotensin system (RAS) generate biologically active peptides that regulate body fluid volume, bradykinin,[1−3] and angiotensins,[4,5] respectively. Therefore, determinations of these peptides are quite important to elucidate the mechanism behind the long-term blood pressure control in the kidney.

Previously, we developed water-soluble fluorogenic derivatization reagents that has a 2,1,3-benzoxadiazole (benzofurazan) structure[6] and reported that 3-(7-fluoro-2,1,3-benzoxadiazole-4-sulfonamido)benzenesulfonic acid (*m*-BS-ABD-F) was successfully applied in the highly sensitive fluorescence detection of bradykinin in rat urine, using the coupled-column HPLC-fluorescence (FL) detection.[7] This method could be useful to distinguish small peptides, which are difficult to determine immunologically due to cross-reactions.

In the next step of our study, we tried to establish an analytical method for angiotensins processed by the RAS. Indeed, there are some difficulties in the evaluation of this endocrine system. First, many studies in the past decade have shown that not only angiotensin II (Ang II) but also several other peptides generated by cleavage of Ang I or Ang II have a variety of biological actions.[8,9] In particular, Ang (1−7), first characterized by Ferrario et al.[10] has been considered to play a significant role in the kidney.[11−13] Therefore, the ratio of the levels of these peptides, or in other words, the balance of the activities of enzymes participating in the cleavage of angiotensins, could be an important parameter. To understand the function of RAS in the kidney, simultaneous

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determinations of angiotensins are indispensable. Second, because these peptides are quite rapidly degraded by a series of enzymes,[14,15] the sampling procedure should be carefully chosen to accurately determine the amount of peptides in the desired area.

To resolve these difficulties, we employed a microdialysis (MD) technique. MD is well known as an effective method for collecting analytes from desired local areas of tissues[16,17] and has been applied to assay various kinds of animal and human tissues, including the kidney.[18−21] MD has also been applied to studies of localized in vivo metabolic events.[22,23] In this study, we attempted to evaluate the enzyme activity of RAS in the rat renal cortex by perfusing Ang I, the crucial substrate of RAS, and collecting angiotensins generated by the enzymes of tissue-specific RAS using an MD probe. It has been reported that proximal tubule cells produce a substantial amount of angiotensinogen and Ang I into proximal tubules[24] and that biologically active angiotensins are found in the proximal tubule fluid and glomerular filtrate at a concentration ∼1000-fold higher than that in the systemic circulation.[25,26] Furthermore, Ang I and Ang II levels in the tubular fluid are much higher than the overall average levels in the kidney.[27] These facts suggest that the evaluation of RAS in the renal cortex, where glomerular cells and proximal tubules are present, greatly helps to elucidate the control mechanism of the body fluid volume and sodium balance. Angiotensins were derivatized with *m*-BS-ABD-F and determined with a coupled-column HPLC with a size exclusion column and an ODS column.

MATERIALS AND METHODS

Chemicals. Ang (1−7) was purchased from Bachem (Bubendorf, Switzerland). All other peptides and phosphoramidon were obtained from Peptide Institute (Osaka, Japan). Captopril was purchased from Wako Pure Chemical Industries (Osaka, Japan).

3-(7-Fluoro-2,1,3-benzoxadiazole-4-sulfonamido)benzenesulfonic acid (*m*-BS-ABD-F) was synthesized according to a method previously reported.[6] Distilled water was further purified with a Milli-Q system (Japan Millipore, Tokyo, Japan). Acetonitrile obtained from Kanto Chemicals Co. (Tokyo, Japan) was of HPLC grade. All other chemicals were of analytical reagent grade.

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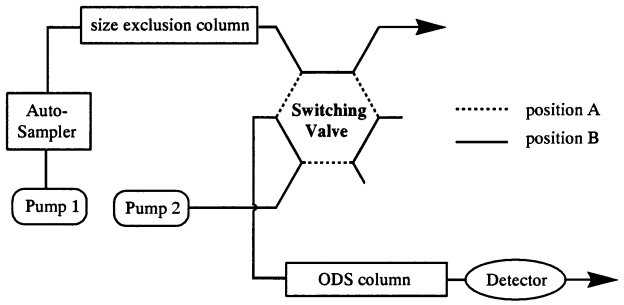

Figure 1. Diagram of the coupled-column HPLC for the determination of angiotensins.

