Biochemical Pharmacology, Vol. 42, No. 10, pp. 1897–1904, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

A SURVEY OF VASOACTIVE PEPTIDE METABOLIZING ENZYMES IN THE RAT MESENTERIC ARTERIAL BED PERFUSATE

EDUARDO B. OLIVEIRA,* MARIA CRISTINA O. SALGADO† and ANTHONY J. TURNER‡§

Departments of *Biochemistry and †Pharmacology, Faculty of Medicine, University of São Paulo, 14049 Ribeirão Preto, Brazil; and ‡Membrane Peptidase Research Group, Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

(*Received* 10 *June* 1991; *accepted* 18 *July* 1991)

Abstract—We have demonstrated that the isolated perfused rat mesenteric arterial bed (MAB) secretes peptidases capable of metabolizing bradykinin and angiotensin I. The major degradative pathway of bradykinin by enzymes found in the rat MAB perfusate was mediated by carboxypeptidase A-like activity, whereas angiotensin 1 degradation followed two main routes, one attributable to a carboxypeptidase A-like enzyme and the other to an endopeptidase. This latter enzyme seems to be a novel serine peptidase capable of releasing angiotensin II directly from both angiotensin I and renin substrate tetradecapeptide. The rat MAB perfusate was also shown to contain additional endo- and exopeptidases that might play a role in the metabolism of other vasoactive peptides. Our finding that isolated rat MAB secretes peptidases into the perfusion medium indicates that peptide processing within the microvasculature environment may be effected by enzymes besides those normally found in plasma or associated with cell membranes.

Several circulating and tissue-associated peptidases have been implicated in the processes of generating and inactivating vasoactive peptides. Plasma and vascular angiotensin I converting enzyme (EC 3.4.15.1) [1] form the potent vasoconstrictor peptide angiotensin II. An important role for both aminopeptidase A (EC 3.4.11.7) and aminopeptidase N (also referred to as aminopeptidase M; EC 3.4.11.2) has been proposed in the metabolism of angiotensins, leading to the formation of angiotensin III and termination of their pressor activities [2, 3]. In addition, vascular preparations containing aminopeptidase N were able to convert or degrade several members of the kinin, tachykinin and opioid peptide families by amino-terminal metabolism [4–6]. Dipeptidyl peptidase IV (EC 3.4.14.5) is a plasma membrane serine peptidase widely distributed in mammalian tissues that is important in the vascular degradation of substance P and other peptides having a proline adjacent to the amino-terminal residue [7]. Carboxypeptidase N (EC 3.4.17.3) degrades biologically active peptides such as kinins [8] and anaphylatoxins [9], and functions as an enkephalin converting enzyme for some C-terminally extended precursors [8].

In the course of our attempts to study the metabolic fate of perfused peptides in the isolated rat mesenteric arterial bed (MAB‖), we realized that relevant enzymic activities were found not only associated with tissue membranes but also free in the perfusing medium. This paper describes some of the properties of a variety of peptidases present in the rat MAB perfusate. The possible significance of such enzymes in relation to vasoactive peptide metabolism is discussed.

§ Address correspondence to: Prof. A. J. Turner, Dept. of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

‖ Abbreviations: MAB, mesenteric arterial bed; Abz, *o*-aminobenzoyl; Ang II-FE, angiotensin II-forming enzyme; EDDn, *N*-(2,4-dinitrophenyl)ethylenediamine; NMec, 4-methyl-7-coumarylamide; QF-ERP, quenched fluorescence-enkephalin related peptide; QF-ERP7-HE, QF-ERP7-hydrolyzing enzyme.

MATERIALS AND METHODS

Materials. Affinity-purified pig kidney angiotensin converting enzyme (EC 3.4.15.1) was prepared as described previously [10]. Carboxypeptidase B (EC 3.4.17.2) was from Worthington (NJ, U.S.A.). Proline iminopeptidase (EC 3.4.11.5) was purchased from Nacalai Tesque (Kyoto, Japan). Angiotensin I, renin substrate tetradecapeptide, bradykinin, hippuryl-Lys, hippuryl-His-Leu, His-Leu, Z-Gly-Phe, Z-Pro-Phe, Dansyl-D-Ala-Gly-Phe (pNO_2)-Gly, diisopropyl phosphofluoridate and *o*-phthalaldehyde, were obtained from the Sigma Chemical Co. (Poole, U.K.). Captopril was obtained from Squibb (Princeton, NJ, U.S.A.). Gly-Pro-NMec, Ala-NMec and Glu-NMec were from Bachem (Bubendorf, Switzerland). QF-ERP5 (Abz-Gly-Gly-Phe-Leu-Arg-EDDn), QF-ERP6 (Abz-Gly-Gly-Phe-Leu-Arg-Arg-EDDn) and QF-ERP7 (Abz-Gly-Gly-Phe-Leu-Arg-Arg-Val-EDDn) were prepared as described previously [11]. Angiotensin II was obtained by quantitative conversion of angiotensin I by treatment with angiotensin converting enzyme. Similarly, des-Arg^9-bradykinin was prepared by incubating bradykinin with carboxypeptidase B.

