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METABOLISM OF VASOACTIVE PEPTIDES BY VASCULAR ENDOTHELIUM AND SMOOTH MUSCLE AMINOPEPTIDASE M

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Abstract—The cellular localization of vascular plasma membrane aminopeptidase M (AmM; EC 3.4.11.2) was examined in cultured porcine aorta endothelium and smooth muscle cells. AmM was 14-fold higher on smooth muscle (117 ± 16 units/mg) than on endothelium (8.4 ± 0.2). Proportional to its cellular distribution, AmM hydrolyzed the N-terminus of kallidin to produce bradykinin, and degraded des(Asp^1)angiotensin I, angiotensin III, hepta(5-11)substance P and Met^5-enkephalin. In contrast, bradykinin, angiotensin II and substance P were resistant to AmM-mediated hydrolysis. Peptide metabolism was optimal at pH 7.0 and was inhibited by *o*-phenanthroline, bestatin (K_i = 2.2 ± 0.1 μM) and amastatin (K_i = 25 ± 5 nM). Des(Asp^1)angiotensin I and angiotensin III had the highest affinity (lowest K_m) for AmM (K_m = 2.2 ± 0.5 and 2.0 ± 0.4 μM respectively), followed by hepta(5-11)substance P (53.9 ± 1.7 μM) and Met^5-enkephalin (75.7 ± 3.5 μM). In contrast, maximal velocities of hydrolysis were higher for Met^5-enkephalin (313 ± 2 nmol/min/mg) than for hepta(5-11)substance P (109 ± 18 nmol/min/mg) or angiotensin III (26.5 ± 1.0 nmol/min/mg). As expected for hydrolysis by a common enzyme, AmM-mediated enkephalin degradation was inhibited competitively by angiotensin III (K_i = 0.34 ± 0.04 μM), hepta(5-11)substance P (43.7 ± 6.3 μM) and kallidin (62 μM). These data suggest that vascular AmM may modulate vasoactive peptide levels *in vivo*, particularly within the microenvironment of endothelial and smooth muscle cell surface receptors.

Both plasma and vasculature contain enzymes that can form, convert and/or inactivate vasoactive peptides. These enzymes include angiotensin I converting enzyme (ACE; EC 3.4.15.1) [1], carboxypeptidase N (CPN; EC 3.4.17.3) and/or a similar enzyme with N-like activity [2, 3], and aminopeptidase A (AmA; EC 3.4.11.7) [4]. In previous studies we found that a plasma membrane fraction purified from porcine aorta or mesenteric artery contains an enzyme immunologically indistinguishable from renal aminopeptidase M (AmM; EC 3.4.11.2) [5]. AmM has also been identified in plasma [5] and in cerebral microvasculature [6–8]. Consistent with its known substrate specificity for hydrolyzing N-terminal basic and neutral amino acids, AmM can hydrolyze several members of the kinin, angiotensin, tachykinin and opioid peptide families [9, 10].

Despite the above, the cellular (endothelium/smooth muscle) distribution of vascular AmM has not been determined. Further, although the kinetics of AmM-mediated hydrolysis of opioid peptides has been investigated [10], no comparable studies have been reported regarding kinin, angiotensin and tachykinin metabolism. Thus, the present studies were conducted to determine the cellular localization of vascular AmM and to investigate the metabolism of these peptides.

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MATERIALS AND METHODS

Materials. Substance P and hepta(5-11)substance P were obtained from Peninsula Laboratories (San Carlos, CA). Kallidin, bradykinin, angiotensin II, des(Asp^1)angiotensin I and angiotensin III, alanyl-, leucyl- and arginyl-2-naphthylamide, alanyl- and α-glutamyl-7-amino-4-trifluoromethyl coumarin (X-AFC), alanyl-4-methoxy-2-naphthylamide (alanyl-MNA), *o*-phenanthroline, amastatin, bestatin, puromycin, phosphoramidon and papain were obtained from the Sigma Chemical Co. (St. Louis, MO). The ACE inhibitor (captopril) and the CPN inhibitor (MERGETPA: D-L-mercaptomethyl-3-guanidinoethylthiopropanoic acid) were obtained from Squibb (Princeton, NJ) and Calbiochem-Behring (San Diego, CA) respectively. Antisera to porcine renal brush border AmM and AmA, obtained as previously described [5], precipitate AmM and AmA, respectively, but do not cross-react with other renal peptidases including ACE, dipeptidyl(amino)-peptidase IV (EC 3.4.14.5), neutral endopeptidase (EC 3.4.24.11) and carboxypeptidase P (EC 3.4.12.-). The MN 300 Uniplates used for TLC were from Analtech, Inc. (Newark, DE) and the amino acid standards, *o*-phthalaldehyde crystals and reagent solution (OPA) used for HPLC were from the Pierce Chemical Co. (Rockford, IL).