Apparatus. A diagram of the coupled-column HPLC system is shown in Figure 1. This system consists of two pumps, an L-6200 and an L-7100 pump (Hitachi, Tokyo, Japan), an AS-8020 autosampler (Tosoh, Tokyo, Japan), a CO-965 column oven (Jasco, Tokyo, Japan), an FP-1520S fluorescence detector (Jasco), an HV-992-01 six-port valve (Jasco), and an 807-IT integrator (Jasco). The fluorescence detector was set at 426 and 564 nm for the excitation and emission wavelengths, respectively. Two different kinds of column, a TSKgel G2000 SW_{XL} (250 × 7.5 mm i.d., 5 μm, Tosoh) and a TSKgel Super ODS (100 × 4.6 mm i.d., 2 μm, Tosoh), were connected with the six-port valve. Sample solutions were injected through the autosampler, which is equipped with a 500-μL loop, into the TSKgel G2000 SW_{XL}, and the fraction that contained the derivatized peptides was introduced into the second column, TSKgel Super ODS, by changing the valve position.

In the microdialysis study, a syringe pump (microinjection pump CMA/100, CMA/Microdialysis, Stockholm, Sweden) was used for the solvent perfusion. We constructed an MD probe as described below, using a hollow fiber dialysis tubing (AN69, 0.22-mm i.d.) kindly provided by Hospal (Tokyo, Japan). Two 25-cm-long fused-silica tubings (0.075-mm i.d., Eicom, Kyoto, Japan) were inserted into both ends of a 15-mm-long AN69 tubing and were fixed by epoxy glue so that the distance between the ends of the fused-silica tubes was 8 mm. The molecular mass cutoff limit of an AN69 membrane is ∼10 000 Da. We also used an MD probe purchased from Bioanalytical Systems (5 mm in length) for comparison with the AN69 equipment.

HPLC Conditions for the Determination of Angiotensins Derivatized with *m*-BS-ABD-F. The column temperature was controlled at 35 °C. The mobile phases for each pump were as follows: pump 1 (A) water/CH_3CN/TFA (980/20/0.5) and (B) water/CH_3CN/TFA (800/200/0.5); and pump 2 (A) water/CH_3CN/TFA (900/100/0.5), (B) water/CH_3CN/TFA (700/300/0.5), and (C) water/CH_3CN/TFA (250/750/0.5). The gradient elution profile for each pump and the time program for the coupled-column HPLC are shown in Table 1. In this program, angiotensin derivatives that eluted from 15 to 18.8 min from the first column were introduced into the second column and were separated. The derivatized peptides were fluorometrically detected.

Standard Procedure for Animal Operation and MD. Male SPF Sprague−Dawley rats were purchased from Charles River Japan Inc. (Kanagawa, Japan) and were housed in an environmentally controlled room with free access to tap water and diet (CE-2, Clea Japan, Tokyo, Japan) for at least 5 days before use. Nine−eleven-week-old rats, weighing 320−410 g, were used in the present study.

Table 1. Gradient Elution Profile and Time Program for Coupled-Column HPLC

Time (min)		*0*	*15*	*18.8*	*20*	*21*	*35*		
Mobile phase for Pump 1	%A	50			50	0	0		
	%B	50			50	100	100		
Total flow rate (ml/min)		0.6			0.6	1.0	1.0		
Valve position		B	A		B				
Time (min)		*0*	*Sample introduction*		*20*	*25*	*35*	*35.5*	*44*
Mobile phase for Pump 2	%A	60			60	25	0	0	0
	%B	40			40	75	100	0	0
	%C[a]	0			0	0	0	100	100
Total flow rate (ml/min)		1.0			1.0	1.0	1.0	1.0	1.0

[a] Mobile phase C was used only for washing.

