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VASCULAR, PLASMA MEMBRANE AMINOPEPTIDASE M

METABOLISM OF VASOACTIVE PEPTIDES

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Abstract—Aminopeptidase M (EC 3.4.11.2), an enzyme present on the cell surface of vascular endothelium and/or smooth muscle, rapidly hydrolyzes leucyl- and arginyl-2-naphthylamides and a number of vasoactive peptides at physiologic pH. Utilizing both thin-layer chromatography and high pressure liquid chromatography, it was found that vascular aminopeptidase M converted kallidin to bradykinin and inactivated des(Asp^1)angiotensin I, angiotensin III, hepta(5-11)substance P and hexa(6-11)substance P. Aminopeptidase M did not, however, hydrolyze bradykinin, angiotensin I, angiotensin II, saralasin, vasopressin, oxytocin or any form of substance P containing a component of the Arg-Pro-Lys-Pro sequence. Both the naphthylamidase and peptidase activities were inhibited similarly by known aminopeptidase M inhibitors including *o*-phenanthroline, amastatin, bestatin and puromycin. However, inhibitors of angiotensin I converting enzyme (captopril), carboxypeptidase N (MERGETPA), neutral endopeptidase (phosphoramidon), post proline cleaving enzyme and dipeptidyl(amino)peptidase IV (diisopropylphosphofluoridate, DFP) were without effect. These results demonstrate that vascular, cell surface aminopeptidase M can selectively metabolize vasoactive peptides and may play a role in modulating their levels in the circulation and/or within the vessel wall.

Numerous studies have established that the vasculature contains a variety of enzymes that can form, convert and/or inactivate vasoactive peptides such as angiotensins, kinins and tachykinins. These vascular enzymes include renin (EC 3.4.99.19) [1, 2], kallikrein (EC 3.4.21.8) [3, 4], angiotensin I converting enzyme (ACE; EC 3.4.15.1) [5, 6] and dipeptidyl(amino)peptidase IV (DAP IV; EC 3.4.14.5) [7]. In recent work [7–9], we have found that a plasma membrane fraction prepared from hog aorta or mesenteric artery is enriched not only in ACE and DAP IV, but also in an enzyme immunologically indistinguishable from renal aminopeptidase M (AmM; EC 3.4.11.2). Although many studies have demonstrated that ACE converts angiotensin I to angiotensin II and inactivates kinins [5, 6, 10] and that DAP IV sequentially converts the undecapeptide substance P to biologically active C-terminal nona(3-11)- and hepta(5-11)-fragments [7, 11, 12], we are not aware of any studies that have specifically examined the differential metabolism of vasoactive peptides by AmM. Such a study may be particularly relevant in view of its vascular plasma membrane localization [9], since such metabolism could occur in the same micro-environment as the surface membrane peptide receptors.

In the present study, plasma membrane of hog aorta and mesenteric artery were prepared as previously described and the capacity of aminopeptidase M to hydrolyze synthetic substrates and vasoactive peptides was determined by fluorometric assay, thin-layer chomatography (TLC) and high pressure liquid chromatography (HPLC). Some of these experiments were briefly summarized in a recent abstract [13].