Cultured endothelium. Primary cultures of endothelium were obtained as previously described [3] by gently scraping endothelial cells from the intimal surface of fresh porcine aorta. Scrapings were suspended in DMEM (Dulbecco's Modified Eagle's

Medium) and spun at 500 *g* (20 min) to separate intact cells. Cells were collected as a pellet in DMEM, resuspended in growth medium [DMEM; 10% (v/v) fetal calf serum; 1% (v/v) penicillin-streptomycin] and incubated in 5% CO_2 at 37°. After 1 hr, medium containing non-adherent cells was replaced with fresh growth medium. Medium was added every 2 days and adherent cells grew to near confluency within 7–10 days.

Endothelia were characterized by cobblestone patterned cells growing in densely packed monolayers. Cells were polygonol in shape with large round nuclei surrounded by relatively little cytoplasm [11]. Examination by transmission electron microscopy revealed features characteristic of endothelium including Weibel–Palade bodies, numerous mitochondria, abundant cytosolic microfilaments, and micropinocytotic vesicles adjacent to the outer membrane [12, 13]. Endothelium contained ACE and released PGI_2 upon stimulation with bradykinin [3].

Cultured smooth muscle. Smooth muscle cells were obtained as previously described [3] from porcine aorta using explants of the medial layer. Explants (1–2 cm^2) were incubated in growth medium until migration of juvenile muscle cells was seen (10–14 days). Juvenile cells had a spindle shape with long, thin cytoplasmic processes extending from one cell to another, and oval or sausage-shaped nuclei containing two or more dense nucleoli. After 4–6 weeks, cells exhibited characteristic features including a ribbon-like appearance, phase-dense cytoplasm and growth in multi-layered arrays which gave the culture a hill and valley appearance characteristic of smooth muscle cells at this stage of growth [14]. Approximately 80% of the cells contracted spontaneously with changes in growth medium [15].

Cell fractionation. For enzyme analysis, cells were washed repeatedly in phosphate-buffered saline to remove culture medium and collected by mechanical scraping with a rubber spatula. After sonication (3 × 10 sec), homogenates were centrifuged at 1000 *g* (10 min) and 10,000 *g* (10 min) to remove unbroken cells and cellular debris. The supernatant fraction was centrifuged at 100,000 *g* (60 min) to obtain the microsomal fraction. Endothelial and smooth muscle cells were also subfractionated to a plasma membrane-enriched fraction as described previously [5]. For comparative studies of AmM, porcine renal brush border was prepared as previously described [16].

Immunoelectrophoresis. Rocket and fused-rocket immunoelectrophoresis of detergent/papain-solubilized membrane against antisera to porcine renal brush border AmM or AmA were carried out in 1% (w/v) agarose gels as described previously [5, 16]. AmM and AmA precipitin lines visualized histochemically using arginyl- and α-glutamyl-MNA respectively [17, 18].

Enzyme assays. Aminopeptidase activity was assayed as the rate of hydrolysis of alanyl-, leucyl- and arginyl-2-naphthylamides [19] or, because of the higher sensitivity that could be obtained, the corresponding X-7-amino-4-trifluoromethyl coumarin (X-AFC) substrates [20]. Specific activities are expressed as units per mg protein where one unit equals the hydrolysis of one nmole substrate per minute (X-2-naphthylamide) or the change in relative fluorescence per minute (X-AFC). Protein was determined according to Bradford [21] using bovine serum albumin as a standard.

Peptide metabolism. Peptidase activity was examined qualitatively by TLC using MN 300 cellulose plates [9]. Quantitative analysis was carried out using HPLC as previously described [9, 10]. Reactions (300–600 μl) were incubated at 37° in 100 mM sodium phosphate buffer (pH 7.0). At sequential time intervals, aliquots (60–120 μl) were immersed in a boiling water bath (5 min) to terminate the reaction, cooled on ice, and centrifuged in a Brinkmann table top centrifuge (3 min); then the supernatant fraction was collected for analysis.

For determination of pH dependence, a 100 mM sodium acetate buffer was used over the pH range 4.0 to 6.0, and the above 100 mM phosphate buffer was used from pH 6.5 to 8.5. For inhibition studies, inhibitors were preincubated with enzyme and buffer for 20 min at 37°. For K_m determinations, measurements of initial velocity were made over a range of substrate concentrations. Data were plotted as $1/V$ vs $1/S$ and fit to the best straight line. For competitive substrate experiments, peptide inhibitors were added simultaneously with the peptide substrate.