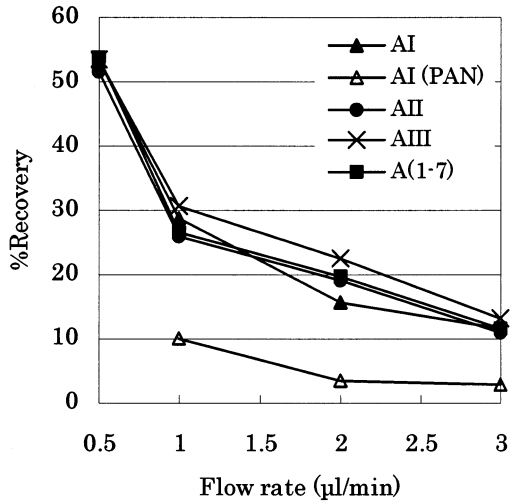

Figure 2. In vitro recoveries of angiotensins from the MD probes. Recovery of Ang I (▲), Ang II (●), Ang III (×), and Ang (1−7) (■) from AN69 (8 mm) and that of Ang I (△) from PAN (5 mm) was determined. The MD probes were immersed in the peptide mixture (5 μM each of angiotensins in PB-R) and perfused with PB-R at a flow rate of 0.5, 1, 2, or 3 μL/min, respectively. Amino acid sequence of peptides: Ang I, DRVYIHPFHL; Ang II, DRVYIHPF; Ang III, RVYIHPF; Ang (1−7), DRVYIHP.

The rats were anesthetized with an intraperitoneal administration of sodium pentobarbital dissolved in saline (50 mg/kg). A heating pad was placed beneath the rats to maintain the body temperature. After a midline abdominal incision, the left kidney was exposed, and the renal capsule was penetrated with a 27-gauge needle, 1−2 mm from the renal surface. One end of the MD probe was tunneled into the needle and was pulled out together with the needle so that only the probe was left implanted in the kidney. After stopping the bleeding, the position of the probe was adjusted so that the dialysis membrane portion was completely buried in the tissue; the probe was fixed with Integran (Nihon-zouki, Osaka, Japan) with glue (Vetbond, 3M, Tokyo, Japan). The outflow tubing was cut to ∼10 cm, and the inflow tubing was connected to a gastight syringe. The probe was perfused at 0.5 μL/min with Ringer's solution that contained 2 mM sodium phosphate buffer, pH 7.0 (PB-R). After preperfusion for 1 h with PB-R, the MD studies were conducted. The system was equilibrated for 30 min whenever the perfusate was changed.

Standard Procedure for Fluorescence Derivatization of Angiotensins with *m*-BS-ABD-F. Siliconized polypropylene tubes (1.5 mL, Assist, Tokyo, Japan) were used for sample collection and the derivatization reaction of the present study.

A 7.5-μL aliquot of an MD sample was mixed with 30 μL each of *m*-BS-ABD-F solution in 95% acetonitrile (20 mM, *m*-BS-ABD-F solution) and 0.2 M sodium phosphate buffer that contained 5 mM EDTA, pH 7.0 (0.2 M PB/EDTA). The mixture was reacted at 70 °C for 2 h and was diluted to 90 μL with 40% acetonitrile.

A 30-μL aliquot of peptide standard mixture that contained 0.25 μM each of Ang I, Ang II, Ang III, and Ang (1−7) in 0.2 M PB/EDTA, equivalent to 7.5 pmol of each peptide, was mixed with 30 μL of *m*-BS-ABD-F solution and 7.5μL of PB-R. The mixture was reacted at 70 °C for 2 h and was diluted to 90 μL with 40% acetonitrile.

From each reaction mixture, 60 μL was injected into the HPLC. Unless described otherwise, we performed the derivatization under the above-described conditions in all experiments.

Validation of the Derivatization Procedure and the HPLC−FL Detection. A 40-μL aliquot of peptide standard mixture in 0.2 M PB/EDTA, which contained 2 μM each of Ang I, Ang II, Ang III, and Ang (1−7), was mixed with 40 μL of *m*-BS-ABD-F solution. The mixture was reacted at 70 °C, and a portion of the reaction mixture was injected into the HPLC. The time course of the derivatization reaction was monitored for up to 2.5 h. Linearity was verified in the range of 0.0125−0.2 (0.25−4 pmol) and 0.1−3.1 μM (2−62 pmol) for four different angiotensins, and the correlation coefficient between the peak area and amount of peptide was determined. The detection limit (signal-to-noise ratio, 3) was determined from the chromatogram obtained with the standard solution (0.5 pmol/injection). Recovery of each peptide (0.25 μM, equivalent to 5 pmol) spiked into the MD sample was determined and was compared with the standard mixture reacted under the same conditions.