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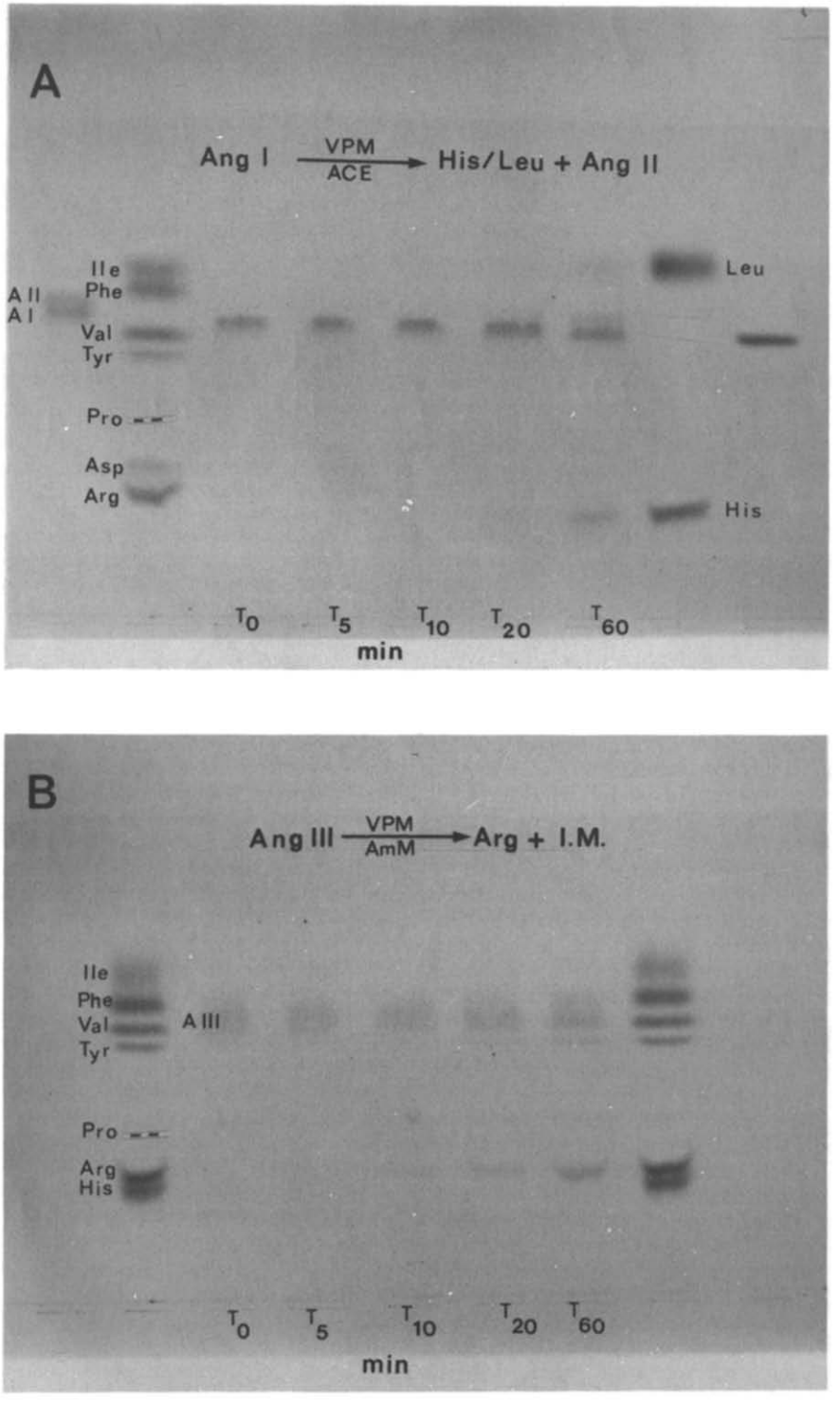
MATERIALS AND METHODS

Materials. All peptides were obtained from Vega Biochemicals (Tuscon, AZ) except for substance P and its lower molecular weight forms which were obtained from Peninsula Laboratories (San Carlos, CA). Naphthylamide substrates, *o*-phenanthroline, bestatin, amastatin, puromycin and phosphoramidon [*N*-(α-rhamnopyranosyloxyhydroxyphosphinyl-L-leucyl-L-tryptophan)] were from the Sigma Chemical Co. (St. Louis, MO). The converting enzyme inhibitor captopril was obtained from Squibb (Princeton, NJ) and the carboxypeptidase N inhibitor MERGETPA (D-L-mercaptoethanol-3-guanidino-ethylthiopropanoic acid) from Calbiochem-Behring (San Diego, CA). Amino acid standards, *o*-phthalaldehyde crystals, and *o*-phthalaldehyde reagent solution (OPA) were from the Pierce Chemical Co. (Rockford, IL) and the MN 300 Uniplates from Analtech, Inc. (Newark, DE).

Plasma membrane preparation. Aorta and mesenteric arteries were obtained from freshly slaughtered hogs, cleaned in 0.9% (w/v) saline, and subfractionated to the plasma membrane-enriched fraction as previously described [7–9]. Enrichments of 5′-nucleotidase (EC 3.1.3.5), ACE, DAP IV and AmM (11- to 17-fold) were comparable to those obtained in previous preparations. Suspensions of plasma membrane were diluted (1–3 mg/ml), aliquoted, and frozen until assayed (1–6 weeks). Storage over these time periods had no significant effect on enzyme activity.

Aminopeptidase M assay. Aminopeptidase M was assayed by the hydrolysis of leucyl-2-naphthylamide [14] as previously described [9]. Where indicated, aminopeptidase hydrolysis of arginyl-, α-glutamyl- and α-aspartyl-2-naphthylamides was also deter-


A
Ang I
VPM
ACE
His/Leu + Ang II
Ile
Phe
A II
A I
Val
Tyr
Pro
Asp
Arg
Leu
His
T0
T5
T10
T20
T60
min
B
Ang III
VPM
AmM
Arg + I.M.
Ile
Phe
Val
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A III
Pro
Arg
His
T0
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T10
T20
T60
min

Fig. 1(A) and (B).