The perfused mesenteric arterial bed. Wistar rats (250–300 g) were anaesthetized with an intra-peritoneal injection of sodium pentobarbital (40 mg/kg, in saline) and the mesenteric arterial bed was

cannulated and removed as described by McGregor [12]. The preparation was transferred to a polypropylene cuvette (15 × 50 mm, such as the body of a 20 mL plastic syringe) maintained at 37° and perfused through the vessels with oxygenated (95% O_2/5% CO_2) Krebs solution (120 mM NaCl, 4.7 mM KCl, 3.0 mM $CaCl_2$, 1.43 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.17 mM KH_2PO_4, 11 mM glucose, pH 7.4). The perfusion rate was 0.5 mL/min and was achieved by means of a peristaltic pump which recirculated the perfusing arterial bed.

Endothelium removal. The endothelium was removed by infusion of sodium deoxycholate (1.0 mg/mL in saline) for 40 sec into the MAB preparation, as described previously [13].

HPLC analysis. The analyses were performed on a Waters HPLC system equipped with an automatic sample injector on a reversed phase column (C-18, μBondapak) at a flow rate of 1.5 mL/min and a 15 min gradient of 5–30% (v/v) acetonitrile in 0.08% (v/v) H_3PO_4, followed by 5 min elution under the final conditions. Peptides and hippuric acid were detected by absorbance at 214 nm.

Molecular weight estimation by gel filtration chromatography. Gel filtration chromatography was carried out on a HR 10/30 Superose-12 fast protein liquid chromatography column (Pharmacia, Piscataway, NJ, U.S.A.), equilibrated and developed with 25 mM Tris–HCl buffer, pH 7.5, containing 150 mM NaCl, at a flow rate of 0.3 mL/min. Molecular weight marker proteins used were cytochrome *c* (12.4 kDa), ovalbumin (45 kDa), aldolase (158 kDa), catalase (232 kDa), ferritin (440 kDa) and thyroglobulin (669 kDa). Whenever necessary, samples were concentrated by ultrafiltration on Amicon YM-10 membrane prior to chromatography.

Enzyme assays. All assays were performed at 37° in 25 mM Tris–HCl buffer, pH 7.5, containing 150 mM NaCl. One unit of enzyme activity was defined as the amount of enzyme releasing 1.0 μmol of product per min, under the described conditions.

QF-ERP7-HE was assayed by a fluorimetric method with 0.1 mM QF-ERP7 as substrate, as described by Juliano *et al.* [11]; the increase in fluorescence (excitation 319 nm, emission 418 nm) in this assay did not discriminate among the several cleavable bonds split in the substrate.

Ang II-FE was assayed with 0.25 mM angiotensin I as substrate by the *o*-phthalaldehyde fluorimetric determination of the dipeptide His-Leu formed [14]; no dipeptidases were demonstrated in the rat MAB perfusate.

Endopeptidase-24.11 was assayed by following the increase in fluorescence (excitation 342 nm, emission 562 nm) concomitant with the hydrolysis of the substrate Dansyl-D-Ala-Gly-Phe (pNO_2)-Gly (50 μM), as described by Florentin *et al.*[15].

Carboxypeptidase N activity was measured using 1.0 mM hippuryl-Lys as substrate and the amount of hippuric acid released, determined by HPLC analysis, as described previously [16].

Carboxypeptidase A-like activity was assayed with 2.5 mM Z-Gly-Phe as substrate and the free Phe was measured on an automated amino acid analsyer equipped with a single column (6 × 180 mm, Beckman W-3 resin); this enzyme activity was named carboxypeptidase A-like solely in consideration of its specificity towards *Z*-Gly-Phe.

Carboxypeptidase P was assayed with 1.0 mM *Z*-Pro-Phe as substrate, with free Phe determined as described in the previous assay.

Aminopeptidase P was assayed using 1.0 mM Gly-Pro-NMec as substrate according to the procedure of Hooper *et al.* [17] in which the fluorimetric detection (excitation 370 nm, emission 442 nm) of free NMec required the coupled action of proline iminopeptidase, in order to release quantitatively the fluorescent moiety from the intermediate (Pro-NMec) formed by the aminopeptidase P.

