online commentary

Matrix metalloproteinase-2 cleavage of adrenomedullin produces a vasoconstrictor out of a vasodilator

Alfredo MARTÍNEZ*[1], Hae-Ryong OH*, Edward J. UNSWORTH†, Claudia BREGONZIO‡, Juan M. SAAVEDRA‡, William G. STETLER-STEVENSON* and Frank CUTTITTA*

*Cell and Cancer Biology Branch and Vascular Biology Faculty, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A., †Laboratory of Neurotoxicology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, U.S.A., and ‡Section of Pharmacology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, U.S.A.

MMPs (matrix metalloproteinases) play a major role in the pathogenesis of hypertension by altering the extracellular matrix during cardiovascular remodelling. In the present study we show that MMP-2, but not MMP-9, cleaves the vasodilator peptide AM (adrenomedullin). Addition of the AM-binding protein, complement factor H, prevents this cleavage, providing a hitherto unknown mechanism of action for this binding protein. We identified the signature cleavage fragments and found some of them in human urine, suggesting that MMP-2 processing of AM may occur *in vivo*. Synthetic AM fragments regulated blood pressure in rats. The larger peptides are vasodilators, as is intact AM, whereas intermediate fragments did not affect blood pressure. In contrast, AM(11–22) elicited vasoconstriction. Studies of AM receptor activation in Rat2 cells confirm that the larger AM cleavage peptides activated this receptor, whereas AM(11–22) did not. The present study defines a new mechanism through which MMP-2 may regulate blood pressure by simultaneously eliminating a vasodilator and generating a vasoconstrictor.

Key words: adrenomedullin, blood pressure, complement factor H, matrix metalloproteinase-2 (MMP-2), peptide fragment, vasoconstrictor.

INTRODUCTION

Hypertension is a major public health problem due to its high prevalence and increased risk of cardiovascular morbidity and mortality [1,2]. Development of hypertension is accompanied by cardiac and arterial remodelling, a process that eventually compromises organ function. While the precise molecular pathogenesis is not fully understood, recent studies suggested that MMPs (matrix metalloproteinases) contribute to this process by remodelling the extracellular matrix [2–4]. The MMP family includes more than 20 members that share structural domains, but differ in substrate specificity, cellular sources and transcriptional regulation. A common characteristic of these enzymes is their ability to degrade components of the extracellular matrix. This feature has relevance to almost every aspect of mammalian biology and pathophysiology [5].

Blood pressure is regulated by the interaction between the sympathetic nervous system and a complex panel of vasoactive peptides. Current translational research involves finding specific inhibitors for the enzymes that either activate hypertensive peptides (as in the case of angiotensin-converting enzyme) or degrade hypotensive peptides (as with neutral endopeptidases which cleave natriuretic peptides) [6,7]. AM (adrenomedullin), a potent and long-lasting vasodilator [8], is becoming increasingly attractive as a potential key mediator of blood pressure homoeostasis. In addition, plasma AM levels are increased in cardiovascular diseases, such as heart failure, hypertension and septic shock, where AM seems to play a protective role [9]. AM is a 52-amino-acid peptide with an internal disulphide bond between amino acids 16 and 21 and with an amidated tyrosine at the carboxy end [8]. We have previously described the existence of a serum-binding protein for AM and characterized it as factor H (complement factor H) [10]. This binding interaction with factor H is able to increase the activity of AM in several experimental models, but so far the molecular mechanism responsible for this enhancing effect is unknown [10]. The functions of AM are mediated through a complex receptor system that requires the presence of a seven-transmembrane domain polypeptide known as CRLR (calcitonin-receptor-like receptor) and the single-transmembrane domain protein, RAMP (receptor-activity-modifying protein) 2 or 3. Receptor activation with nanomolar concentrations of AM results in intracellular elevation of cAMP levels [11].

Previous analysis of AM degradation by cell membrane extracts indicated a potential role for aminopeptidases and MMPs, with a pattern different from those involved in angiotensin II formation or atrial natriuretic peptide degradation [12]. Considering the importance of AM and the gelatinase subfamily of the MMPs on hypertension, we decided to study whether there is an interaction between both regulatory systems.

In the present study we report on the specific degradation of AM by MMP-2, but not by MMP-9. We also present the protective effect that factor H exerts on the half-life of AM in the presence of MMP-2, thus providing an explanation for the observed increase of AM activity in the presence of its binding protein. Furthermore, we identified some MMP-2-specific digestion products of AM in the urine of normal individuals, indicating that this process occurs *in vivo*. In addition, one of the peptide products exhibited a delayed vasoconstrictor activity in rats without interacting with CRLR/RAMP2, suggesting that other independent receptor systems may be involved in this activity. Therefore specific MMP-2 inhibitors may be useful in maintaining local AM levels and thus mitigate hypertension.

Abbreviations used: AM, adrenomedullin; CGRP, calcitonin-gene-related peptide; CRLR, calcitonin-receptor-like receptor; ET, endothelin; factor H, complement factor H; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; RAMP, receptor-activity-modifying protein.

[1] To whom correspondence should be addressed (email martinea@mail.nih.gov).