A Waters high performance liquid chromatograph system was employed for the HPLC analysis. Standards and unknowns (20–100 μl) were automatically derivatized with *o*-phthalaldehyde (20 μl) 3 min prior to chromatography (Pre-column Derivatization Program, Waters 710B WISP™ Autosampler) and subsequently separated on a reverse phase column (Waters, 10 micron, C_{18}-Radial-PAK™, 8 mm × 10 cm) at a constant flow rate of 5.0 ml/min utilizing a linear gradient from 100% Buffer A to 40% Buffer A/60% Buffer B. Buffer A was 10 mM sodium phosphate (pH 7.0) and Buffer B was a 50/50 (v/v) mixture of Buffer A and acetonitrile. Integration of sample peak areas and quantitation of metabolites against the last-run standards were calculated automatically by the data module. Standards were run every sixth injection.

RESULTS

Peptide metabolism—TLC. Incubation of smooth muscle plasma membrane with kallidin and separation of metabolites by TLC demonstrated the rapid production of Lys and bradykinin and the disappearance of kallidin. In contrast, no N-terminal hydrolysis of bradykinin was seen. In the presence of captopril (100 μM) and MERGETPA (100 μM) to inhibit endothelial ACE and carboxypeptidase [3] activities, N-terminal hydrolysis of kallidin was also seen during incubation with an equivalent amount (protein) of endothelial plasma membrane. However, the rate of metabolism was considerably slower.

No N-terminal Asp metabolite was observed when smooth muscle membrane was incubated with either angiotensin I or angiotensin II. Incubation of smooth muscle with angiotensin III resulted in the production of N-terminal Arg. Des(Asp^1)angiotensin I, having the same N-terminus, was similarly metabolized. Using an equivalent amount of endo-

Table 1. Ratios of synthetic substrate hydrolysis by renal brush border, smooth muscle and endothelium aminopeptidase activities

	Enzyme source		
	Ratios of synthetic substrate hydrolysis		
Substrate	RBB	SM	Endo
Alanyl-2NA	100	100	100
Leucyl-2NA	69 ± 7	62 ± 6	180 ± 28
Arginyl-2NA	37	42 ± 4	50 ± 1

Ratios of alanyl-, leucyl- and arginyl-2-naphthylamidase (-2NA) activities by renal brush border (RBB), smooth muscle (SM) and endothelial (Endo) membrane are shown. Absolute values of alanyl-2-naphthylamidase activity (2250, 107 and 3.0 nmol/min/ml, respectively) were set at 100 and the leucyl- and arginyl-2-naphthylamidase activities were calculated accordingly. Values are means ± SEM (N = 3) or the average of two determinations.

thelium, N-terminal Arg production from angiotensin III was substantially slower. Further, the simultaneous production of Phe indicated that significant hydrolysis of the C-terminal Pro-Phe bond was also occurring.

Incubation of Met^5-enkephalin with smooth muscle membrane evidenced production of Tyr from the N-terminus. Endothelial-mediated hydrolysis was similar though less rapid.

Since previous studies have shown that vascular cell surface dipeptidyl(amino)peptidase IV (DAP IV) converts substance P to hepta-(5-11)substance P [22–24], this biologically active metabolite [25] was also incubated with smooth muscle and endothelial plasma membrane. Incubation with smooth muscle resulted in production of N-terminal Gln. Endothelium produced the same pattern except that, as above, hydrolysis was significantly slower.

Endothelial and smooth muscle aminopeptidase M. The above data demonstrated that significant (though not necessarily exclusive) N-terminal peptide metabolism occurred on both smooth muscle and endothelium. Since preliminary studies (not shown) demonstrated complete inhibition of this metabolism by the aminopeptidase inhibitor amastatin (100 μM), experiments were conducted to determine whether membrane-bound aminopeptidases such as AmM or AmA were present on these cultured cells.

As shown in Table 1, renal brush border AmM hydrolyzed alanyl-, leucyl- and arginyl-2-naphthylamides in a ratio of 100:69:37, and a similar ratio was found for smooth muscle membrane. In contrast, endothelium, although having a similar ratio of alanyl- to arginyl-naphthylamidase activity (100:50), contained significantly higher levels of leucyl-2-naphthylamidase activity (180).

As shown in Fig. 1, fused-rocket immunoelectrophoresis of smooth muscle membrane against antibody to renal AmM produced a precipitant line which fused with that produced by partially purified AmM (Sigma). No evidence of spurring or crossover in the fused region could be detected. Endothelium also produced a fused rocket-shaped precipitate (not shown), whereas the fetal calf serum used for cell culture did not contain antigens recognized by the AmM antisera.

In contrast, no immunoreactive AmA could be detected on either smooth muscle or endothelium. Further, consistent with the lack of hydrolysis of N-terminal Asp from angiotensin II, no significant hydrolysis of the AmA substrates aspartyl- and α-glutamyl-2-naphthylamide was found.