Aminopeptidases A and N, and dipeptidyl peptidase IV, were assayed by fluorimetric measurement (excitation 370 nm, emission 442 nm) corresponding to the release of free NMec from 1.0 mM substrate solutions of Glu-NMec, Ala-NMec and Gly-Pro-NMec, respectively [16]. The assay of dipeptidyl peptidase IV by this procedure was made possible because the diisopropylphosphofluoridate-treated MAB perfusate had no detectable aminopeptidase P activity.

RESULTS

The partial purification of the proteins in the rat MAB perfusate achieved by gel filtration enabled several proteolytic enzyme activities to be identified unambiguously (Fig. 1). Two endopeptidases, Ang II-FE and QF-ERP7-HE were recovered among the high molecular weight proteins and were free of exopeptidase activities. The major peak of QF-ERP7-HE activity eluted at a volume corresponding to a M_r value greater than 670 kDa, whereas that of Ang II-FE activity corresponded to the molecular weight range of 500–650 kDa.

The contribution of the different peptidases to the metabolism of bradykinin and angiotensin was assessed by HPLC analysis of the fragments generated by incubating 25 μL aliquots of each fraction with 15 nmol of either peptide. Fractions corresponding to the carboxypeptidase N-II peak (Fig. 1) accounted for nearly all the bradykinin degradation, with des-Arg^9-bradykinin being the only product formed. The enzymic activities corresponding to aminopeptidase N and carboxypeptidase N-I (Fig. 1) displayed less than 3% of the kininase activity shown by carboxypeptidase N-II (Fig. 1), generating the octapeptides des-Arg^1-bradykinin and des-Arg^9-bradykinin, respectively.

Two major enzymic activities were responsible for the degradation of angiotensin I. The first is referred to as Ang II-FE and could be monitored either by measuring the formation of angiotensin II by HPLC, or by following the release of His-Leu using a specific fluorimetric reaction [14]; the latter procedure was used to determine the Ang II-FE activity shown in Fig. 1, being particularly reliable because the MAB perfusate was devoid of dipeptidases capable of hydrolysing His-Leu [18]. Evaluation of angiotensin I degrading enzymes, based on HPLC analyses of fragments, failed to demonstrate any activity overlapping with that of aminopeptidases shown in Fig. 1. It should be mentioned, however, that small amounts of unidentified angiotensin I fragments were