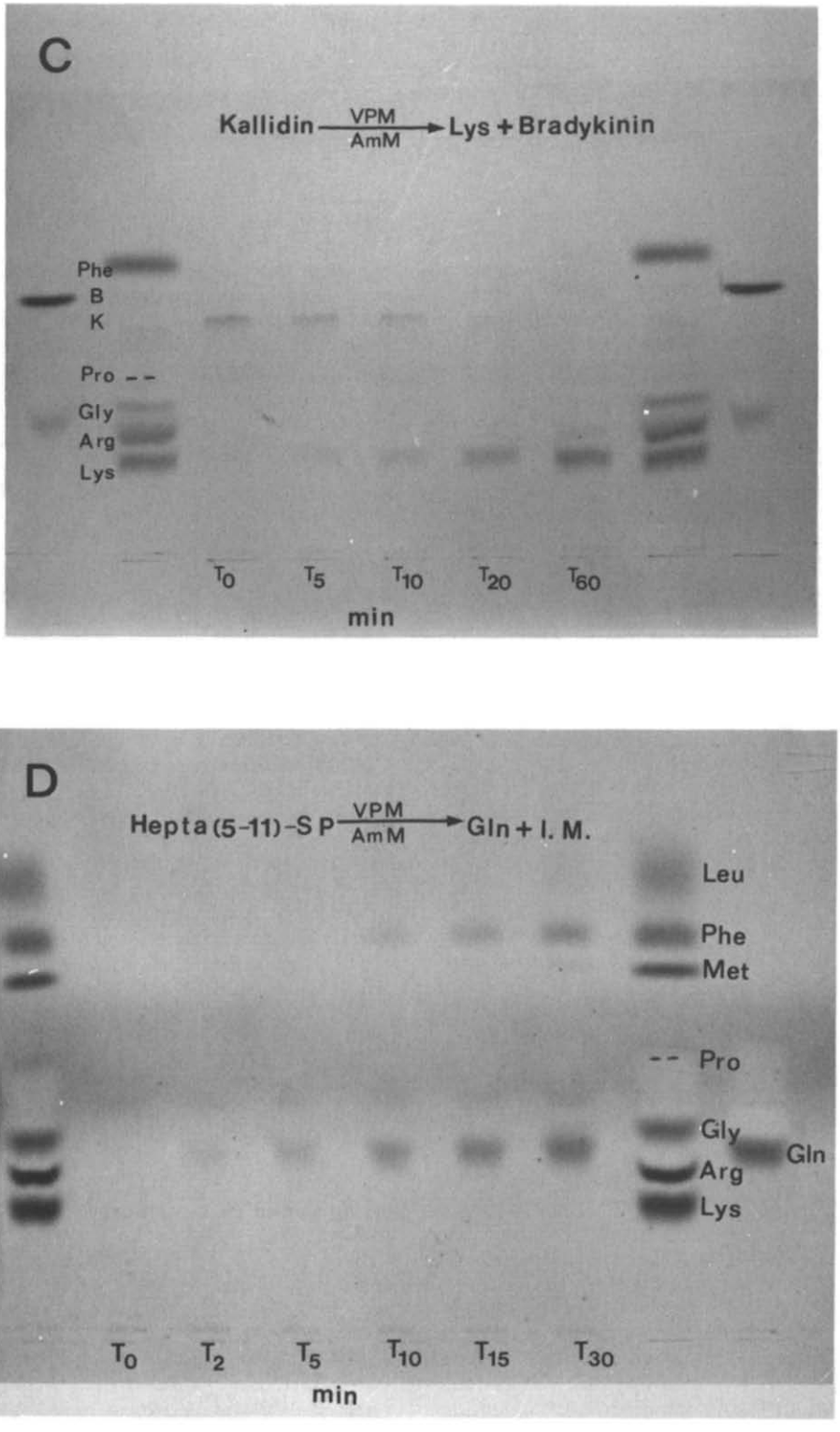

Fig. 1. Metabolism of angiotensin I (A), angiotensin III (B), kallidin (C) and hepta(5-11)substance P (D) by vascular plasma membrane (VPM) angiotensin I converting enzyme (ACE) or aminopeptidase M (AmM) during 30- to 60-min incubations. Metabolites and known standards were prepared and separated by thin-layer chromatography as described in Materials and Methods. Identified standards include angiotensin I (A I), angiotensin II (A II), angiotensin III (A III), kallidin (K) and bradykinin (B). I.M. denotes inactive metabolites.

mined as above. Enzyme specific activity is expressed as units/mg protein where one unit equals one nanomole substrate hydrolyzed per minute. Protein was determined with the protein assay kit of BioRad (Richmond, CA) using bovine serum albumin as a standard [15].

Peptide metabolism—TLC. Qualitative analysis of peptide metabolism was carried out by TLC on MN 300 cellulose plates. The standard incubation consisted of mixing the peptide (500 μM final concentration) in 40 μl buffer (100 mM Tris/HCl, pH 7.0) with 10 μl of the membrane fraction. At sequential time intervals, 5 μl aliquots were spotted on the plate and immediately dried. Plates were developed in butanol–acetic acid–water (4:1:5), and the products were visualized by staining with 0.4% (w/v) ninhydrin in acetone according to Toennies and Kolb [16]. In selected cases, the rate of product formation was quantified against known amino acid standards using a Photovolt recording densitometer with integrator.