In Vitro Recovery of Peptides from MD Probes. An MD probe was immersed in a peptide mixture which contained 5 μM each of Ang I, Ang II, Ang III, and Ang (1−7) in PB-R) and was perfused with PB-R at a flow rate of 0.5, 1, 2, and 3 μL/min. The

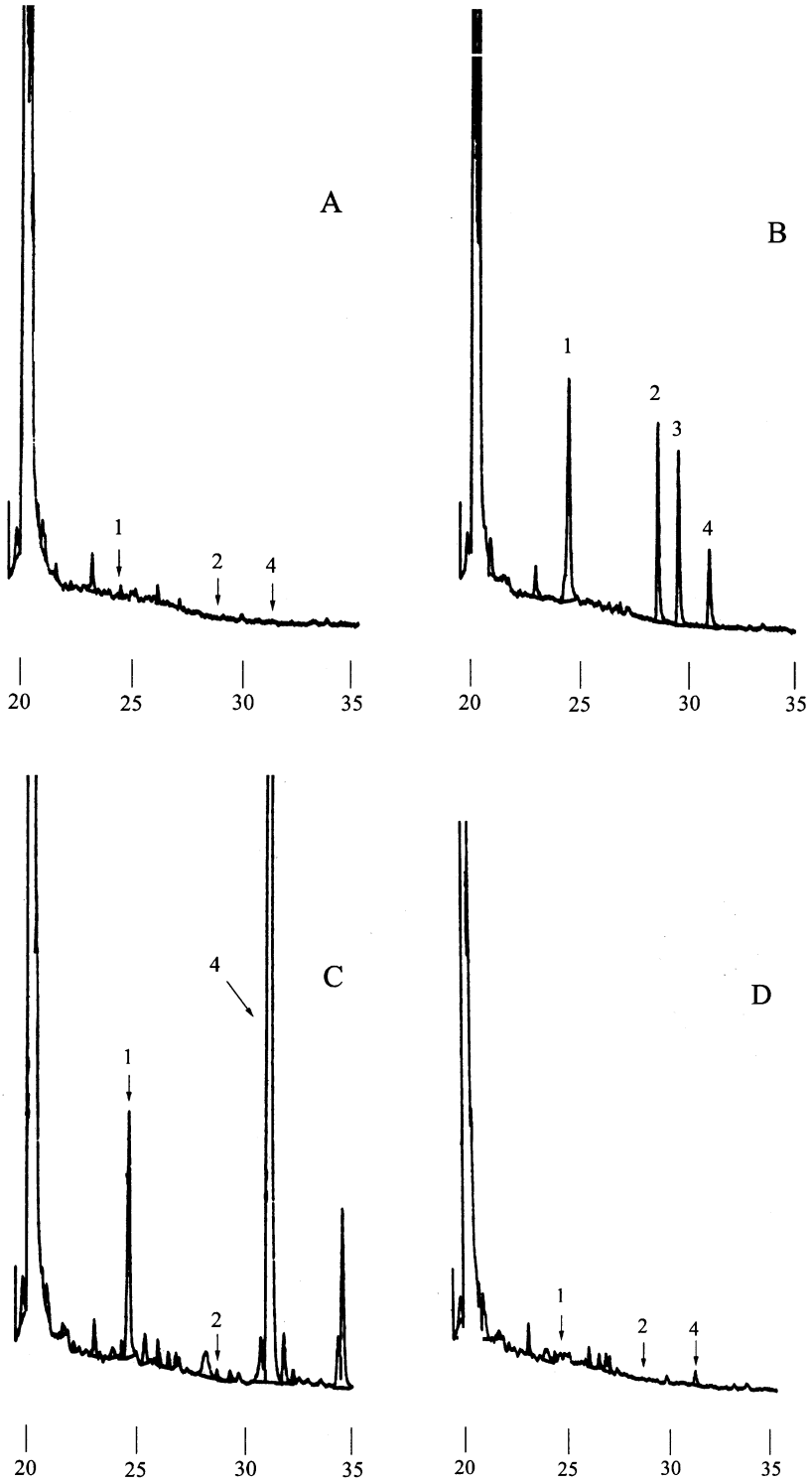

Figure 3. Chromatograms of dialysate samples obtained with the perfusion of MD probes implanted into the rat renal cortex: peak 1, Ang (1−7); peak 2, Ang II; peak 3, Ang III; peak 4, Ang I. (A) Dialysate sample obtained with PB-R perfusion. (B) Synthetic angiotensins (Ang I, Ang II, Ang III, and Ang (1−7), 2.5 pmol) spiked to (A). (C) Dialysate sample obtained with Ang I (20 μM) perfusion. (D) Dialysate sample obtained with PB-R perfusion after switching the perfusate from Ang I solution (20 μM) to PB-R again.