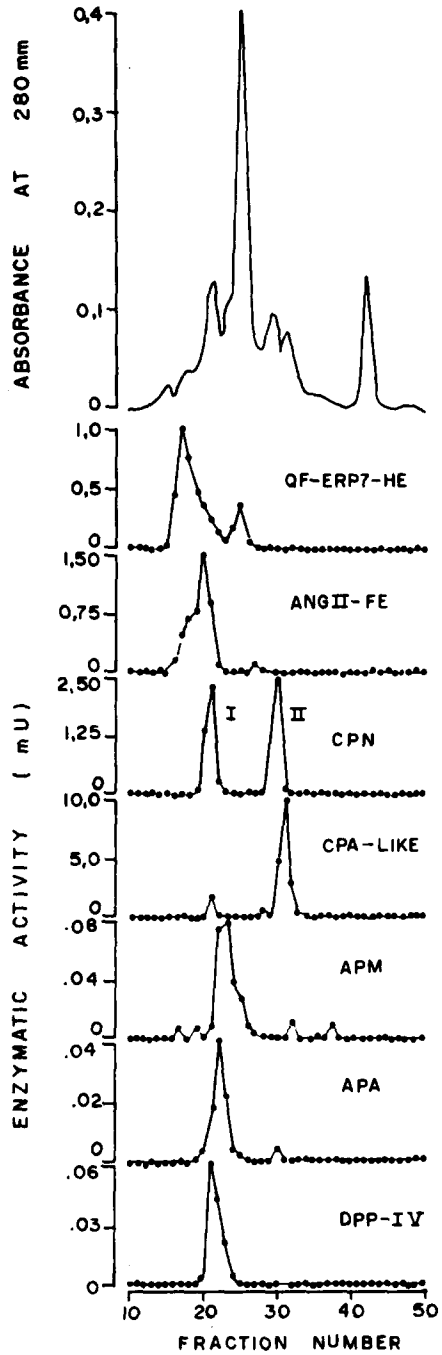

Fig. 1. Gel filtration chromatography of rat MAB perfusate: distribution of peptidase activities in the fractionated effluent volume. Two hundred μL of 50-fold concentrated perfusate, corresponding to the material released by the rat MAB during 20 min approximately, were injected into a HR 10/30 Superose-12 column (Pharmacia) equilibrated and developed with 25 mM Tris–HCl pH 7.5, containing 150 mM NaCl at room temperature at flow rate of 0.3 mL/min. Fractions of 0.5 mL were collected and the indicated enzymic activities measured as described in Materials and Methods. The Ang II-FE activity shown was determined by measuring the amount of His-Leu released in the conversion of angiotensin I into angiotensin II. Enzymic activities corresponding to endopeptidase-24.11, aminopeptidase P and carboxypeptidase P could not be demonstrated in any of the fractions. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of product per min, under the described conditions. The protein profile (top) was obtained by continuous A_{280nm} measurement of the column output. DPP, dipeptidyl peptidase.

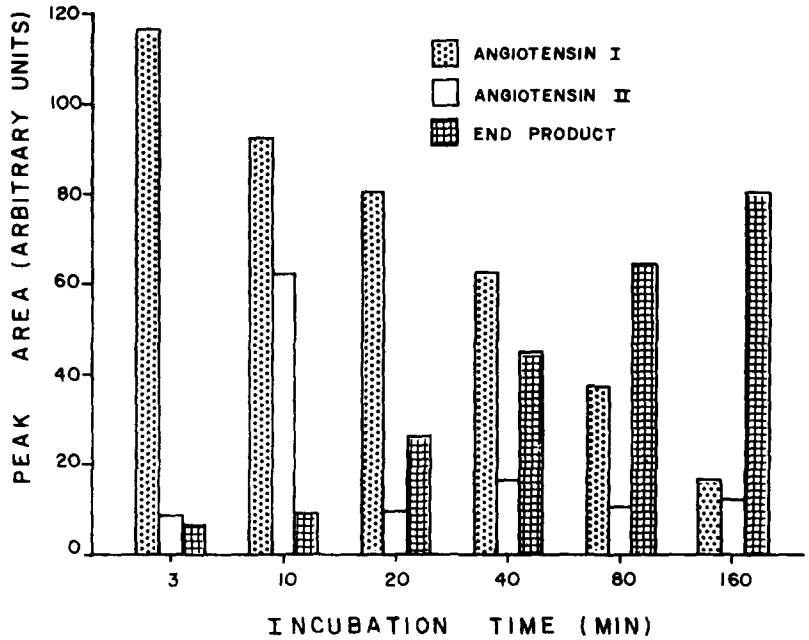

Fig. 2. Time course of angiotensin I degradation by rat MAB perfusate. Twenty nmol of angiotensin I in 0.1 mL of 25 mM Tris–HCl buffer, pH 7.5, containing 150 mM NaCl, were incubated with 10 μL of 50-fold concentrated rat MAB perfusate for the indicated periods of time. After stopping the digestion by acidification with 10 μL of 1% H_3PO_4, each reaction mixture was analysed by reversed phase HPLC, as described in Materials and Methods. The amounts of remaining angiotensin I and the two peptides formed in the reaction mixtures are expressed as peak areas (arbitrary units) and indicated for each incubation time.