Peptide metabolism—HPLC. Quantitative analysis of peptide metabolism was carried out by HPLC. The standard incubation consisted of mixing the peptide (25 μM final concentration) in 490 μl buffer (50 mM sodium phosphate, pH 7.0) with 10 μl of a dilution of the plasma membrane fraction. At sequential time intervals, 60 μl aliquots were immersed in a boiling water bath (5 min) to terminate the reaction and centrifuged in a Brinkmann table top centrifuge (3 min); the supernatant fraction was collected for analysis. Control experiments established that recovery of all peptide and amino acid metabolites was greater than 95%. Reaction rates, calculated during the first 15% of the reaction, were linear with time of incubation and proportional to the amount of enzyme used. Inhibitors were preincubated with enzyme and buffer for 20 min before addition of substrate.

A high pressure liquid chromatograph (Waters Associates; Milford, MA) consisting of two model 6000A pumps, a model 730 Data Module, a model 721 System Controller, a model 710B WISP Autosampler and a model 420 Fluorescence Detector were employed for the HPLC analysis. Standards and unknowns (10–60 μl) were automatically derivatized with OPA solution (20 μl) 3 min prior to chromatography (Pre-column Derivatization Program, Waters 710B WISP Autosampler) [17] and subsequently separated on a reverse phase column (Waters, 5 μm, C_{18}-Radial-PAK, 8 mm × 10 cm) at a constant flow rate of 2.5 ml/min utilizing a linear gradient from 100% Buffer A to 40% Buffer A/60% Buffer B (Gradient 1). Subsequently the column was washed with 100% Buffer B and then re-equilibrated in 100% Buffer A. For measurement of lysine, the above gradient was modified to a linear gradient from 100% Buffer A to 100% Buffer B (Gradient 2). Integration of sample peak areas and quantitation of metabolites against the most recent standard peak areas were automatically calculated by the data module. Standards were run every fifth injection.

The OPA solution was made fresh daily by mixing 3.8 ml *o*-phthaladehyde reagent solution, 0.1 ml methanol, 0.1 ml β-mercaptoethanol and 16.8 mg *o*-phthalaldehyde crystals. Buffer A was 100 mM sodium phosphate (pH 7.0) and Buffer B was a 50/50 (v/v) mixture of Buffer A and acetonitrile. Prior to use, both buffers were degassed by filtration through a Millipore AP Pre-Filter.

RESULTS

As previously found [9], the vascular plasma membrane AmM hydrolyzed leucyl-2-naphthylamide at a rate of 20.5 to 29.8 nmoles/min/mg. Arginyl-2-naphthylamide was also hydrolyzed although only at one-third the rate (7.3 to 9.4 units/mg). However, even during extended incubations, no aminopeptidase A-like hydrolysis of α-aspartyl- or α-glutamyl-2-naphthylamides could be detected. Hydrolysis of leucyl-2-naphthylamide was optimal at pH 7.2 with little activity below pH 6.5 or above pH 9.0.

Angiotensins. Incubation of the vascular plasma membrane with angiotensin I and subsequent separation of metabolites by TLC revealed that no N-terminal Asp product was produced. Rather, after 20 min and more clearly after 60 min, a metabolite co-migrating with angiotensin II appeared above angiotensin I (Fig. 1A). The only other metabolites were His and Leu. Since earlier experiments had established that the plasma membrane fraction contained angiotensin I converting enzyme and dipeptidase activities [7, 9], we confirmed that this observed reaction was true conversion of angiotensin I to angiotensin II via converting enzyme by repeating the incubation in the presence of captopril (10^{-4} M). Under these conditions, nearly complete inhibition was observed. The lack of N-terminal hydrolysis of angiotensin I and angiotensin II is consistent with the reported specificity of renal AmM for N-terminal basic and neutral amino acids compared to N-terminal acidic residues and our earlier observation that no significant α-glutamyl- and α-aspartyl-2-naphthylamidase activities were present. Finally, the angiotensin II antagonist saralasin (Sar^1-Ala^8-angiotensin II) was only marginally hydrolyzed when incubated with the plasma membrane fraction (not shown).

Angiotensin III, however, was metabolized by plasma membrane AmM, producing the initial N-terminal Arg metabolite within 5 min (Fig. 1B). By 60 min, Val and Tyr metabolites were also produced indicating sequential N-terminal hydrolysis. Des(Asp^1)angiotensin I, having the same N-terminus as angiotensin III, was also rapidly metabolized (not shown). Thus, plasma membrane AmM is an effective angiotensinase of both angiotensin III and des(Asp^1)angiotensin I, whereas angiotensin I, angiotensin II and saralasin are resistant to such inactivation.

Kinins. As shown in Fig. 1C, incubation of kallidin (Lys-bradykinin) with the plasma membrane resulted in (within 5 min) a metabolite which co-migrated with the basic amino acid lysine. A metabolite co-migrating with bradykinin (which stains only faintly) was also detectable. By 60 min, Phe and Arg metabolites were also produced. These results represent rapid hydrolysis of the Lys–Arg bond of kallidin by aminopeptidase M to produce lysine and bradykinin. Simultaneously, although at a slower

rate, the Pro[8]–Phe[9] bond of kallidin was hydrolyzed by converting enzyme into inactive metabolite and Phe-Arg (detected as Phe and Arg). To confirm this interpretation, the same incubation carried out with captopril (10^{-4} M) resulted in bradykinin and lysine metabolites only.

