



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

INHIBITION OF AMINOPEPTIDASES N, A AND W

A RE-EVALUATION OF THE ACTIONS OF BESTATIN AND INHIBITORS OF ANGIOTENSIN CONVERTING ENZYME

STEPHEN TIEKU and NIGEL M. HOOPER*

Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

(Received 29 June 1992; accepted 4 August 1992)

Abstract—The effects of a range of metallopeptidase inhibitors on the activities of the porcine kidney cell surface zinc aminopeptidases, aminopeptidase A (AP-A; EC 3.4.11.2), aminopeptidase N (AP-N; EC 3.4.11.7) and aminopeptidase W (AP-W; EC 3.4.11.16), have been directly compared. Amastatin and probenstin were effective against all three aminopeptidases, with the concentration of inhibitor required to cause 50% inhibition (I_{50}) in the low micromolar range ($I_{50} = 1.5\text{--}20\ \mu\text{M}$), except for probenstin with AP-N which displayed an I_{50} of 50 nM. Actinonin failed to inhibit significantly either AP-A or AP-W, and thus can be considered a relatively selective inhibitor ($I_{50} = 2.0\ \mu\text{M}$) of AP-N. In contrast, bestatin was a relatively poor inhibitor of AP-N ($I_{50} = 89\ \mu\text{M}$) and failed to inhibit AP-A, but was more potent towards AP-W ($I_{50} = 7.9\ \mu\text{M}$). Thus, some of the observed chemotherapeutic actions of bestatin may be due to inhibition of cell-surface AP-W. A number of other metallopeptidase inhibitors, including inhibitors of endopeptidase-24.11 (EC 3.4.24.11) and membrane dipeptidase (EC 3.4.13.11), and the carboxylalkyl and phosphoryl inhibitors of angiotensin converting enzyme (EC 3.4.15.1) failed to inhibit significantly AP-A, AP-N or AP-W. However, AP-W was inhibited with I_{50} values in the micromolar range by the sulphhydryl converting enzyme inhibitors rentiapril ($I_{50} = 1.6\ \mu\text{M}$), zofenoprilat ($I_{50} = 7.0\ \mu\text{M}$) and YS 980 ($I_{50} = 17.7\ \mu\text{M}$). Neither AP-A nor AP-N were affected by these sulphhydryl compounds. Inhibition of AP-W may account for some of the side effects noted with the clinical use of the sulphhydryl converting enzyme inhibitors. The availability of compounds which are totally selective for AP-W over any of the other mammalian cell surface zinc aminopeptidases may aid in identifying endogenous substrates, and thus physiological or pathophysiological role(s) of AP-W.

Mammalian cell surface peptidases are involved in the metabolism of a range of biologically active peptides, including peptide hormones, neuropeptides and dietary peptides [1]. Some of them are proving to be therapeutic targets in a variety of disease states, including heart disease, inflammation and metastasis [2, 3]. Several cell surface peptidases have recently been identified as cluster differentiation (CD[†]) antigens. For example, endopeptidase-24.11 (EC 3.4.24.11) has been shown to be identical to the common acute lymphocytic leukaemia antigen (CALLA; CD10) [4], aminopeptidase N (AP-N; EC 3.4.11.2) to CD13 [5], dipeptidyl peptidase IV (EC 3.4.14.5) to CD26 [6] and aminopeptidase A (AP-A; EC 3.4.11.7) to the murine β -lymphocyte differentiation antigen BP1/6C3 [7]. The role(s) of these peptidases in metastasis is unclear, although the termination or generation of peptide signals affecting the proliferation of transformed and normal cells is a possibility. In addition, AP-N has been identified recently as a receptor in both pigs and humans for the coronaviruses TGEV and 229E, respectively [8, 9].

The zinc aminopeptidases constitute a relatively large subdivision of the mammalian cell surface

peptidase family. To date four distinct enzymes have been characterized: AP-N, AP-A, aminopeptidase P (AP-P; EC 3.4.11.9) and aminopeptidase W (AP-W; EC 3.4.11.16). AP-P is somewhat different from the other three in being anchored in the plasma membrane by a glycosyl-phosphatidylinositol moiety [10] and in having a strict requirement for Pro in the penultimate position of susceptible substrates [11]. AP-N, the best characterized of this class, displays a broad substrate specificity [12] and has been identified as the major activity releasing the N-terminal Tyr from the enkephalins [13]. The enzyme may also play a role in the metabolism of cholecystokinin-8 [14] and neurokinin A [15]. AP-A hydrolyses acidic residues from the N-terminus of susceptible substrates and may be involved in the *in vivo* conversion of angiotensin II to angiotensin III [16]. AP-W preferentially hydrolyses short peptides and exhibits maximal rates towards dipeptides in which the P₁' residue is aromatic [17, 18]. So far, AP-W has not been implicated in the metabolism of any biologically active peptide, due in part to the lack of a selective inhibitor. The specificities of these latter three aminopeptidases overlap to a certain extent often leading to difficulties in identifying unequivocally the enzyme responsible for the metabolism of a particular peptide.