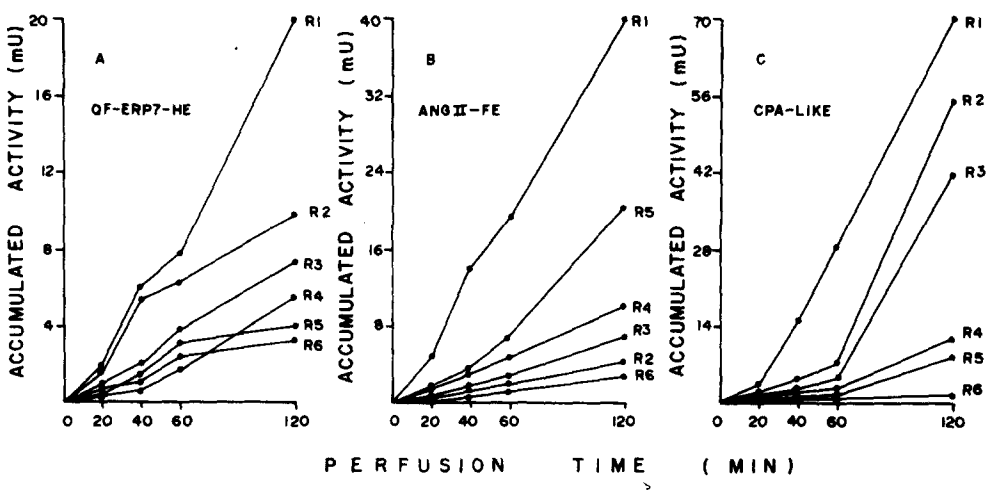

Fig. 3. Time course of accumulation of three proteolytic activities in the rat MAB perfusate obtained by recirculating the perfusion medium. Six intact MAB preparations were individually maintained at 37° and perfused with oxygenated (95% O_2/5% CO_2) Krebs solution at flow rate of 0.5 mL/min. The initial volume of recirculating medium was 10 mL, and samples of 200 μL were withdrawn at the indicated times for enzymic assays. The QF-ERP7 hydrolysing enzyme (A), the Ang II-FE (B) and the carboxypeptidase A-like enzyme (C) were assayed as described in Materials and Methods and the corresponding activities plotted against perfusion time. MAB preparations from individual rats are indicated by R1–R6.

formed by enzymes collected in fractions 25 and 26 (Fig. 1), and that angiotensin II generated by the action of Ang II-FE in fractions 21 and 22 (Fig. 1) was partially degraded by contaminant exopeptidases, as expected from the elution pattern of these enzymes outlined in Fig. 1. The second major angiotensin I degrading activity found in the MAB perfusate, and revealed by HPLC analysis of the reaction mixture, could be ascribed to a carboxypeptidase A-like enzyme. It eluted in fractions 30–32 (Fig. 1), overlapping with the peptidase revealed by its ability to cleave *Z*-Gly-Phe (carboxypeptidase A-like; Fig. 1), and the angiotensin fragment formed had a retention time on reversed phase chromatography slightly shorter than angiotensin II, and compatible with that of the heptapeptide angiotensin 1-7.

The renin substrate tetradecapeptide was also cleaved by the partially purified Ang II-FE, with angiotensin II and a peptide of shorter retention time being the only two products detected by HPLC analysis. Under identical incubation conditions (0.25 mM initial substrate concentration), this enzyme formed 3.2 times more angiotensin II when incubated with angiotensin I than with renin substrate tetradecapeptide. In parallel control assays, affinity purified renal angiotensin converting enzyme [10] failed to cleave the renin substrate.

Analysis of the angiotensin I hydrolysates obtained after incubation with MAB perfusate for up to 160 min, corresponding to about 85% substrate consumption (Fig. 2), demonstrated that the disappearance of angiotensin I was accompanied by a steady accumulation of the end product, with angiotensin II being an intermediate in the degradative pathway. Since the end product of this reaction was identical to that formed when purified carboxypeptidase A-like enzyme (Fig. 1) was incubated with angiotensin I, it should correspond to the fragment angiotensin 1-7; thus, the results presented in Fig. 2 probably reflect the occurrence of two distinct pathways, one in which the intermediate angiotensin II was formed by two successive carboxypeptidase-A-like-catalysed cleavages of the substrate, and the other in which the intermediate was the product of the Ang II-FE action. In both routes the last hydrolytic step would have been catalysed by the carboxypeptidase A-like enzyme, leading to the accumulation of the same end product with proline as the carboxyl-terminal residue.

The data presented in Fig. 3 indicate the rates of accumulation of three peptidases in the recirculating medium of six individual rat MAB preparations. No external stimulus was applied to the MAB either before or during the perfusion period. There are three aspects worth considering by comparing the pattern of accumulation of a given enzymic activity among the six MAB preparations and the patterns of different enzymes in a particular MAB preparation. First, the rate of accumulation of the enzymes was not always a linear function of perfusion time; secondly, there were great differences in the accumulation rate of a given enzymic activity among the various MAB preparations; and thirdly, individual preparations displayed distinct relative rates of accumulation when any two enzymic activities were considered. The results shown in Fig. 3 support the

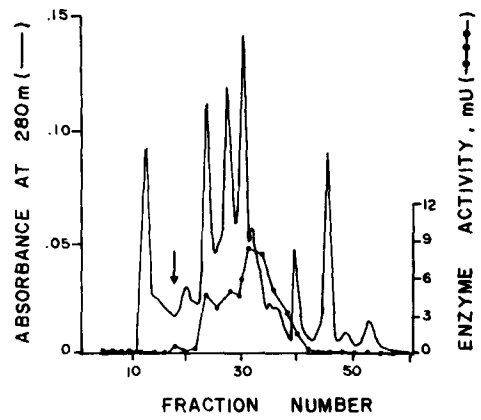

Fig. 4. Gel filtration chromatography of the perfusate obtained from sodium deoxycholate-treated (endothelium-denuded) rat MAB. Two hundred μL of 20-fold concentrated MAB perfusate (collected for 60 min following detergent treatment) were chromatographed on a Superose-12 column under the conditions described in the legend to Fig. 1. The QF-ERP7-hydrolysing activity was measured as described in Materials and Methods and is expressed as enzyme units. The arrow indicates the expected elution position of QF-ERP7-HE obtained from perfusate of an intact rat MAB, as shown in Fig. 1.

conclusion that all three enzymes studied were constitutively secreted into the perfusion media but they are insufficient, as yet, to indicate the mechanism of secretion and the reasons underlying the distinct patterns of appearance of each enzymic activity in the MAB perfusates.