system was equilibrated for 10 min after each change of flow rate. The effluent was collected for 20 min and was evaporated to dryness. Then, an aliquot (30 μL) of each 0.2 M PB/EDTA and *m*-BS-ABD-F solution was added and the resulant mixture was reacted under the conditions described above. A portion of the diluted reaction mixture was injected into the HPLC, and the concentration of each peptide in the effluent was determined. The relative recovery (%) of each peptide was calculated as a ratio of the concentration of each peptide in the effluent to that in the initial outer solution (5 μM).

Collection of Endogeneous Angiotensins from Rat Renal Cortex with the MD Probe. An MD probe implanted in the rat renal cortex was perfused with PB-R at a flow rate of 0.5 μL/min. The dialysate sample of 30 min was evaporated to dryness and

Table 2. Effect of Neprilysin Inhibition on the Generation of Ang (1−7) and Ang II during the MD Perfusion of Ang I

phosphoramidon concn(mM)	Ang (1−7)[a] (μM) mean ± SE	Ang II[a] (μM) mean ± SE	notes
0	0.78 ± 0.19	0.025 ± 0.006	$n = 4$
0.5	0.23 ± 0.02[b]	0.033 ± 0.010	$n = 3$
5	0.15 ± 0.02[b]	0.021 ± 0.005	$n = 3$

[a] The concentrations of angiotensins in the dialysates when 100 μM Ang I was perfused with phosphoramidon. [b] $P < 0.05$ on one-sided t-test.

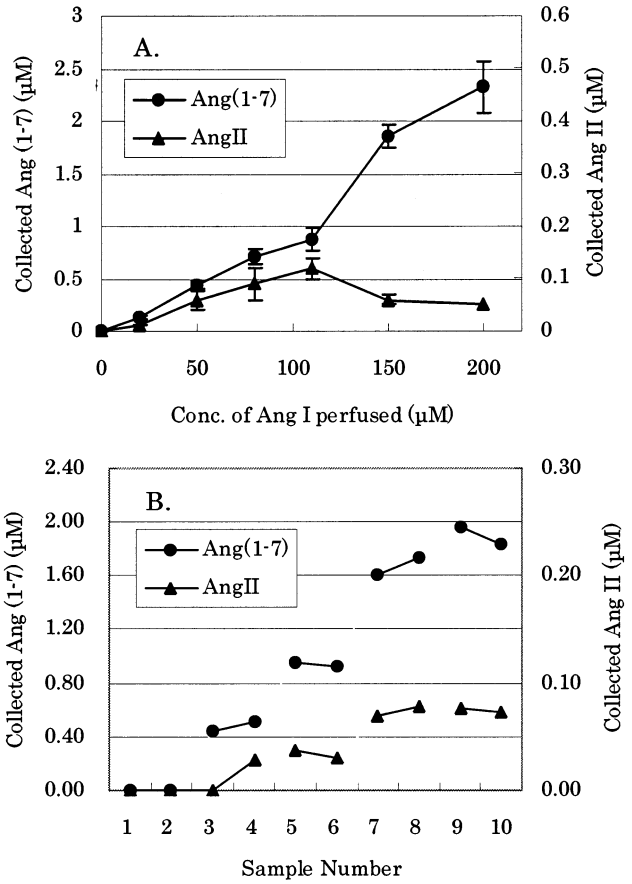

Figure 4. Relationship between the concentration of perfused Ang I and its metabolites, Ang (1−7) (●) and Ang II (▲), in the dialysate samples. The concentration of Ang I was increased stepwise from 20 to 200 μM (20, 50, 80, 110, 150, and 200 μM). (A) Averaged results ($n = 4$, mean ± SE). (B) The concentration of Ang (1−7) (●) and Ang II (▲) in the individual dialysate samples continuously collected from the rat renal cortex.

derivatized with *m*-BS-ABD-F under the conditions described above.