EXPERIMENTAL

Chemicals

Synthetic human AM was purchased from Peninsula Laboratories (San Carlos, CA, U.S.A.). Purified human factor H was obtained from Sigma (St. Louis, MO, U.S.A.). Predicted AM fragments were synthesized by Princeton Biomolecules (Langhorne, PA, U.S.A.) with a purity higher than 95 %. Human recombinant MMP-2 and MMP-9 were obtained and activated as described previously [13]. At the end of the activation period enzyme samples were diluted and assayed for activity using the thiopeptolide assay, as described previously [14].

Digestion reactions

Synthetic AM (240 μg/reaction) was exposed to 11.5 μg of MMP-2 or MMP-9 in low-salt collagenase buffer (50 mM Tris, pH 7.5, 50 mM NaCl and 0.02 % Brij) for various periods of time in the presence or absence of 6 mg of factor H. These amounts correspond to an approximate molar ratio of 1:250:250 (enzyme/AM/factor H). The reaction was stopped with EDTA. Digestion reactions were run in 16 % Tricine gels (Invitrogen, Carlsbad, CA, U.S.A.) under reducing conditions and the gels were stained with Gel Code Blue Stain Reagent (Pierce, Rockford, IL, U.S.A.).

HPLC

Digestion reactions were also loaded into an analytical reverse phase R2H HPLC column (5 mm × 100 mm; Poros, Applied Biosystems, Foster City, CA, U.S.A.) in a 10–60 % acetonitrile gradient over 10 min, and the protein peaks eluted from the column were detected with a wavelength of 230 nm.

MS

The protein peaks identified by HPLC were further characterized by MS. One microlitre of each HPLC fraction was mixed with 1 μl of α-cyano-4-hydroxycinnamic acid. One microlitre of each mixture was applied to a MALDI (matrix-assisted laser-desorption ionization) plate and allowed to air dry. The plate was loaded into a PerSeptive Biosystems Voyage DE mass spectrometer (ABI, Foster City, CA, U.S.A.). The instrument was calibrated with angiotensin I using a two-point calibration, angiotensin I at *m/z* 1296.7 and the matrix dimer at *m/z* 379.0. The laser intensity used to observe the peptides was 2721 with an accelerating voltage of 25 000 V. The extraction delay time was 50 ns. One hundred laser shots comprised a spectrum. The resulting data were analysed using Voyager Data Explorer (ABI).

Detection of AM fragments in urine

Urine (250 ml) from healthy male volunteers was extracted through C-18 Sep-Pak cartridges (Waters Corporation, Milford, MA, U.S.A.). The eluate was freeze dried, resuspended in 5 ml of 5 % acetonitrile in water plus 0.1 % trifluoroacetic acid, and fractionated by HPLC in a C-18 preparative column (30 mm × 30 cm; Delta Pack, Waters Corporation) using a 5–60 % acetonitrile gradient over 155 min at a flow rate of 15 ml/min. The column had been previously standardized with the synthetic peptides (see Figure 3a). Fractions were freeze dried, resuspended in sample buffer (Invitrogen) and run on 12 % Bis/Tris gels (Invitrogen) under reducing conditions. Peptides were transferred on to nitrocellulose filters, and Western blotting was performed with a previously characterized antibody against AM [15] and a chemiluminescence kit (ECL Plus™ Western Blotting Detection System, Amersham Biosciences, Piscataway, NJ, U.S.A.).

Blood pressure measurements

The effects of AM peptide fragments on blood pressure were measured as described previously [16]. Male 10-week-old Lewis/ssncr rats (SAIC, Frederick, MA, U.S.A.) were anaesthetized with 3 % halothane, intubated and maintained with 1 % halothane in 70 % nitrous oxide/30 % oxygen (VMS Anesthesia Machine, Matrix Medical Inc., Orchard Park, NY, U.S.A.) at 82 strokes/min. Rectal temperature was monitored throughout the experiment. A PE50 catheter was placed on to the right femoral artery and arterial blood pressure was recorded through a P23XL transducer (Grass Instruments, Quincy, MA, U.S.A.). Peptides were injected into the right femoral vein through another catheter. To confirm that changes in blood pressure were not an artifact of the anaesthesia, the experiments were repeated in conscious animals. After catheters were placed under anaesthesia, the animals were allowed to recover for 24–48 h before taking blood pressure measurements. All procedures were performed under a protocol approved by the National Institutes of Health.

Measurement of cAMP response

The rat fibroblast cell line Rat2 was obtained from the A.T.C.C. (Manassas, VA, U.S.A.) and kept in RPMI 1640 medium supplemented with 10 % fetal calf serum (Invitrogen). Accumulation of intracellular cAMP was measured as described previously [10]. Cells were seeded into 24-well plates at 2×10^4 cells/well and incubated at 37 °C in 5 % CO_2/95 % air until they reached 80 % confluency. Before the assay, cells were incubated for 15 min in TIS medium (RPMI 1640 plus 10 μg/ml transferrin, 10 μg/ml insulin and 50 nM sodium selenite) containing 1 % BSA, 1 mg/ml bacitracin and 100 μM isobutylmethylxanthine. Peptides were applied in the same medium for 5 min at the indicated concentrations in a volume of 250 μl. The reaction was terminated by adding an equal volume of ice-cold ethanol. cAMP contents were measured using the Biotrac cAMP radioimmunoassay (Amersham).