Although the N-terminal Arg of bradykinin is a basic amino acid, aminopeptidase M was ineffective in hydrolyzing its Arg-Pro N-terminal bond (not shown), presumably due to the presence of proline in the penultimate position [14]. Thus, vascular plasma membrane aminopeptidase M is a kinin converting enzyme but does not degrade bradykinin itself.

Tachykinins. Since we have shown previously that the vascular plasma membrane contains DAP IV, which sequentially converts the undecapeptide substance P to the biologically active nona(3-11)- and hepta(5-11)-forms of substance P [7], we found, as expected, that no N-terminal hydrolysis of substance P (Arg-Pro N-terminus) or nona(3-11)substance P (Lys-Pro N-terminus) occurred in the presence of diisopropylphosphofluoridate (DFP), a DAP IV inhibitor. Further, no N-terminal hydrolysis of deca(2-11)substance P or octa(4-11)substance P occurred due to the presence of proline at their N-termini (not shown). However, hepta(5-11)substance P was rapidly hydrolyzed, producing the initial N-terminal Gln metabolite within 2 min (Fig. 1D). By 10 min, a Phe metabolite was also produced, indicating sequential N-terminal hydrolysis. Hexa(6-11)substance P was also hydrolyzed at the N-terminus (not shown). Thus, AmM effectively inactivated both hepta(5-11)- and hexa(6-11)substance P, whereas any component of the Arg-Pro-Lys-Pro sequence at the N-terminus protected the peptide from degradation.

Cyclic peptides. As expected due to their cyclic structures (CyS–SCy), no significant hydrolysis of vasopressin or oxytocin occurred during incubation with the plasma membrane fraction.

Peptide metabolism—HPLC. To quantitatively study the metabolism of the above peptides at low substrate concentrations (25 μM), incubations were carried out and the metabolites were separated and quantitated by HPLC as described in Materials and Methods. Since control experiments had established that the rate of production of N-terminal amino acid was equivalent to the rate of peptide hydrolysis, that OPA-derivatives of amino acids fluoresce much more intensely than OPA-peptide derivatives [18, 19], and that some des(amino acid)peptide metabolites were subject to further hydrolysis [e.g. des(Arg[1])angiotensin III], quantitation of peptide hydrolysis was determined as the rate of N-terminal amino acid production over time. Utilizing Gradient 1, the retention times of Gln, Arg and Phe were 13.95, 18.24 and 20.55 min, respectively, whereas the peptide substrates and des(amino acid)metabolites eluted during the column wash with 100% Buffer B (Fig. 2). When conversion of kallidin to lysine and bradykinin was studied, Gradient 2 was used and the rate of Lys (retention time 24.6 min) formation determined (not shown). Injection of known amounts of amino acid standards over a range of 20–240 pmoles established that the OPA-derivatives of Gln, Arg, Phe and Lys could be accurately quantitated under these conditions (Table 1).

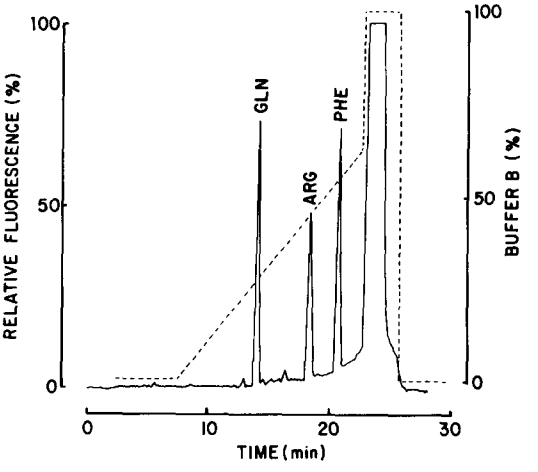

Fig. 2. Separation of OPA-derivatized amino acids (100 pmoles) by high pressure liquid chromatography utilizing Gradient 1 as described in Materials and Methods. Gradient (hatched line) is given as percent Buffer B.

As shown in Fig. 3, kallidin was converted to bradykinin (Lys product) at a rate of 8.3 nmoles/min/mg protein. Hepta(5-11)substance P (Gln product) was also rapidly hydrolyzed at a similar rate (7.1 units/mg) as were hexa(6-11)- and penta(7-11)-substance P (not shown). Angiotensin III (Arg product) was inactivated at a substantially slower rate (2.2 units/mg).