The observation that about 2% of the lining endothelial cells of blood vessels *in vivo* are dead [19] led us to compare the protein and enzymic gel filtration profiles of perfusates obtained from normal and deoxycholate-treated MABs. Perfusion of rat MAB with deoxycholate solution under controlled conditions disrupts the endothelial cells while preserving the integrity and functionality of inner cell layers [13]. Both the protein and the QF-ERP7-HE activity profiles obtained with perfusate of deoxycholate-treated MAB (Fig. 4) bore little resemblance to those obtained with perfusate of intact MAB (see Fig. 1). These observations are an argument against the possible cytoplasmic origin of the QF-ERP7-HE found in perfusates of intact MAB and lend support to the suggestion that rat MAB actively secretes proteolytic enzymes into the perfusing medium.

Table 1 summarizes some features of the two endopeptidases found in the rat MAB perfusate, regarding substrate specificity and sensitivity to inhibitors. These data were useful not only in providing the initial biochemical characterization of these enzymes but also in distinguishing them from previously described endopeptidases with which they share some similarities.

DISCUSSION

During our studies of peptide metabolism in perfused rat MAB, the so-called McGregor's preparation [12], we found several soluble proteolytic

Table 1. Sensitivity to inhibitors and substrate specificity of the endopeptidases Ang II-FE and QF-ERP7-HE

	Ang II-FE	QF-ERP7-HE
Inhibitor	Relative activity (% control)	
pHMB (1 mM)	100	100
EDTA (1 mM)	100	100
Dip-F (1 mM)	3	2
Captopril (5 μM)	100	100
Phosphoramidon (5 μM)	ND	100
Dynorphin A_{1-13} (5 μM)	ND	94
Substrate	Products	
Bradykinin	None	None
Angiotensin I	Ang II, His-Leu*	None*
Renin substrate 14P	Ang II, no His-Leu	ND
Hippuryl-His-Leu	None	ND
QF-ERP7- or QF-ERP6	None*	Positive*
QF-ERP5	ND	None

Enzyme preparations were obtained as described in the legend to Fig. 1 (*). In these reactions enzymes were prepared by rechromatography over Superose-12 column, whereby fractions displaying only Ang II-FE or QF-ERP7-HE activity were obtained. All the inhibitors were preincubated with enzyme for 30 min at room temperature. Angiotensin II release was followed by HPLC analysis and His-Leu by fluorimetric reaction with *o*-phthaldialdehyde. Detailed procedures for enzymic measurements are described in Materials and Methods. ND, not determined; renin substrate 14P, renin substrate tetradecapeptide; pHMB, *p*-hydroxymercuribenzoate; Dip-F, diisopropylphosphofluoridate.

enzymes in the perfusate that could be isolated and partially characterized. Previous reports indicated that cultured endothelial cells were capable of producing and secreting substances such as coagulating factors stored in the Weibel–Palade granules [20] and angiotensins, but not renin and angiotensinogen, present in the cells [21]. We demonstrated that the MAB endothelial cells contained proteins and enzymes distinct from those found in the perfusate of the intact MAB, suggesting also that the peptidases we isolated were secreted selectively into the perfusate and not released nonspecifically as a result of cell damage.

We identified both endo- and exopeptidases among the proteins recovered from the MAB perfusate, whose rates of appearance in the perfusion media seemed to be regulated independently, as judged by the time course of accumulation of three representative enzymes. Their contribution to the metabolic fate of two biologically active peptides was ascertained by studying the action of the whole perfusate and its fractions obtained by gel filtration. Bradykinin was almost exclusively converted into des-Arg^9-bradykinin after incubation with whole MAB perfusate; it is worth noting that, of the two equally active carboxypeptidases N activities revealed by the synthetic substrate after gel filtration, only the low molecular weight form was effective in destroying bradykinin. Small amounts of des-Arg^1-bradykinin were also detected in the hydrolysate, reflecting the low relative abundance of aminopeptidases in the MAB perfusate.