RESULTS

AM is a substrate for MMP-2

MMP-2 rapidly cleaves synthetic AM in a time-dependent manner as demonstrated by the progressive appearance of lower-molecular-mass bands in polyacrylamide gels (Figure 1, lanes 1–4). The resistance of AM to degradation by MMP-9 (Figure 1, lanes 9–12) underscores the specificity of MMP-2 degradation, considering that both enzyme preparations (MMP-2 and MMP-9) are able to efficiently digest a variety of common substrates, including gelatin and thiopeptolide (results not shown). Factor H, the serum-binding protein for AM, is not a substrate for either one of the MMPs (results not shown), but addition of factor H completely prevents MMP-2-mediated degradation of AM (Figure 1, lanes 5–8). In contrast, factor H does not interfere with the ability of MMP-2 to degrade thiopeptolide (results not shown), demonstrating that prevention of AM degradation by MMP-2 is dependent on the specific protein–protein interaction between AM and factor H.

Analysis of these digestion reactions by reverse-phase chromatography reveals a rapid decrease in the area of the peak representing the intact peptide (arrow in Figure 2a) and the concomitant progressive appearance of additional new peaks

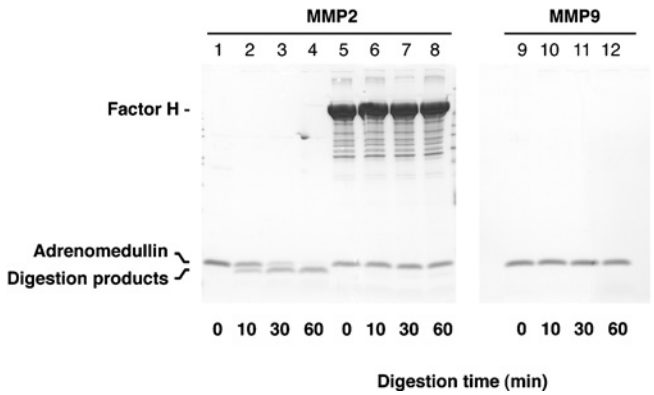

Figure 1 MMP-2, but not MMP-9, degraded AM in the absence of factor H

Synthetic AM was exposed to MMP-2 (lanes 1–8) or MMP-9 (lanes 9–12) in the presence (lanes 5–8) or absence (lanes 1–4 and 9–12) of factor H. Individual reactions were stopped at the indicated times and the resulting peptides separated by electrophoresis in 16 % polyacrylamide gels. Lanes 1–4 show a progressive degradation of the original peptide and a concomitant appearance of digestion products.

(Figures 2b–2e). MS analysis identifies the AM fragments generated by MMP-2 digestion. These include AM(8–52), AM(11–52), AM(23–52), AM(29–52), AM(11–28) and AM(11–22). The amino acid patterns at the cleavage sites are compatible with the predicted motifs for MMP-2 targets [17]. Two of the AM cleavage peptides, AM(8–52) and AM(11–52), maintain both the intramolecular disulphide ring structure and the α-amide, two characteristics required for AM receptor activation [18]. Two other fragments, AM(23–52) and AM(29–52), retain only the terminal amide; whereas the remaining two fragments, AM(11–28) and AM(11–22), have the loop but not the carboxy end of the molecule. We synthesized all these peptides to further characterize their biological activities.

Predicted fragments of MMP-2-digested AM are found *in vivo*

To investigate whether some of the fragments obtained by *in vitro* digestion of AM are also present in a biological fluid, we assayed urine samples from healthy human volunteers for signature AM peptide fragments generated by MMP-2 cleavage. Urine is an abundant source for both MMP-2 [19] and AM [8]. First, we standardized a preparative C-18 HPLC column using the synthetic peptides as markers and identified the fractions in which particular peptides eluted from the column (Figure 3a, arrows). Using the same conditions, we fractionated the equivalent of 250 ml of urine and analysed selected fractions by Western blotting with a well-characterized antibody against AM [15]. Chemiluminescent detection reveals a moiety of approx. 6 kDa (that co-migrates with synthetic AM) in fractions 95 and 96, and smaller fragments in fractions 57, 58, 63 and 64 (Figure 3b). Comparison of these results with the elution profile of the synthetic peptides identifies the low-molecular-mass band observed in fractions 57 and 58 as AM(29–52), the one in fractions 63 and 64 as AM(23–52), and the larger peptide of fractions 95 and 96 as undigested AM. As our detection antibody recognizes the carboxy end of AM [15], only the fragments containing this region could be detected by Western blot analysis.

Peptide fragments exhibit both hypotensive and hypertensive activity

Vasodilatation is the best characterized function of AM [8,9]. To understand the physiological implications of the digestion of