Angiotensin I Perfusion Study in Rat Renal Cortex with the MD Probe. First, following the MD perfusion with PB-R described above, a 20 μM Ang I solution in PB-R was perfused at a flow rate of 0.5 μL/min, and the dialysate was collected for 30 min. After that, the dialysate was switched again to PB-R, and the MD perfusion was performed again as described above.

Separately, the concentration of Ang I was changed stepwise from 20 to 200 μM (four to six steps), and a 15-min sample collection was conducted twice for each concentration of Ang I. Angiotensins in the dialysate samples were derivatized as described above and were determined with the HPLC-FL system.

Effect of Enzyme Inhibition on the Ang I Perfusion MD Study. Phosphoramidon and captopril were used as inhibitors of neprilysin and angiotensin-converting enzyme (ACE), respectively. Ang I solutions (100 μM in PB-R), containing phosphoramidon (0, 0.5 or 5 mM) or captopril (0, 10 mM), were perfused into the rat renal cortex with the MD probe at a flow rate of 0.5 μL/min. A 15-min sample collection was conducted twice for each perfusate. Angiotensins in the dialysate samples were derivatized as described above and were determined by the HPLC-FL system.

RESULTS AND DISCUSSION

Fluorogenic Derivatization of Angiotensins with *m*-BS-ABD-F. We previously showed that *m*-BS-ABD-F reacts with the N-terminal amino residue of bradykinin to afford fluorescent derivatives under neutral pH.[7] The angiotensins determined in this study, Ang I, Ang II, Ang III, and Ang (1−7), were also successfully derivatized with this reagent, and the derivatization reactions at 70 °C/pH 7.0 reached a plateau within 2 h. Derivatization with this reagent showed a single peak for each peptide, which showed that angiotensins were stable under this condition and were derivatized without degradation or racemization.

HPLC Conditions for the Determination of Angiotensins Derivatized with *m*-BS-ABD-F. MD samples collected through tissues generally contain a large number of low molecular weight amino compounds at high concentrations, which are expected to interfere with the determination of peptides. Therefore, we used a coupled-column HPLC equipped with a size exclusion column (TSKgel G2000 SW_{XL}) to remove interfering compounds and an ODS column (TSKgel Super ODS) to separate angiotensin derivatives. Derivatives of angiotensins that have a molecular mass of 1250−1500 Da were eluted together from the first column, the size exclusion column, at earlier retention times than the low molecular weight compounds. By introducing the effluent from the first column between 15 and 18.8 min after the sample injection, the four angiotensin derivatives could be loaded onto the second column, the ODS column, with high recovery (>96%); compounds with interfering peaks were effectively removed. These peptide derivatives that were effectively concentrated on the top of the second column were successfully separated by the gradient elution in the second column. By using this system, the simultaneous determination of four kinds of angiotensins was possible.

Validation Data for the Derivatization Procedure and the HPLC-FL Detection. For the four peptides, the derivatization reaction reached a plateau within 2 h, and good linearity was obtained between the amount of peptide and the peak area obtained by FL detection in the range from 0.0125 to 0.2 ($R > 0.999$) and 0.1 to 3.1 μM ($R > 0.99$). The detection limits (signal-to-noise ratio, 3) for Ang I, Ang II, Ang III, and Ang (1−7), were 94, 44, 47, and 83 fmol, respectively. Recoveries of Ang I, Ang II,