Although several inhibitors of the aminopeptidases are available, most of these are effective on more than one enzyme. We observed recently that certain compounds designed as supposedly selective inhibitors of angiotensin converting enzyme (EC

* Corresponding author. Tel. (0532) 333163; FAX (0532) 333167.

† Abbreviations: AP-A, aminopeptidase A; AP-N, aminopeptidase N; AP-W, aminopeptidase W; AP-P, aminopeptidase P; CD, cluster differentiation antigen; I_{50} , concentration of inhibitor required to cause 50% inhibition.

3.4.15.1) also inhibited AP-P with I_{50} values in the micromolar range [19]. In the present study we have extended this work by directly comparing the effect of a range of converting enzyme inhibitors on the activities of AP-A, -N and -W in an attempt to identify compounds which could be used to discriminate between these activities and thus be useful tools for delineating the role of a particular peptidase in the metabolism of a biologically active peptide. In addition, we have reassessed the inhibitory profile of a number of compounds which have been characterized as inhibitors of the zinc aminopeptidases.

MATERIALS AND METHODS

Materials. AP-N and AP-W were purified from pig kidney cortex as described previously [17, 20]. Both enzyme preparations were apparently homogenous as assessed by SDS-PAGE. AP-N had a specific activity of $41.2 \mu\text{mol}$ 4-methyl-7-coumarylamide/min/mg of protein, and AP-W a specific activity of $15.03 \mu\text{mol}$ PheNH₂/min/mg of protein. Microvillar membranes were prepared from pig kidney cortex as described by Booth and Kenny [21], except that the 15,000 g centrifugation steps were each extended from 12 to 15 min. Enalaprilat (MK 422), lisinopril (MK 521), L155,212, L155,502 and L155,524 were gifts from Merck, Sharp and Dohme Research Laboratories (Rahway, NJ, U.S.A.). Spiraprilat (SCH 33861) was a gift from Schering-Plough Research (Bloomfield, NJ, U.S.A.). Pentoprilat (CGS 13934) was a gift from Ciba-Geigy Corporation (Summit, NJ, U.S.A.). Quinaprilat (PD 109548-1) and indolaprilat (PD 110021-0) were gifts from Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI, U.S.A.). Cilazaprilat (Ro 31-3113/000) was a gift from Roche Products Ltd (Welwyn Garden City, U.K.). Rentiapril (SA 446) and YS 980 were gifts from Santen Pharmaceutical Co. Ltd (Osaka, Japan). Ramiprilat was a gift from Hoechst Pharmaceutical Research Laboratories (Milton Keynes, U.K.).

Zofenoprilat (SQ 26,703), fosinoprilat (SQ 27,519), ceranopril (SQ 29,852) and captopril (SQ 14,225) were gifts from Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ, U.S.A.). Probestin was a gift from Dr T. Aoyagi (Tokyo, Japan). Arphamenines A and B and Asp-PheNH₂ were obtained from the Sigma Chemical Co. (Poole, U.K.). All other materials were from sources noted previously.

Enzyme assays. AP-A was assayed in pig kidney microvillar membranes (50 μg protein) with α -Glu-4-methyl-7-coumarylamide (0.2 mM) as substrate in 0.1 M Tris-HCl, 1 mM CaCl₂, pH 7.4 at 37°. The product, 4-methyl-7-coumarylamide, was detected fluorimetrically at an excitation wavelength of 370 nm and an emission wavelength of 442 nm [22]. AP-N (100 ng) was assayed with L-Ala-4-methyl-7-coumarylamide (0.2 mM) as substrate in 0.1 M Tris-HCl, pH 7.4 at 37° [22]. AP-W (100 ng) was assayed with Asp-PheNH₂ (1 mM) as substrate in 0.1 M Tris-HCl, pH 7.4 at 37°. The product, PheNH₂, was separated from the substrate and was quantified by reverse phase HPLC as described for Gly-D-Phe [23]. Incubations were performed in duplicate with each concentration of inhibitor. Enzyme and inhibitors were preincubated for 15 min at 4°.