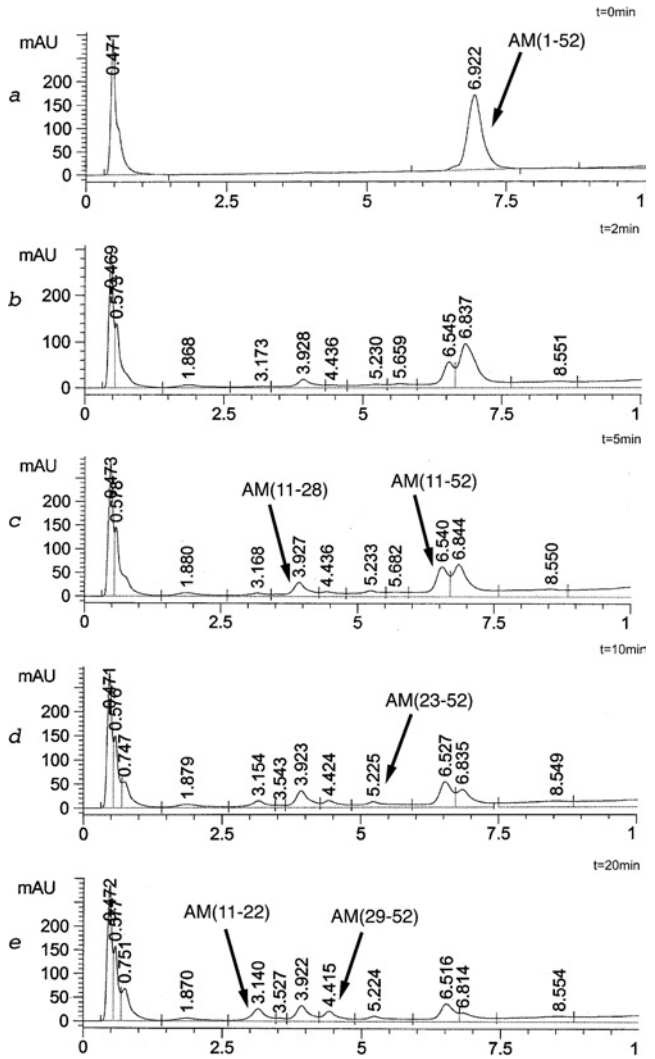

Figure 2 MMP-2 digestion of AM generated novel peptide fragments

Digestion reactions were stopped at the indicated times and then analysed by HPLC in a reverse-phase column. The single peak at time = 0 corresponds to the intact AM peptide. This peak progressively diminished over time, whereas additional peaks began to appear. The fractions exhibiting new peaks were analysed by MS and some of them are identified in the Figure.

AM by MMP-2, we studied the impact of the AM fragments on blood pressure regulation in anaesthetized rats. Untreated animals had a systolic blood pressure of 125 ± 10 mmHg ($n = 5$). Intact AM (Figure 4a) and the peptide fragments containing both the intramolecular disulphide loop and the final tyrosine amide (Figure 4b) induced a deep and long-lasting hypotension (reduction of 55 ± 5 mmHg, $n = 5$). In contrast, AM(23–52), AM(29–52) and AM (11–28) do not have any discernible effect on blood pressure regulation (Figure 4c). Interestingly, the fragment AM(11–22) showed a vasopressor effect (71 ± 24 mmHg over basal levels, $n = 5$, Figure 4d), indicating that MMP-2 degradation of AM not only attenuated the hypotensive effect of AM, but that it also generated a hypertensive fragment. Furthermore, the hypotensive and hypertensive peptides exhibited very different modes of action, with the vasodilator molecules acting almost immediately following injection, and the vasoconstrictor peptide needing 4–5 min before eliciting its effect. This divergence in timing suggests that AM(11–22) may be acting through a receptor system independent of the one used by AM and the larger peptide

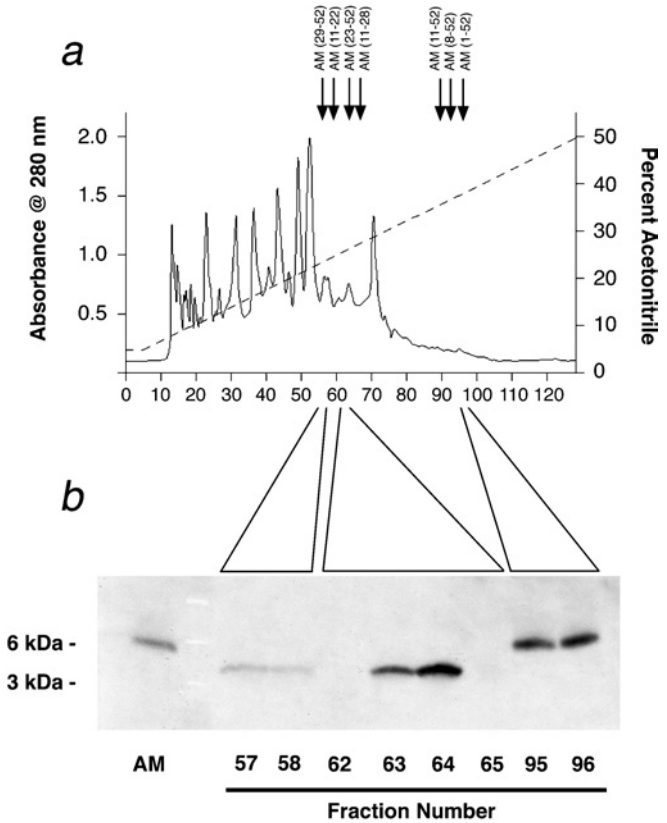

Figure 3 Urine from normal volunteers contained products of MMP-2-dependent AM degradation

After an initial C-18 cartridge extraction, the equivalent of 250 ml of urine was fractionated through a C-18 HPLC preparative column following an acetonitrile gradient (broken line) (**a**). Select fractions were loaded on to a 12 % polyacrylamide gel, transferred on to nitrocellulose, labelled with a polyclonal antibody against AM and developed by chemiluminescence (**b**). Synthetic AM (3 ng) was added in the first lane (AM) as a positive control.

fragments. To demonstrate that these effects on blood pressure are not influenced by the anaesthesia, the synthetic peptides were also tested in conscious animals. Given the complexity of the technique, only one animal per peptide was used and therefore no quantification is possible, but in all cases the individual values were within the range obtained in anaesthetized animals.