When angiotensin I was incubated with whole MAB perfusate under conditions limiting the substrate consumption to about 85%, the peptides found in the hydrolysate could be ascribed to the action of two enzymes: the Ang II-FE and the carboxypeptidase A-like enzyme. The end product that accumulated as the angiotensin I was degraded was the heptapeptide angiotensin 1-7, as inferred from its retention time on HPLC and from the specificity of the enzymes involved in the reaction. Two metabolic pathways could be assigned leading to the same end product, one catalysed by the carboxypeptidase A-like enzyme alone and the other resulting from the successive action of the Ang II-FE followed by the carboxypeptidase A-like enzyme. Kohara *et al.* [22] have shown recently that angiotensin 1-7 is present in the blood and is formed from angiotensin I through an ACE-independent pathway.

Although some other enzymes were detected in the MAB perfusate, especially aminopeptidases, they did not seem to make any important contribution to the initial steps of bradykinin or angiotensin I degradation. Based on measurements using synthetic substrates for the quantification of peptidases separated by gel filtration, the carboxypeptidases were several-fold more active than the aminopeptidases.

Some biochemical features of the two endopeptidases found in the MAB perfusate are outlined in Table 1. The presence of the QF-ERP7-HE was demonstrated by its ability to hydrolyse the intramolecularly quenched fluorescent enkephalin related heptapeptide QF-ERP7, a selective substrate for endo-oligopeptidase A found in some tissue homogenates [11]. In contrast to endo-oligopeptidase A, an enzyme capable of hydrolysing bradykinin and inhibited by both *p*-hydroxymercuribenzoate and dynorphin A_{1-13}, the QF-ERP7-HE was shown to be

a serine proteinase inactive towards bradykinin. Well known inhibitors of the angiotensin converting enzyme (EC 3.4.15.1) and of endopeptidase-24.11, had no inhibitory effect on this enzyme. The lack of reactivity of QF-ERP7-HE towards the substrate QR-ERP5 suggested that it might have its specificity directed to paired basic amino acids, a distinguishing feature of enzymes involved in the processing of various peptide hormone precursors. However, assignment of the biological role of this novel serine proteinase awaits further experimental work.

The second endopeptidase found in the rat MAB perfusate was a high molecular weight serine proteinase named Ang II-FE. It was capable of releasing angiotensin II directly from both angiotensin I and renin substrate tetradecapeptide, but the rate was 3.2 times higher with the former substrate. The data presented in Table 1 emphasize the differences between the Ang II-FE and the angiotensin I converting enzyme since the latter hydrolyses hippuryl-His-Leu and bradykinin, and is inhibited by ethylenediaminetetraacetic acid (EDTA) and captopril [16]. The enzymological properties of other angiotensin II-producing enzymes have been presented recently in a report demonstrating the non-identity of angiotensin II-producing enzyme III with cathepsin G, tonin and calcium-independent carboxypeptidase [23]; comparison of such properties with those described in Table I for the Ang II-FE clearly indicates differences between these enzymes by more than one criterion. Two additional activities associated with serine proteinases have been described as being capable of generating angiotensin II: the chymostatin-sensitive enzyme from dog isolated renal artery [24] and the major angiotensin II-forming enzyme from human heart (25). Although this latter enzyme has been well characterized, the information presently available on the Ang II-FE from rat MAB perfusate seems insufficient to allow a clear distinction between the two enzymes, leaving the possibility that they might correspond to the membrane-bound and the soluble forms of the same enzymic entity.

Our findings that endo- and exopeptidases were secreted into the perfusate of rat mesenteric bed disclosed a hitherto unrecognized peptide metabolizing potential within the microvasculature environment. The broad range of specificities detected and the secretion rates attained by some of the activities were suggestive of a prominent role for these enzymes in local peptide metabolism. However, the widespread occurrence of these enzymes, the mechanism of their secretion and their participation in physiological processes, remain to be established.

Acknowledgements—This work was supported by the U.K. Medical Research Council and the Wellcome Trust, and was carried out, in part, under the auspices of the USP-BID program for the development of scientific research.