To confirm that the above reactions were due to the plasma membrane AmM, parallel reactions were carried out in the presence of known aminopeptidase M inhibitors including *o*-phenanthroline, amastatin,

Table 1. Quantitation of known amounts of amino acids after pre-column derivatization with *o*-phthalaldehyde*

Amounts injected (pmoles)	Amounts determined (pmoles)			
	Gln	Arg	Phe	Lys
20	19.7 ± 0.1	20.7 ± 0.2	20.4 ± 0.1	20.3 ± 0.1
40	39.9 ± 0.1	40.5 ± 0.2	41.0 ± 0.5	37.3 ± 2.8
80	81.0 ± 0.6	83.0 ± 1.4	81.6 ± 1.2	77.9 ± 1.9
240	243 ± 5	256 ± 4	248 ± 5	236 ± 40

* Samples were derivatized, separated by high pressure liquid chromatography, and quantitated as described in Materials and Methods. Values given are the mean ± S.E.M. of three determinations.

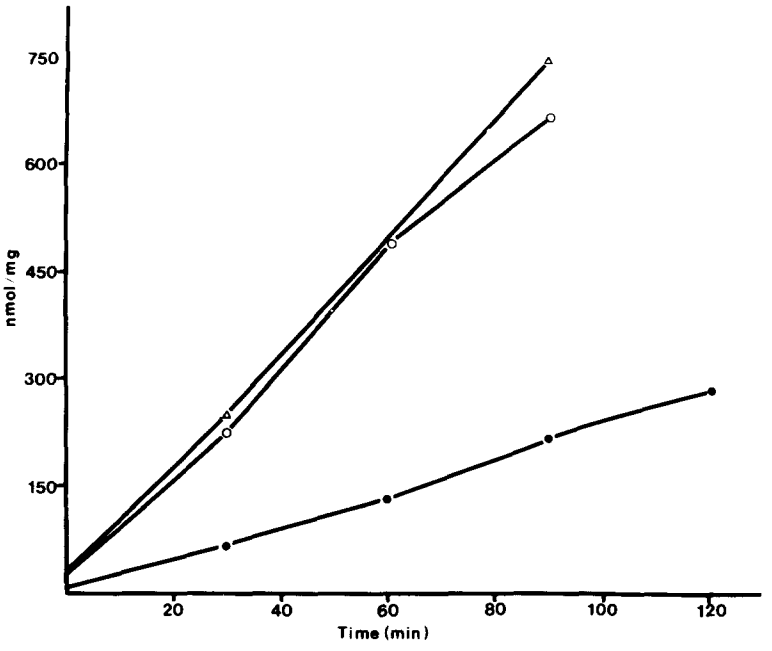

Fig. 3. Conversion of kallidin to bradykinin (△) and degradation of hepta(5-11)substance P (○) and angiotensin III (●) by vascular plasma membrane aminopeptidase M. The N-terminal amino acid product of each reaction, Lys, Gln and Arg respectively, was separated and quantified by high pressure liquid chromatography as described in Materials and Methods

bestatin and puromycin [20–22]. As shown in Table 2, the hydrolysis of leucyl-2-naphthylamide, kallidin, hepta(5-11)substance P and angiotensin III were all similarly inhibited. However, inhibitors of angiotensin I converting enzyme (captopril), carboxypeptidase N (MERGETPA) [23], neutral endopeptidase (phosphoramidon) [20, 24], dipeptidyl-(amino)peptidase IV and post proline cleaving enzyme (DFP) [25] had no significant effect.

As shown in Fig. 4, the competitive substrate angiotensin III was also a potent inhibitor of AmM (determined by the hydrolysis of leucyl-2-naphthylamide) with an I_{50} of approximately 1 μM. Although the previous TLC experiments had established that peptides such as angiotensin I, angiotensin II and saralasin were not substrates for AmM, nevertheless, all three peptides were also effective (though less potent) inhibitors.

DISCUSSION

Three specific peptidases are established as playing a role in the processing of vasoactive peptide *in vivo*. Angiotensin I converting enzyme, by hydrolysis of C-terminal dipeptides, converts angiotensin I to angiotensin II and inactivates kallidin and brady-

Table 2. Inhibition profile of aminopeptidase M hydrolysis of leucyl-2-naphthylamide, kallidin, hepta(5-11)substance P and angiotensin III*

Drug	Concn (M)	Inhibition (%)			
		Leu-2-Naph	Kallidin	Hepta(5-11)-substance P	Angiotensin III
Control		0	0	0	0
o-Phenanthroline	10^{-3}	100	100	99	97
Amastatin	10^{-5}	95	85	89	81
Bestatin	10^{-5}	48	23	30	21
Puromycin	5×10^{-5}	33	33	34	18
Captopril	10^{-4}	0		0	12
MEGETPA	10^{-4}	0	8	11	6
Phosphoramidon	10^{-5}	7	5	0	19
DFP	10^{-5}	0	0	0	0

* Inhibitors were preincubated with enzyme and buffer for 20 min prior to addition of substrate. Values given are the averages of two to five determinations.