The vasoconstrictor peptide does not act via a classic AM receptor mechanism

To determine if AM peptide fragments elicit their hypotensive and hypertensive effects via the AM receptor, we examined their cAMP response in fibroblasts. Rat2 is a fibroblast cell line that contains a well-characterized AM receptor and does not bind other members of the AM peptide family, such as CGRP (calcitonin-gene-related peptide) [20,21]. AM(8–52) and AM(11–52) elicited an intracellular elevation of cAMP in Rat2 cells equal to the one induced by the intact AM molecule, whereas the rest of the fragments did not elevate cAMP levels over basal values (Figure 5a). These observations confirmed a previous report that indicated loss of either the disulphide bond or the terminal amide results in inability of receptor binding [18]. We also studied whether the peptides that did not elicit a cAMP response are in fact competitors for AM binding to its receptor. This seemed not to be the case, since increasing concentrations of these peptides did not have any effect on the induction of the cAMP response

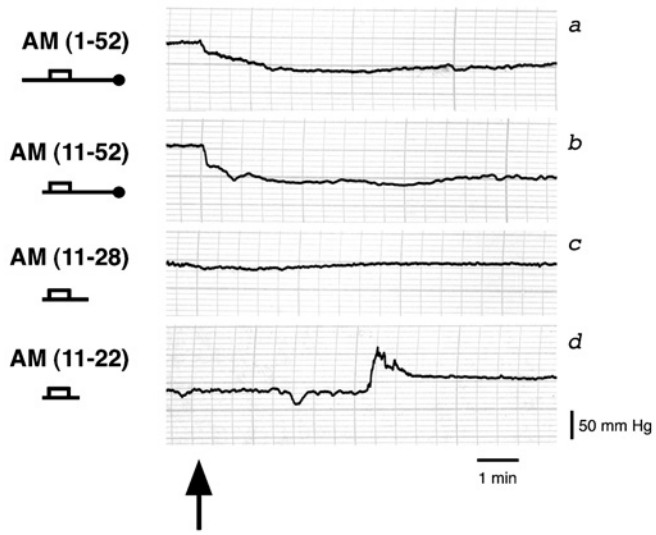

Figure 4 One of the new fragments elevated blood pressure in rats

Typical recordings of the blood pressure modifications elicited by intact AM (**a**) and its fragments (**b**–**d**) in anaesthetized rats. The peptides AM(8–52) and AM(11–52) induced hypotension, and only the effect of the latter is shown (**b**). The fragments AM(23–52), AM(29–52) and AM(11–28) did not have any effect, and only the effect of the AM(11–28) is shown (**c**). The small peptide AM(11–22) induced vasoconstriction several minutes after injection (**d**). The arrow indicates the time when the peptides were injected. The horizontal bar represents 1 min. The vertical bar represents 50 mmHg. Schematic drawings of the structure of the AM peptides are provided underneath their name. The solid circle represents the amide group at the carboxy end and the rectangle indicates the intramolecular disulphide bond.

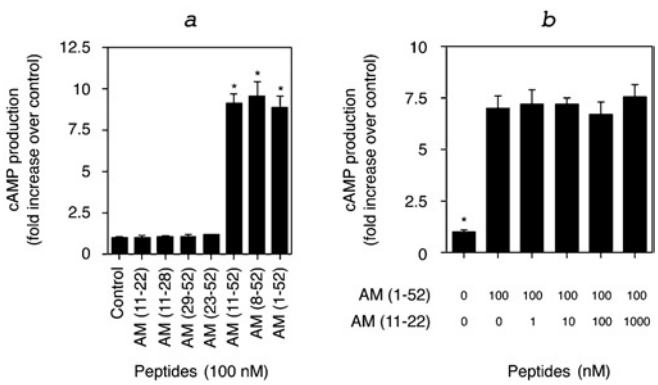

Figure 5 Some digestion products were no longer able to activate the AM receptor

Intracellular levels of cAMP were quantified by radioimmunoassay as an indirect measurement of AM receptor activation in Rat2 cells. (**a**) Intact AM and the two larger fragments induced a significant elevation of cAMP when compared with the addition of PBS (Control), whereas the rest of the test peptides did not have any effect on the levels of cAMP (*$P < 0.001$). (**b**) Addition of different concentrations of AM(11–22) did not affect the response elicited by the intact peptide AM(1–52). The control value is significantly different from all the treatments (*$P < 0.001$), but these are not statistically significant among themselves. The results are expressed as the means ± S.D. for 8 independent measurements.

by AM, as exemplified by AM(11–22) (Figure 5b). This was also compatible with the vasoconstrictor activity of AM(11–22) being mediated through a different receptor system.