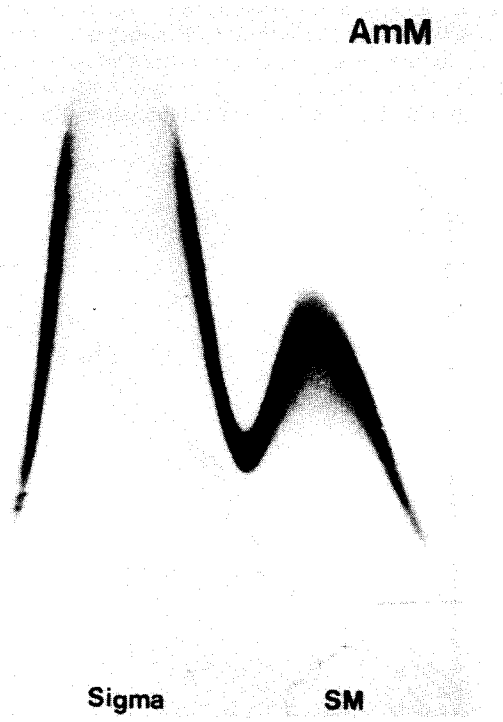

Fig. 1. Fused-rocket immunoelectrophoresis of partially purified (Sigma) renal aminopeptidase M (AmM) and detergent/papain-solubilized smooth muscle membrane (SM). After 1 hr for diffusion, samples were vertically electrophoresed into gel containing anti-porcine AmM immunoglobulin at 2 V/cm for 18 hr. Precipitin lines were visualized histochemically by staining for alanyl-4-methoxy-2-naphthylamide (alanyl-MNA) hydrolysis.

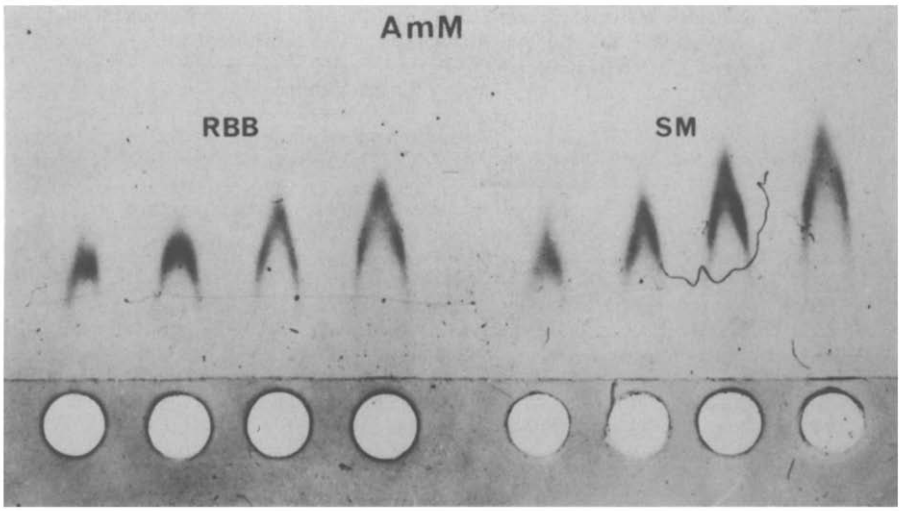

Fig. 2. Rocket immunoelectrophoresis of increasing amounts of detergent/papain-solubilized renal brush border (RBB) and smooth muscle membrane (SM). Samples were vertically electrophoresed into gel containing antiporcine AmM immunoglobulin at 15 V/cm for 1 hr. Precipitin lines were visualized histochemically by staining for alanyl-MNA hydrolysis.

Quantitative rocket immunoelectrophoresis of renal brush border and smooth muscle membrane (Fig. 2; typical experiment) and endothelial membrane (not shown) against antibody to AmM produced rocket-shaped precipitates with peak areas proportionate to the amount of membrane protein used. When peak areas (immunoreactive AmM; iAmM) were plotted against the units of alanyl-2-naphthylamidase activity inoculated onto the gels, the relationships of iAmM to alanyl-2-naphthylamidase activity were the same in all three preparations (Fig. 3A). In contrast, when the level of iAmM was replotted versus leucyl-2-naphthylamidase, only smooth muscle generated a line comparable to brush border AmM (Fig. 3B). Thus, unlike smooth muscle, endothelium contained more leucyl-2-naphthylamidase activity than could be accounted for by the level of iAmM.