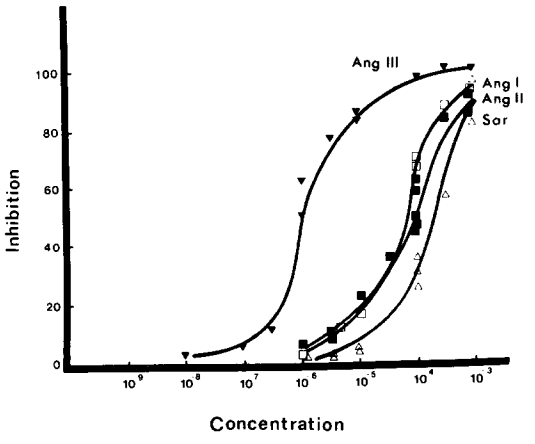

Fig. 4. Inhibition (%) of vascular plasma membrane aminopeptidase M leucyl-2-naphthylamide hydrolysis by a range of concentrations of angiotensin III (Ang III), angiotensin I (Ang I), angiotensin II (Ang II) and saralasin (Sar).

kinin [5, 6, 10]. Carboxypeptidase N (EC 3.4.17.3) hydrolyzes both kallidin and bradykinin at their C-termini [6] to produce the des(Arg)-forms of the peptides which are reported to have potent effects on B_1 receptors [26–28]. Aminopeptidase A (EC 3.4.11.7), which specifically hydrolyzes N-terminal acidic amino acids [29], is thought to play a role in converting angiotensin II to angiotensin III [30, 31].

Based on their reported substrate specificities, a group of peptidases including aminopeptidase M, leucine aminopeptidase (EC 3.4.11.2), alanine aminopeptidase (EC 3.4.11.–) and aminopeptidase B (EC 3.4.11.6) have the capacity to metabolize vasoactive peptides and are often collectively referred to as "angiotensinases". However, little progress has been made in determining their specific physiologic roles since definitive data concerning identification and localization has been lacking.

Recent work has established that vascular AmM exists as an intrinsic cell surface peptidase, and a circulating form of the enzyme is present in plasma [9]. The plasma membrane localization of AmM, unlike the cytosolic localization of leucine aminopeptidase, alanine aminopeptidase and aminopeptidase B [32], puts it in an excellent position to affect the local levels of susceptible peptides in the microenvironment of vascular cell surface peptide receptors. However, since the vascular plasma membrane preparation used contains surface membrane from both endothelium and smooth muscle [7–9], the cellular localization of AmM cannot be determined directly from these investigations. Nevertheless, recent studies in our laboratory have identified AmM in cultured vascular smooth muscle cells [33], and its presence in cultured endothelium is presently under investigation. In spite of its relatively broad specificity, the present study demonstrates that the N-terminal sequences or cyclic structures of most of the recognized vasoactive peptides make them resistant to AmM hydrolysis. Such resistant peptides include angiotensin I, angiotensin II, bradykinin, vasopressin, oxytocin, substance P and its deca(2-11)-, nona(3-11)- and octa(4-11)-forms. Aminopeptidase M does, however, rapidly convert kallidin to bradykinin and inactivate des(Asp^1)angiotensin I, angiotensin III and the hepta(5-11)- and hexa-(6-11)-forms of substance P (Fig. 5).

In view of the results of the present and previous studies, it is tempting to speculate that AmM may play a physiologically significant role in the processing of vasoactive peptides *in vivo*. Angiotensin III and des(Asp^1)angiotensin I were metabolized by vascular AmM and this hydrolysis may account for the short half-life of angiotensin III *in vivo* and may relate to the report of Gaynes *et al.* [34] that rapid inactivation of des(Asp^1)angiotensin I makes it an unlikely substrate for direct formation of angiotensin III *in vivo*. The absence of aminopeptidase A-like activity or amino-terminal hydrolysis of angiotensin I or II in the vascular plasma membrane fraction would indicate that conversion of angiotensin II to angiotensin III does not occur at the cell surface of large vessels and may be limited to the vasculature of specialized organs as has been reported in the adrenal gland [31]. If AmM is present in the same adrenal microvasculature, it may play a role in limiting local angiotensin III effects. Interestingly, the only angiotensin II hydrolysis which we could detect

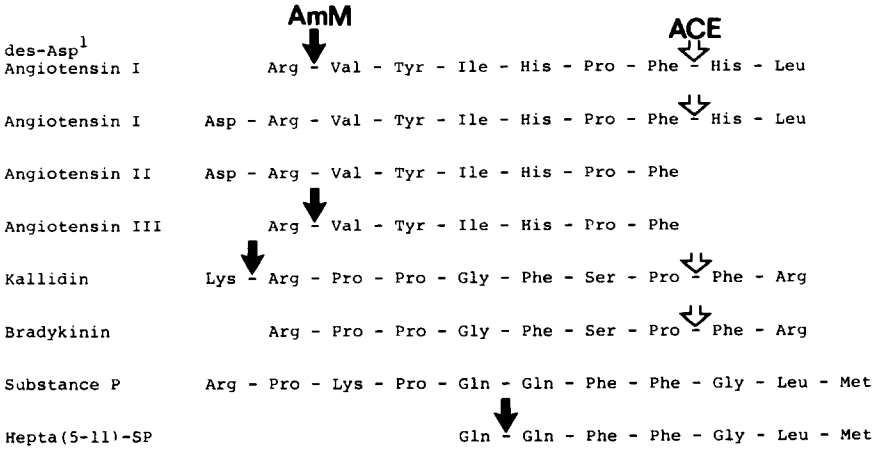

Fig. 5. Hydrolysis of angiotensins, kinins and tachykinins by vascular plasma membrane aminopeptidase (M) (AmM) and angiotensin I converting enzyme (ACE).