DISCUSSION

In the present study we have shown that AM is rapidly cleaved by MMP-2 and that as a result smaller peptides are sequentially

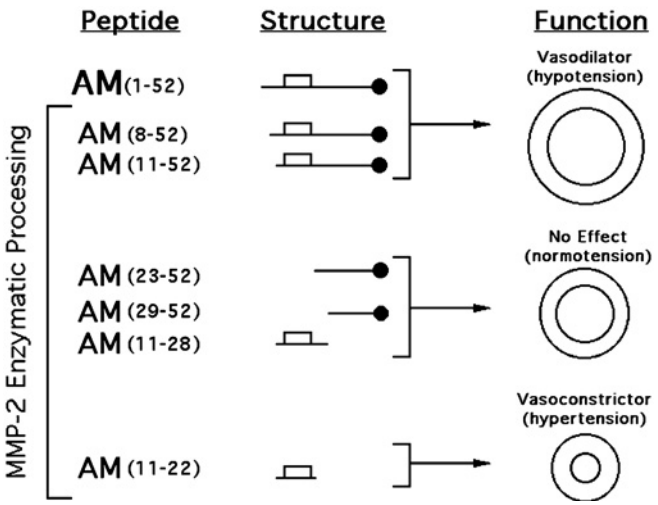

Figure 6 Diagram of the sequential degradation of AM into smaller peptides and the physiological implications of the process

The larger peptides maintain the vasodilator capability characteristic of intact AM, whereas intermediate peptides lack vasomotor activity, and the small peptide AM(11–22) is a vasoconstrictor. Schematic diagrams are as in Figure 4.

produced. The specificity of the reaction was demonstrated by the fact that another gelatinase, MMP-9, did not affect AM integrity. Interestingly, the AM-binding protein factor H efficiently blocked MMP-2 degradation of AM, while it did not inhibit catabolism of other MMP-2 substrates. Also, factor H was not a substrate for MMP-2. Unique peptide fragments, consistent with MMP-2-mediated degradation of AM, were found in a biological fluid, suggesting that the *in vivo* catabolism of AM is predicted, at least in part, by MMP-2 activity, although at this moment we cannot exclude that other MMPs may also process AM *in vivo*. The peptide products that retained the amidated end of the molecule and the internal disulphide loop induced vasodilatation *in vivo* and elevated cAMP levels in a cell line known to express the specific AM receptor, whereas peptides lacking either of these features did not. The smallest peptide fragment, which contains little more than the internal disulphide loop, elicited a hypertensive response without influencing AM receptor activation. These observations suggest that MMP-2 activity may contribute to the hypertensive phenotype both by reducing the levels of the potent vasodilator AM and by generating a new hypertensive peptide (Figure 6).

In a previous study, Lewis et al. [12] exposed synthetic human AM to cell membrane preparations from sheep kidney, adrenal and lung tissues. Among the series of peptide fragments generated in this manner, one of the most abundant was AM(8–52), which we have also found as one of the first products resulting from MMP-2-mediated degradation. In the discussion of that article [12], the authors predicted the need for metalloproteinase and aminopeptidase activities to explain all the fragmentary peptide products observed.

We had previously reported that factor H enhances AM function in several experimental model systems, such as induction of cAMP in Rat2 fibroblasts [10], growth promotion of breast cancer cell lines [10] and reduction of insulin secretion by rat isolated pancreatic islets [22]. We also determined that this enhancing effect was not due to changes in the kinetics of AM binding to its receptor [10]. In the present study we have shown that factor H was able to prevent the degradation of AM by MMP-2, therefore defining a mechanism by which the binding protein prolongs the half-life of AM and thus increases its biological effects. This regulatory process may be very relevant in understanding the biology of AM in regulating vascular tone. Current radioimmunoassay protocols used to determine the circulating levels of AM require a preliminary purification step through a C-18 cartridge. An unanticipated consequence of this step is the removal of factor H and the fraction of AM that is bound to it [10]. As a result, these protocols measure only free AM and the reported concentrations are extremely low, varying from 1 to 10 pM [8]. Obviously, these levels are insufficient for receptor activation and have raised doubts about the endocrine effects of AM. On the other hand, the existence of a serum AM-binding protein, which circulates at a high concentration (500 μg/ml) [23] and protects AM from proteolytic degradation, suggests that the most important pool of AM may be the one that circulates bound to factor H, rather than the free fraction. Additional studies are needed to address this provocative question in disease states.

The finding of a vasoconstrictor peptide as a result of the digestion of AM by MMP-2 was intriguing and is in accordance with a previous study [24] where different fragments of AM were arbitrarily synthetized and assayed for blood pressure regulation activity in anaesthetized rats. Although the fragment AM(11–22) was not studied in that report [24], the structurally related peptide acetyl-AM(16–21) showed vasopressor activity. Another similar fragment, AM(11–26), was purified from bovine adrenal medulla, but, although the peptide induced vasopressor activity, the elevation in blood pressure lasted only about 70 s [25]. The involvement of the catecholamine system and α-adrenergic receptors has been suggested as a mechanism to explain the pressor activity of these particular fragments of AM [24,26]. Our observations of the delay between the administration of AM(11–22) and the onset of the vasopressor response, together with the disconnection of AM(11–22) and the AM receptor system, is consistent with the existence of an alternative mechanism responsible for the reported effects. Treatment with α-adrenergic receptor blockers, such as phentolamine or reserpine, may resolve whether AM(11–22) affects this pathway. AM has been previously found in urine and its physiological impact in renal homoeostasis has been extensively studied [8]. Our finding of MMP-2-induced fragments of AM being in the urine suggests that renal physiology may also be modulated by these peptide fragments, either by affecting directly glomerular/tubular function or by altering renal hemodynamics. Further investigation in this field is warranted.