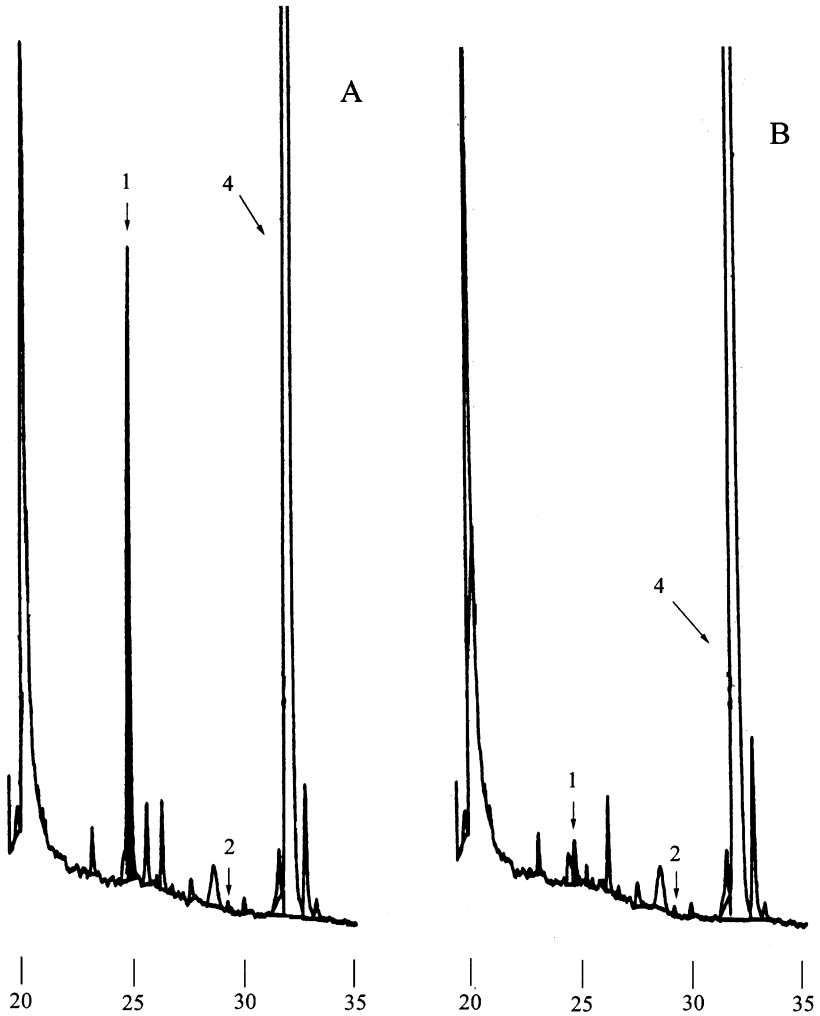

Figure 5. Effect of neprilysin inhibition on the generation of Ang (1−7) and Ang II during the MD perfusion of Ang I. Ang I was perfused with or without phosphoramidon, the neprilysin inhibitor: peak 1, Ang (1−7); peak 2, Ang II; peak 4, Ang I. (A) Chromatogram obtained with the perfusion of Ang I (100 μM). (B) Chromatogram obtained with the co-perfusion of Ang I (100 μM) and phosphoramidon (0.5 mM).

Ang III, and Ang (1−7) spiked into the MD sample (5 pmol) were 91.6, 93.9, 94.9, and 94.9%, respectively. From these results, we confirmed the high sensitivity and good precision obtained with our HPLC-FL system.

In Vitro Recovery of Peptides from MD Probes. In MD studies, probes made with commercially available polyacrylonitrile (PAN) are widely used. Although those probes have been successfully applied for the sampling of low molecular weight compounds, recovery with these probes is not high enough for higher molecular weight compounds such as peptides. A hollow fiber dialysis tubing made with AN69, acrylonitrile−sodium methallyl sulfonate, has also applied to the MD study.[19,21] Because of its high hydrophilicity and large pore size, this membrane shows high water flux. Therefore, good recoveries of peptides were expected. In this study, we constructed MD probes with AN69 (8 mm in length) and examined the usefulness of this membrane for the sampling of angiotensins. The results are depicted in Figure 2. Not only was a high percent recovery obtained (∼50% at a flow rate of 0.5 μL/min) for Ang I, Ang II, Ang III, and Ang (1−7) but these values were also in good agreement among each other, which was considered to be a strong advantage in MD studies because the amount of peptides collected from the probes can be compared with each other without correction. On the other hand, the recovery in Ang I obtained from the probe made with PAN (5 mm in length) was obviously lower than that obtained from AN69 even though the shorter length of the probe was taken into consideration (Figure 2).

Accordingly, we selected AN69 in the following studies, and the length of the probe was set at 8 mm, considering that it will be used for rat renal MD. The flow rate of the perfusate was set at 0.5 μL/min in all the studies described below.