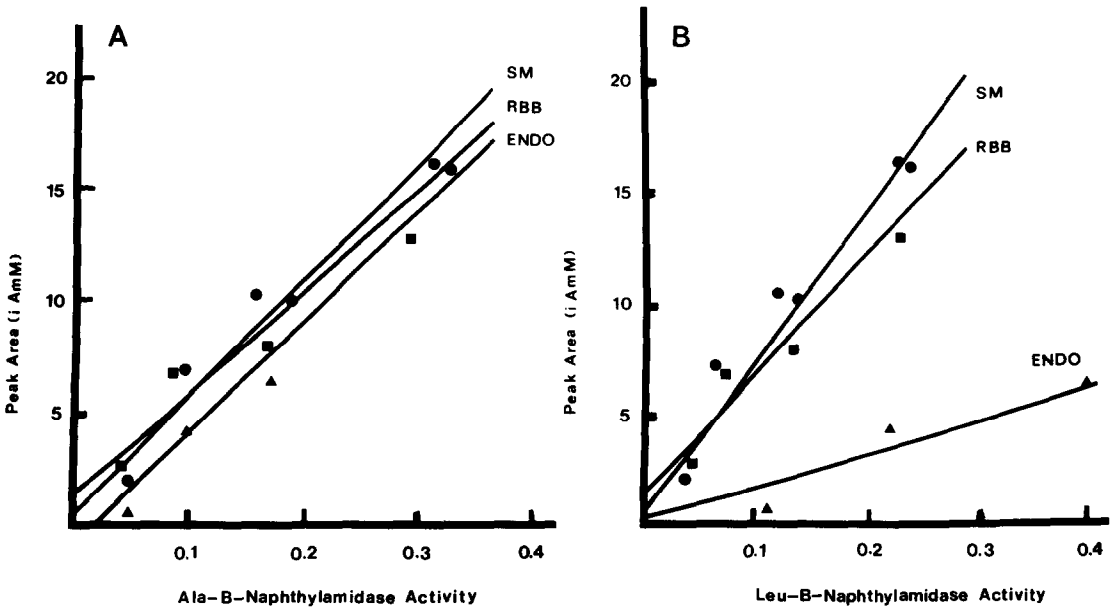

Fig. 3. Relationships of peak area (immunoreactive AmM) to (A) alanyl-2-naphthylamidase and (B) leucyl-2-naphthylamidase activities in renal brush border (RBB), smooth muscle (SM) and endothelium (Endo). Dilutions of each sample were vertically electrophoresed into gel containing antiporcine AmM immunoglobulin at 15 V/cm for 1 hr. Precipitin lines were visualized by histochemical stain as described in the legend of Fig. 2.

Inhibition profile. Amastatin, bestatin and puromycin were dose-dependent inhibitors of renal brush border, endothelial and smooth muscle AmM alanyl-2-naphthylamidase activities with similar inhibition profiles for all three preparations. Amastatin was the most potent inhibitor (IC_{50} = 20–80 nM) followed by bestatin (IC_{50} = 1–3 μM) and puromycin (IC_{50} = 20–50 μM). All three preparations were also inhibited by *o*-phenanthroline (1 mM), whereas inhibitors of ACE (captopril), CPN (MERGETPA), neutral endopeptidase (phosphoramidon) and DAP IV (diprotin A) had no effect (less than 10% inhibition at a final concentration of 10 μM). When these studies were repeated for endothelial leucyl-2-naphthylamidase activity, a similar inhibition profile was obtained.

Cellular localization. In view of the presence of AmM on both endothelium and smooth muscle, quantitative studies were conducted to compare the cellular distribution of AmM with that of N-terminal peptide metabolism. AmM was assayed with alanyl-AFC and N-terminal peptide metabolism was quantitated by HPLC (see Materials and Methods).

The pH optima for alanyl-AFC hydrolysis and the N-terminal hydrolysis of angiotensin III, hepta(5-11)substance P and Met^5-enkephalin by both endothelium and smooth muscle were at or near 7.0. AmM hydrolysis of alanyl-AFC was 14-fold more rapid on smooth muscle (116.7 ± 16.0 units/mg) than on endothelium (8.4 ± 0.2 units/mg). Similarly, as suggested by the earlier qualitative TLC studies, smooth muscle N-terminal inactivation of angiotensin III (1.56 ± 0.47 nmol/min/mg) was 14-fold greater than endothelium (0.11 ± 0.05 nmol/min/mg). Smooth muscle N-terminal degradation of hepta(5-11)substance P (2.49 ± 0.50 nmol/min/mg) and Met^5-enkephalin (18.9 ± 7.2 nmol/min/mg) was also comparably faster than on endothelium (0.27 ± 0.02 and 1.27 ± 0.18 nmol/min/mg respectively). Leucyl-AFC was also hydrolyzed more rapidly by smooth muscle (90.1 ± 17.9 units/mg) than by endothelium (15.0 ± 0.9 units/mg).