There are precedents where MMP-2 can also cleave other vasoactive substances. For instance, this enzyme digests the vasoconstrictor big endothelin-1, ET-1(1–38), yielding the smaller peptide ET-1(1–32), which is also a vasoconstrictor [27]. Furthermore, CGRP is also degraded by MMP-2, but, in contrast with our observations on AM, the resulting vasoconstriction is just a consequence of the reduction in the levels of the vasodilator CGRP [28]. All these observations suggest that MMP-2 inhibitors may be useful as regulators of high blood pressure.

The ability of MMP-2 to radically change the physiological action of a substrate has been demonstrated before in the case of MCP-3 (monocyte chemoattractant protein-3). Full-length MCP-3 induces chemotaxis of mononuclear inflammatory cells, but the cleavage products act as general chemokine antagonists and dampen inflammation [29].

MMP-2 is also a neutral endopeptidase and investigators are intensively studying clinical applications for inhibitors of this enzyme family [6,7]. There is evidence that three different neutral endopeptidase inhibitors (candoxatrilat, thiorphan and SCH32615) enhance clinical aspects attributed to AM [30–32]. Whether this enhancement is due to MMP-2 blockade or to other endopeptidases remains to be determined. Specific inhibitors of MMPs are being used to prevent extracellular matrix remodelling in cardiovascular diseases, with encouraging results [33]. It should

be interesting to investigate whether AM, a protective agent in cardiovascular disorders [9], is responsible for some of the beneficial actions generated by MMP inhibitors in cardiovascular disease states. Our observation that MMP-2-mediated digestion of AM generates a vasoconstrictor out of the original vasodilator peptide defines a new mechanism by which MMP-2 further contributes to regulation of vasomotor tone, and suggests that application of MMP-2 inhibitors may regulate blood pressure.