REFERENCES

1. Soffer RL, Angiotensin converting enzyme and the regulation of vasoactive peptides. *Annu Rev Biochem* **45**: 73–94, 1976.
2. Ahmad S and Ward PE, Role of aminopeptidase activity in the regulation of the pressor activity of circulating angiotensins. *J Pharmacol Exp Ther* **252**: 643–650, 1990.
3. Ward PE, Benter IF, Dick L and Wilk S, Metabolism of vasoactive peptides by plasma and purified renal aminopeptidase M. *Biochem Pharmacol* **40**: 1725–1732, 1990.
4. Palmieri FE, Bausback HH and Ward PE, Metabolism of vasoactive peptides by vascular endothelium and smooth muscle aminopeptidase M. *Biochem Pharmacol* **38**: 173–180, 1989.
5. Bausback HH and Ward PE, Degradation of low molecular weight opioid peptides by vascular plasma membrane aminopeptidase M. *Biochim Biophys Acta* **882**: 437–444, 1986.
6. Sanderink G-J, Artur Y and Siest G, Human aminopeptidases: A review of the literature. *J Clin Chem Clin Biochem* **26**: 795–807, 1988.
7. Palmieri FE and Wad PE, Mesentery vascular metabolism of substance P. *Biochim Biophys Acta* **755**: 522–525, 1983.
8. Palmieri FE, Bausback HH, Churchill L and Ward PE, Kinin and enkephalin conversion by an endothelium plasma membrane carboxypeptidase. *Biochem Pharmacol* **35**: 2749–2756, 1986.
9. Huey R, Bloor CM, Kawahara MS and Hugli TE, Potentiation of the anaphylatoxin *in vivo* using an inhibitor of serum carboxypeptidase N (SCPN). I. Lethality and pathologic effects on pulmonary tissue. *Am J Pathol* **112**: 48–60, 1983.
10. Hooper NM and Turner AJ, Isolation of two differentially glycosylated forms of peptidyl peptidase A (angiotensin converting enzyme) from pig brain: a re-evaluation of their role in neuropeptide metabolism. *Biochem J* **241**: 625–633, 1987.
11. Juliano L, Chagas JR, Hirata IY, Carmona E, Sucupira M, Oliveira ES, Oliveira EB and Camargo ACM, A selective assay for endooligopeptidase A based on the cleavage of fluorogenic substrate structurally related to enkephalin. *Biochem Biophys Res Commun* **173**: 647–652, 1990.
12. McGregor DD, The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels in rat. *J Physiol Lond* **177**: 21–30, 1965.
13. Synetos EP, Sideri E, Catravas JD and Maragoudakis ME, Endothelium removal does not abolish angiotensin converting enzyme activity from the mesenteric arterial bed of the rat. *Biochem Pharmacol* **40**: 1149–1151, 1990.
14. Yang H-YT and Neff NH, Distribution and properties of angiotensin converting enzyme of rat brain. *J Neurochem* **19**: 2443–2450, 1972.
15. Florentin D, Sassi A and Roques BP, A highly sensitive fluorimetric assay for enkephalinase, a neutral metalloendopeptidase that releases Tyr-Gly-Gly from enkephalins. *Anal Biochem* **141**: 62–69, 1984.
16. Turner AJ, Hooper NM and Kenny AJ, Neuropeptide-degrading enzymes. In: *Neuropeptides: a Methodology* (Eds. Fink G and Harmar AJ), pp. 189–223. John Wiley and Sons Ltd, New York, 1989.
17. Hooper HM, Hryszko J and Turner AJ, Purification and characterization of pig kidney aminopeptidase P. *Biochem J* **267**: 509–515, 1990.
18. Hooper NM, Angiotensin converting enzyme: how reliable is the fluorimetric assay with benzoyl-Gly-His-Leu as substrate? *Biochem J* **270**: 840–841, 1990.
19. Schwartz SM, Disturbances in endothelial integrity. *Ann NY Acad Sci* **401**: 228–233, 1982.
20. Wagner DD, Olmsted JB and Marder VJ, Immunolocalization of van Willebrand protein in Weibel–Palade bodies of human endothelial cells. *J Cell Biol* **95**: 355–360, 1982.

21. Kifor I and Dzan VJ, Endothelial renin-angiotensin pathway: evidence for intracellular synthesis and secretion of angiotensin. *Circ Res* **60**: 422–428, 1987.
22. Kohara K, Brosnihan KB, Chappell MC, Khosla MC and Ferrario CM, Angiotensin-(1–7): a member of circulating angiotensin peptides. *Hypertension* **17**: 131–138, 1991.
23. Haas E, Lewis L, Koshy TJ, Varde AU, Renerts L and Bagai RC, Angiotensin II-producing enzyme III from acidified serum of nephrectomized dogs. *Am J Hypertens* **2**: 696–707, 1989.
24. Okamura T, Okunishi H, Ayajiki K and Toda N, Conversion of angiotensin I to angiotensin II in dog isolated renal artery: role of two different angiotensin II-generating enzymes. *J Cardiovasc Pharmacol* **15**: 353–359, 1990.
25. Urata H, Kinoshita A, Misono KS, Bumpus FM and Husain A, Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. *J Biol Chem* **265**: 22348–22357, 1990.