Table 2. Kinetics of N-terminal peptide hydrolysis by smooth muscle membrane aminopeptidase M

	K_m (μM)	V_{max} (nmol/min/mg)
des(Asp^1)Angiotensin I	2.2 ± 0.5 (N = 5)	32.5 ± 6.0
Angiotensin III	2.0 ± 0.4 (N = 7)	26.5 ± 1.0
Hepta(5-11)-substance P	53.9 ± 1.7 (N = 5)	109 ± 18
Met^5-Enkephalin	75.7 ± 3.5 (N = 4)	313 ± 2

Values are means ± SEM.

Kinetics of aminopeptidase M-peptide metabolism. Since smooth muscle did not contain any aminopeptidase activity beyond that which could be accounted for by the level of iAmM, smooth muscle plasma membrane was used to examine the kinetics of AmM-mediated N-terminal peptide metabolism. As shown in Table 2, the K_m values of des(Asp^1)angiotensin I and angiotensin III for AmM were 2.2 and 2.0 μM respectively. The $K_m$value obtained for hepta(5-11)substance P was significantly higher (53.9 μM) as was that for Met^5-enkephalin (75.7 μM). The maximal rates of peptide hydrolysis were 32.5 ± 6.0 and 26.5 ± 1.0 nmol/min/mg for des(Asp^1)angiotensin I and angiotensin III respectively (Table 2). Hepta(5-11)substance P and Met^5-enkephalin were hydrolyzed considerably faster (109 ± 18 and 313 ± 2 nmol/min/mg respectively).

Since the above data indicated that angiotensin III (K_m = 2 μM) would be a preferred substrate for AmM at low substrate concentrations, experiments were conducted to test this expectation. In individual reactions at low substrate concentrations (8 μM), hepta(5-11)substance P was hydrolyzed more rapidly than angiotensin III (Fig. 4A). However, when incubated together (8 μM each), the rate of angiotensin

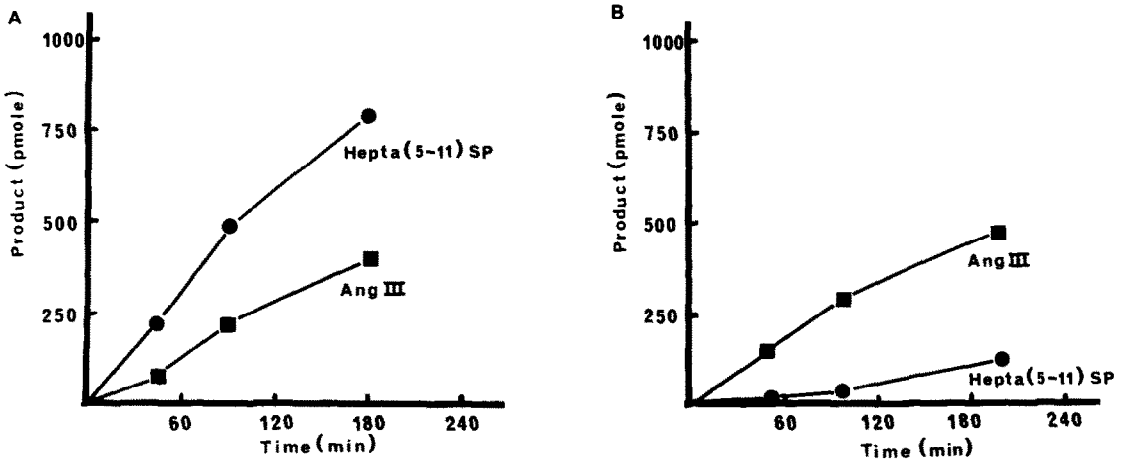

Fig. 4. N-terminal metabolism of hepta(5-11)substance P and angiotensin III with smooth muscle plasma membrane AmM. Peptides were incubated separately (A) or together (B) at a final substrate concentration of 8 μM. The metabolism of hepta(5-11)substance P and angiotensin III was determined as the rates of N-terminal Gln and Arg production respectively.

Table 3. Peptide inhibition of smooth muscle membrane aminopeptidase M-mediated Leu^5-enkephalin hydrolysis

Substrate	Competitive substrate	K_i (μM)
Leu^5-Enkephalin		
Leu^5-Enkephalin	Angiotensin III	0.34 ± 0.04 (N = 3)
Leu^5-Enkephalin	Hepta(5-11)-substance P	43.7 ± 6.3 (N = 3)
Leu^5-Enkephalin	Kallidin	62 (61, 64)
Leu^5-Enkephalin	Bradykinin	50

The rates of Leu^5-enkephalin hydrolysis were determined at three substrate concentrations in the presence of a range of concentrations of peptide (competitive substrate) inhibitors. Values are means ± SEM or the average of two determinations (kallidin) or a single determination (bradykinin).