REFERENCES

1 Yildirir, A., Batur, M. K. and Oto, A. (2002) Hypertension and arrhytmia: blood pressure control and beyond. Europace **4**, 175–182
2 Mulvany, M. J. (2002) Small artery remodeling and significance in the development of hypertension. News Physiol. Sci. **17**, 105–109
3 D'Armiento, J. (2002) Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. Trends Cardiovasc. Med. **12**, 97–101
4 Intengan, H. D. and Schiffrin, E. L. (2001) Vasopeptidase inhibition has potent effects on blood pressure and resistance arteries in stroke-prone spontaneously hypertensive rats. Hypertension **38**, 581–587
5 Brinckerhoff, C. E. and Matrisian, L. M. (2002) Matrix metalloproteinases: a tail of a frog that became a prince. Nat. Rev. Mol. Cell Biol. **3**, 207–214
6 Nawarskas, J., Rajan, V. and Frishman, W. H. (2001) Vasopeptidase inhibitors, neutral endopeptidase inhibitors, and dual inhibitors of angiotensin-converting enzyme and neutral endopeptidase. Heart Dis. **3**, 378–385
7 Corti, R., Burnett, J. C., Rouleau, J. L., Ruschitzka, F. and Luscher, T. F. (2001) Vasopeptidase inhibitors: a new therapeutic concept in cardiovascular disease? Circulation **104**, 1856–1862
8 López, J. and Martínez, A. (2002) Cell and molecular biology of the multifunctional peptide, adrenomedullin. Int. Rev. Cytol. **221**, 1–92
9 Eto, T. (2001) A review of the biological properties and clinical implications of adrenomedullin and proadrenomedullin N-terminal 20 peptide (PAMP), hypotensive and vasodilating peptides. Peptides **22**, 1693–1711
10 Pío, R., Martínez, A., Unsworth, E. J., Kowalak, J. A., Bengoechea, J. A., Zipfel, P. F., Elsasser, T. H. and Cuttitta, F. (2001) Complement factor H is a serum-binding protein for adrenomedullin, and the resulting complex modulates the activities of both partners. J. Biol. Chem. **276**, 12292–12300
11 McLatchie, L. M., Fraser, N. J., Main, M. J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M. G. and Foord, S. M. (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature **393**, 333–339
12 Lewis, L. K., Smith, M. W., Brennan, S. O., Yandle, T. G., Richards, A. M. and Nicholls, M. G. (1997) Degradation of human adrenomedullin(1–52) by plasma membrane enzymes and identification of metabolites. Peptides **18**, 733–739
13 Fridman, R., Fuerst, T. R., Bird, R. E., Hoyhtya, M., Oelkuct, M., Kraus, S., Komarek, D., Liotta, L. A., Berman, M. L. and Stetler-Stevenson, W. G. (1992) Domain structure of human 72-kDa gelatinase/type IV collagenase. Characterization of proteolytic activity and identification of the tissue inhibitor of metalloproteinase-2 (TIMP-2) binding regions. J. Biol. Chem. **267**, 15398–15405
14 Ye, Q. Z., Johnson, L. L., Yu, A. E. and Hupe, D. (1995) Reconstructed 19 kDa catalytic domain of gelatinase A is an active proteinase. Biochemistry **34**, 4702–4708
15 Martínez, A., Miller, M. J., Unsworth, E. J., Siegfried, J. M. and Cuttitta, F. (1995) Expression of adrenomedullin in normal human lung and in pulmonary tumors. Endocrinology **136**, 4099–4105
16 Martínez, A., Julián, M., Bregonzio, C., Notari, L., Moody, T. W. and Cuttitta, F. (2004) Identification of vasoactive non-peptidic positive and negative modulators of adrenomedullin using a neutralizing antibody-based screening strategy. Endocrinology **145**, 3858–3865
17 Turk, B. E., Huang, L. L., Piro, E. T. and Cantley, L. C. (2001) Determination of protease cleavage site motifs using mixture-based oriented peptide libraries. Nat. Biotechnol. **19**, 661–667
18 Eguchi, S., Hirata, Y., Iwasaki, H., Sato, K., Watanabe, T. X., Inui, T., Nakajima, K., Sakakibara, S. and Marumo, F. (1994) Structure–activity relationship of adrenomedullin, a new vasodilatory peptide, in cultured rat vascular smooth muscle cells. Endocrinology **135**, 2454–2458
19 Thrailkill, K. M., Kumar, S., Rosenberg, C. K., Auten, K. J. and Fowlkes, J. L. (1999) Characterization of matrix metalloproteinases in human urine: alterations during adolescence. Pediatr. Nephrol. **13**, 223–229
20 Coppock, H. A., Owji, A. A., Austin, C., Upton, P. D., Jackson, M. L., Gardiner, J. V., Ghatei, M. A., Bloom, S. R. and Smith, D. M. (1999) Rat-2 fibroblasts express specific adrenomedullin receptors, but not calcitonin-gene-related-peptide receptors, which mediate increased intracellular cAMP and inhibit mitogen-activated protein kinase activity. Biochem. J. **338**, 15–22
21 Choksi, T., Hay, D. L., Legon, S., Poyner, D. R., Hagner, S., Bloom, S. R. and Smith, D. M. (2002) Comparison of the expression of calcitonin receptor-like receptor (CRLR) and receptor activity modifying proteins (RAMPs) with CGRP and adrenomedullin binding in cell lines. Br. J. Pharmacol. **136**, 784–792
22 Martínez, A., Pío, R., López, J. and Cuttitta, F. (2001) Expression of the adrenomedullin binding protein, complement factor H, in the pancreas and its physiological impact on insulin secretion. J. Endocrinol. **170**, 503–511
23 Whaley, K. and Ruddy, S. (1976) Modulation of the alternative complement pathways by β1H globulin. J. Exp. Med. **144**, 1147–1163
24 Watanabe, T. X., Itahara, Y., Inui, T., Yoshizawa-Kumagaye, K., Nakajima, K. and Sakakibara, S. (1996) Vasopressor activities of N-terminal fragments of adrenomedullin in anesthetized rat. Biochem. Biophys. Res. Commun. **219**, 59–63
25 Kitamura, K., Matsui, E., Kato, J., Katoh, F., Kita, T., Tsuji, T., Kangawa, K. and Eto, T. (2001) Adrenomedullin(11–26): a novel endogenous hypertensive peptide isolated from bovine adrenal medulla. Peptides **22**, 1713–1718
26 Champion, H. C., Friedman, D. E., Lambert, D. G., Murphy, W. A., Coy, D. H. and Kadowitz, P. J. (1997) Adrenomedullin(16–31) has pressor activity in the rat but not the cat. Peptides **18**, 133–136
27 Fernandez-Patron, C., Radomski, M. W. and Davidge, S. T. (1999) Vascular matrix metalloproteinase-2 cleaves big endothelin-1 yielding a novel vasoconstrictor. Circ. Res. **85**, 906–911
28 Fernandez-Patron, C., Stewart, K. G., Zhang, Y., Koivunen, E., Radomski, M. W. and Davidge, S. T. (2000) Vascular matrix metalloproteinase-2-dependent cleavage of calcitonin gene-related peptide promotes vasoconstriction. Circ. Res. **87**, 670–676
29 McQuibban, G. A., Gong, J. H., Tam, E. M., McCulloch, C. A., Clark-Lewis, I. and Overall, C. M. (2000) Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. Science **289**, 1202–1206
30 Lisy, O., Jougasaki, M., Schirger, J. A., Chen, H. H., Barclay, P. T. and Burnett, J. C. (1998) Neutral endopeptidase inhibition potentiates the natriuretic actions of adrenomedullin. Am. J. Physiol. Renal Physiol. **44**, F410–F414
31 Wilkinson, I. B., McEniery, C. M., Bongaerts, K. H., MacCallum, H., Webb, D. J. and Cockcroft, J. R. (2001) Adrenomedullin (ADM) in the human forearm vascular bed: effect of neutral endopeptidase inhibition and comparison with proadrenomedullin NH_2-terminal 20 peptide (PAMP). Br. J. Clin. Pharmacol. **52**, 159–164
32 Rademaker, M. T., Charles, C. J., Cooper, G. J., Coy, D. H., Espiner, E. A., Lewis, L. K., Nicholls, M. G. and Richards, A. M. (2002) Combined angiotensin-converting enzyme inhibition and adrenomedullin in an ovine model of heart failure. Hypertension **39**, 93–98
33 Creemers, E. E., Cleutjens, J. P., Smits, J. F. and Daemen, M. J. (2001) Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? Circ. Res. **89**, 201–210

Received 2 June 2004/22 July 2004; accepted 13 August 2004
Published as BJ Immediate Publication 13 August 2004, DOI 10.1042/BJ20040920