Collection of Intrinsic Angiotensins from Rat Renal Cortex with the MD Probe. First, we tried to collect intrinsic peptides in the rat renal cortex with the MD probe by perfusing Ringer's solution that contained 2 mM sodium phosphate buffer, pH 7.0 (PB-R). However, we could not detect any peaks for Ang I, Ang II, Ang III, or Ang (1−7) (Figure 3A and B), suggesting that the concentration of these peptides in this area was not high enough for direct detection by this method. This result was expected because the reported concentration of these peptides in the proximal tubule is ∼5−30 nM.[24−26]

Ang I Perfusion Study in Rat Renal Cortex with the MD Probe. Following the PB-R perfusion study described above, we perfused a 20 μM Ang I solution in PB-R. In the chromatogram of the dialysate samples (Figure 3C), a large peak of Ang (1−7) and a small peak corresponding to Ang II were found in addition

to the blank peaks. Because the original Ang I solution did not give any of those peaks, Ang (1−7) and Ang II found in the dialysate samples were thought to have been generated during the MD perfusion. The results indicate that Ang I provided to the local area of the tissue with the MD probe was processed by the surrounding enzymes to generate biologically active peptides, which diffused into the inside of the probe.

After the Ang I perfusion, the perfusate was switched again to PB-R, and the effluent was collected after the 30-min equilibration. The chromatogram showed good agreement with that observed before Ang I perfusion (Figure 3D), in which all the peaks observed during the perfusion had almost completely disappeared, even after the high concentration of Ang I (20 μM) was perfused. This result suggests that the transfer of peptides could rapidly equilibrate in the vicinity of the probe.

Relationship between the Concentration of Perfused Ang I and Its Metabolites Collected with the Probe. Next, we changed stepwise the Ang I concentration in the perfusate in the range from 20 to 200 μM. In all the experiments (n = 4), the peaks of Ang (1−7) and Ang II were found after the Ang I perfusion. The amount of Ang (1−7) in the dialysate samples increased with increasing Ang I concentration. Although the amount of Ang II tended to vary, it also increased and reached saturation at ∼100 μM Ang I (Figure 4A). Furthermore, the concentration of Ang (1−7) and Ang II in the dialysate samples well-reflected the rapid change in concentration of Ang I (Figure 4B).

It is well known that the proximal tubules have an abundant source of neprilysin, an endopeptidase that cleaves Ang I to Ang (1−7) and of ACE.[24,28] Furthermore, Ang (1−7) is considered to be a renal main product of Ang I.[11] In this experiment, we also found Ang (1−7) and Ang II in the dialysate samples and Ang (1−7) was the main peptide. These results suggested that the processing pathway of angiotensins in this area was well-monitored by our MD system.

Effect of Enzyme Inhibition on the Ang I Perfusion MD Study. Pathways of processing of angiotensins are quite complicated due to the presence of many kinds of enzymes related to RAS. In this experiment, we administrated enzyme inhibitors through MD probes and attempted to identify the enzymes that process Ang I into Ang (1−7) or Ang II.

First, we perfused phosphoramidon, a neprilysin inhibitor, with 100 μM Ang I (n = 3). The results are shown in Table 2 and Figure 5. The concentration of Ang (1−7) in the dialysate was drastically decreased by co-perfusion of 0.5 and 5 mM phosphoramidon. These results suggest that Ang (1−7) was mainly formed from the cleavage by neprilysin in the rat renal cortex. Furthermore, the fact that the production of Ang (1−7) was not completely inhibited by neprilysin inhibition suggests the contribution of other enzymatic pathways for the generation of this peptide. The concentration of Ang II was not significantly changed throughout the experiment.

Second, captopril, an ACE inhibitor, was perfused at the concentration of 10 mM in the same way. Although a decrease in Ang II was expected in this experiment, no significant change was found in the level of Ang II (data not shown). Although further improvement of our HPLC-FL system in sensitivity would be required for the more detailed study, this result suggests the possibility that enzymes other than ACE contribute to the generation of Ang II in the rat renal cortex.

CONCLUSION

We have developed an evaluation system for enzyme activities related to RAS in the rat renal cortex. Our *m*-BS-ABD-F/HPLC-FL system was convenient and showed good specificity for the determination of angiotensins. Production of Ang (1−7) and Ang II was observed by Ang I perfusion using MD probes, and neprilysin inhibition suppressed the production of Ang (1−7). The results obtained suggested that our MD system combined with HPLC-FL detection has a big potential for the studies of local angiotensin-processing pathways in vivo. In addition, the MD technique that has the possibility of being applied to free-moving animals was considered to be effective for the study of long-term regulation systems such as the renal RAS, which contributes to the long-term control of arterial pressure.

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