III hydrolysis was not affected significantly, whereas little hepta(5-11)substance P was metabolized (Fig. 4B).

Inhibition by competitive peptide substrates/products. To quantitate the ability of each peptide substrate (or product) to inhibit N-terminal peptide metabolism, the initial velocities of enkephalin hydrolysis were determined at three substrate concentrations in the presence of various concentrations of peptide inhibitors added simultaneously. As shown in Table 3, all four peptides were effective competitive inhibitors with angiotensin III being the most potent (K_i = 0.34 μM). The K_i of hepta(5-11)substance P was 43.7 μM, while both the AmM substrate (kallidin) and product (bradykinin) were similarly effective (K_i = 62 and 50 μM respectively).

Inhibition by amastatin, bestatin and puromycin. Inhibitor studies of AmM enkephalin degradation demonstrated that both amastatin (Fig. 5) and bestatin were potent competitive inhibitors of enkephalin degradation by smooth muscle plasma membrane AmM with K_i values of 25 ± 5 nM and 2.2 ± 0.1 μM respectively (N = 3). Puromycin was significantly less potent (41 μM; average of two determinations).

DISCUSSION

AmM was identified previously in porcine aorta and mesenteric arteries as an intrinsic cell surface peptidase [5, 9]. More recent studies have also identified AmM in isolated porcine, rabbit [6] and rat [7, 8] cerebral microvessels. In the present study, immunoreactive AmM was found on both cultured endothelium and smooth muscle. Both cell types hydrolyzed alanyl-2-naphthylamide (or -AFC) (the

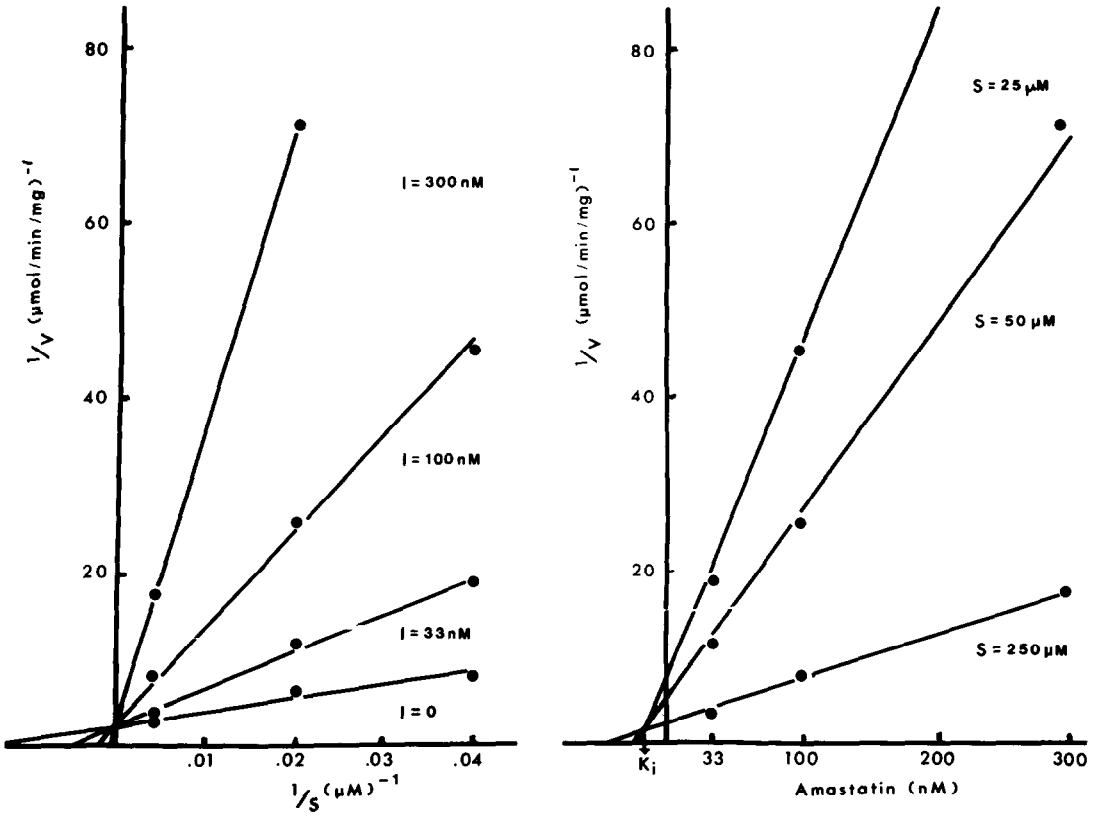

Fig. 5. Inhibition of smooth muscle AmM degradation of enkephalin (S) by a range of concentrations of amastatin (I). Amastatin was preincubated for 20 min (37°) with enzyme and buffer before addition of substrate.

preferred substrate for AmM) proportionate to the level of iAmM detected. The relationship of enzyme activity to iAmM in both cell types was the same as that found for renal brush border AmM. Further, endothelium, smooth muscle and renal AmM activities were similarly inhibited by amastatin, bestatin, puromycin and *o*-phenanthroline.