occurred by C-terminal release of phenylalanine, and this observation is consistent with the report of Magnan and Regoli [35] that the rabbit aorta bioassay preparation rapidly metabolized angiotensin II at the carboxy-terminus.

As noted earlier, AmM is present not only in the vasculature but also in plasma [9]. An aminopeptidase with a similar substrate and inhibition profile and which converts kallidin to bradykinin has also been reported in human plasma by Guimaraes and co-workers [36]. Although conversion of kallidin to bradykinin could be expected to have little physiologic affect on B_2 receptors, such a conversion could be significant regarding B_1 receptors where kallidin is nearly ten times as potent as bradykinin [27]. Glandular kallikreins, which produce kallidin, have been reported in both plasma [37, 38] and vascular tissues [3, 4] and B_1 receptors have been identified in numerous vessels including aorta and mesenteric vasculature [27]. Thus, although AmM does not degrade bradykinin, its conversion of vascular and/or circulating kallidin to bradykinin would significantly decrease vascular effects mediated by B_1 receptors. Such a role could be significant in view of the reports that B_1 receptors increase in response to tissue injury, particularly fever, inflammatory and allergic reactions [27].

Substance P and some of its C-terminal fragments are potent hypotensive/natriuetic peptides which dilate arterial and constrict venous smooth muscle by interacting with specific receptors which are common for the C-terminal fragments [39]. Substance P is present in the circulation and in substance P containing neurons innervating numerous vessels [40, 41]. Blumberg and Teichberg [42] have suggested that the N-terminal Arg-Pro-Lys-Pro sequence of substance P protects the peptide from inactivation. If circulating substance P and/or substance P released from neurons present in the vasculature have effects on vascular tone, mechanism for terminating its action would be required. Nakata *et al.* [43] have reported that substance P is released from nerve terminals but only the hepta(5-11)-form is subject to active re-uptake. Since we have found previously that substance P and nona(3-11)substance P are hydrolyzed by vascular plasma membrane DAP IV to the hepta(5-11)-form [7], it is possible that the plasma membrane DAP IV may be responsible for conversion of substance P to a form that can be removed (re-cycled?) by the neuron. Aminopeptidase M, in turn, could degrade any of the heptapeptide which escaped re-uptake.

Different vascular beds demonstrate different orders of potency to substance P and its C-terminal fragments which may indicate the presence of subtypes of substance P receptors [39]. Differences in potency may also be related to differential metabolism. For example, the high potency of octa(4-11)-substance P on the rabbit mesenteric vein [compared to substance P and hepta(5-11)substance P] might be related to the presence of proline at the N-terminus which could effectively stabilize it from hydrolysis by both AmM and DAP IV.

The present studies have demonstrated that vascular AmM has both the appropriate substrate specificity and localization to metabolize particular vasoactive peptides. Although these peptides were hydrolyzed at rates comparable to the hydrolysis of kallidin and angiotensin I by ACE, the relative rates of such metabolism *in vivo* remain to be determined. The concentration of such peptides in the vascular wall is not known, but their concentration in plasma is significantly below that used in the present studies (25 μM). Thus, further experiments will need to be conducted to determine the relative affinity (K_m) of AmM for kallidin, angiotensin III and hepta(5-11)-substance P. For example, although ACE hydrolyzes both kallidin and angiotensin I, its higher affinity for kinins, compared to angiotensin I, is associated with preferential degradation of kinins under *in vivo* conditions [5]. Further, although the present studies have excluded numerous vasoactive peptides as AmM substrates, the possibility that other known or unknown endogenous peptides may also be hydrolyzed by AmM should not be ruled out.

In summary, the cell surface membrane of vascular smooth muscle contains aminopeptidase M, an enzyme that can selectively hydrolyze a variety of vasoactive peptides which are potential modulators of vascular tone and permeability. The specificity and inhibition profiles of aminopeptidase M differentiate it from other vascular peptidases thought to play a role in modulating the levels of vasoactive peptides including angiotensins I converting enzyme, carboxypeptidase N, aminopeptidase A and dipeptidyl(amino)peptidase IV. Nevertheless, further studies will be required on the relative affinity, and cellular (endothelial/smooth muscle) and vascular (adrenal, coronary, etc.) distribution of aminopeptidase M, along with the identification of specific inhibitors which can be used *in vivo*, before the significance of vasoactive peptide metabolism by vascular aminopeptidase M can be determined.

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