Consistent with the presence of iAmM, N-terminal hydrolyses of kallidin, des(Asp^1)angiotensin I, angiotensin III, Met^5-enkephalin and hepta(5-11)substance P were significant (though not necessarily exclusive) routes of peptide metabolism on both cell types. Further, the cellular distribution of this N-terminal peptide metabolism was directly proportional to that of AmM-mediated alanyl-AFC hydrolysis.

Since the above aminopeptidase inhibitors are not specific for AmM, other aminopeptidases may have contributed to the observed N-terminal peptide metabolism. This is likely to be the case for endothelium since, although no AmA was present, more leucyl-aminopeptidase activity was detected than could be accounted for by the level of iAmM. Whether this activity is due to contamination or represents a peripheral membrane protein as has been reported in preparations of brain synaptosomes [26] remains to be determined. Nevertheless, since comparative inhibition and cellular distribution studies could not clearly differentiate this activity from AmM, subsequent studies of AmM-mediated peptide metabolism were conducted with smooth muscle membrane which did not contain aminopeptidase activity beyond that accounted for by the level of iAmM.

Met^5-enkephalin was degraded more rapidly by smooth muscle AmM than any other peptide tested, and the K_m value obtained (75 μM) was similar to that found previously for cerebral microvessel AmM [6]. A somewhat similar K_m value was obtained for hepta(5-11)substance P (54 μM), although it was degraded only 30% as fast. Despite their even slower rates of hydrolysis, angiotensin III and des(Asp^1)-angiotensin I had the highest affinities for AmM. The K_m of angiotensin III for AmM (2 μM) was lower than that reported for angiotensin I with ACE (30 μM) [27] and comparable to that of bradykinin (1 μM), the preferred substrate for ACE [28]. High affinity was also evident in competitive substrate experiments where angiotensin III was the most effective peptide inhibitor tested (K_i = 0.34 μM).

The previously established cell surface localization of vascular AmM puts it in an excellent position to affect the local levels of vasoactive peptides in the microenvironment of cell surface receptors [5]. Although endothelial AmM conversion of kallidin to bradykinin is probably not physiologically significant, N-terminal metabolism of des(Arg^{10})kallidin could reduce B_1 kinin receptor activation since des(Arg^{10})kallidin is an order of magnitude more potent than des(Arg^9)bradykinin in stimulating B_1 receptors [29].

Despite the lower levels of AmM on endothelium, N-terminal hydrolysis was the only detectable route of degradation of enkephalins and hepta-(5-11)substance P on both endothelium and smooth muscle. Since the half-life of opioid peptides *in vivo* is increased by amastatin and bestatin [30], it is reasonable to suggest that endothelial and smooth muscle AmM may play a significant role in degrading locally released and/or plasma opioids. Although the N-terminal Arg^1-Pro^2-Lys^3-Pro^4 sequence of substance P makes it resistant to hydrolysis by most aminopeptidases including AmM [9], this sequence is removed by endothelial and smooth muscle DAP IV [22–24]. Thus, the co-localization of both DAP IV and AmM suggests a sequential pathway for metabolism of substance P.

Angiotensins I and II are converted by AmA into des(Asp^1)angiotensin I and angiotensin III respectively. Although no AmA was detected in aortic cells, AmA has been identified in plasma and microvasculature [4, 17]. Both des(Asp^1)angiotensin I and angiotensin III were degraded either in part (endothelium) or exclusively (smooth muscle) by N-terminal hydrolysis. The high affinity (low K_m) of both peptides for AmM suggests preferential metabolism at low (physiologic) substrate concentrations. A physiologic role for AmM is supported by recent studies where bestatin potentiated the *in vivo* effects of angiotensin III [31]. In addition, amastatin, the most potent inhibitor of AmM-mediated peptide degradation (K_i = 25 nM), increases the vasopressor effect of angiotensin III *in vivo* (studies in progress).

The K_i of angiotensin III as an inhibitor of enkephalin hydrolysis (0.34 μM) was lower than the K_m of angiotensin III for AmM (2 μM). These data suggest that both angiotensin III and its metabolite (des-Arg^1-angiotensin III) are potent inhibitors of AmM and that they may potentiate the levels of other peptides *in vivo* by inhibiting AmM-mediated degradation. Such an interpretation is consistent with the report that angiotensin III was the most potent peptide tested (K_i = 0.66 μM) in inhibiting aminopeptidase-mediated enkephalin degradation in human CSF [32].

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