RESULTS

The effect on the activity of AP-A, -N and -W of specific metallopeptidase inhibitors (at a final concentration of 0.1 mM) was examined (Table 1). AP-A was inhibited significantly (>69%) only by amastatin and probestin. Actinonin and arphamenine B caused partial inhibition of AP-A. AP-N was inhibited significantly (>91%) by actinonin, amastatin and probestin, but less so (52%) by bestatin. AP-W was inhibited significantly (>89%) by amastatin, bestatin and probestin. Arphamenine A also caused partial inhibition of AP-W. The inhibitors of endopeptidase-24.11, phosphoramidon, thiorphan and L155,524, and the inhibitor of membrane dipeptidase, cilastatin, failed to inhibit

Table 1. Effect of metallopeptidase inhibitors on the activities of AP-A, -N and -W

Class of peptidase	Inhibitor	Relative activity (%) at an inhibitor concentration of 0.1 mM		
		AP-A	AP-N	AP-W
Aminopeptidase	None	100.0	100.0	100.0
	Actinonin	75.0 ± 2.4	9.0 ± 1.0	104.0 ± 1.1
	Amastatin	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Arphamenine A	100.0 ± 3.0	74.0 ± 4.7	50.0 ± 3.0
	Arphamenine B	68.8 ± 2.0	105.0 ± 8.2	93.0 ± 6.2
	Bestatin	100.0 ± 2.5	48.0 ± 11.0	11.0 ± 1.0
	Probestin	31.0 ± 6.8	0.0 ± 0.0	6.8 ± 2.0
Endopeptidase	Phosphoramidon	100.0 ± 0.0	95.0 ± 0.0	97.3 ± 3.0
	Thiorphan	105.0 ± 2.0	84.0 ± 3.2	70.0 ± 14.0
	L155,524	100.0 ± 0.0	93.0 ± 0.0	86.0 ± 2.0
Dipeptidase	Cilastatin	103.0 ± 2.6	103.0 ± 4.0	83.0 ± 4.4

The aminopeptidases were assayed with the appropriate substrate as described in Materials and Methods.

The results are the means ± SEM of three independent determinations with each inhibitor.

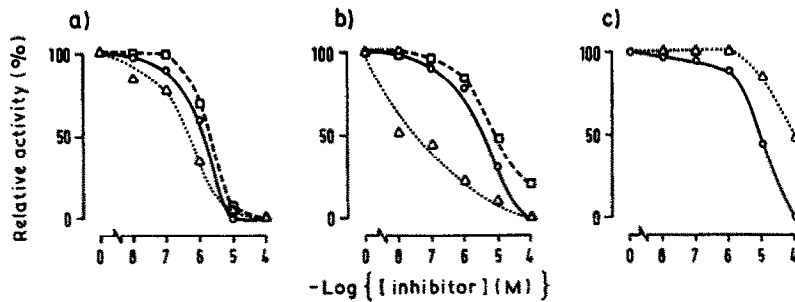


Fig. 1. Inhibition of AP-A, -N and -W by amastatin, bestatin and probestin. The aminopeptidases were assayed with the appropriate substrate as described in Materials and Methods. Each point is the mean of triplicate determinations. (a) Amastatin; (b) probestin; (c) bestatin. (□) AP-A; (Δ) AP-N; (○) AP-W.

Table 2. Effect of angiotensin converting enzyme inhibitors on the activities of AP-A, -N and -W

Class of inhibitor	Inhibitor	Relative activity (%) at an inhibitor concentration of 0.1 mM		
		AP-A	AP-N	AP-W
Sulphydryl	None	100.0	100.0	100.0
	Captopril	88.2 ± 0.0	94.2 ± 2.0	43.6 ± 3.0
	Rentiapril	87.2 ± 1.7	90.0 ± 0.4	0.0 ± 0.0
	YS 980	94.0 ± 3.4	92.0 ± 1.0	20.0 ± 2.0
Carboxylalkyl	Zofenoprilat	94.0 ± 0.0	95.0 ± 0.0	17.6 ± 6.6
	Benazeprilat	98.0 ± 6.1	100.0 ± 0.0	92.0 ± 2.6
	Cilazaprilat	110.0 ± 5.0	88.0 ± 2.0	73.0 ± 4.6
	Enalaprilat	120.0 ± 0.0	86.0 ± 3.4	83.9 ± 3.5
	Indolaprilat	104.0 ± 3.0	70.0 ± 0.5	80.5 ± 2.0
	Lisinopril	103.0 ± 2.6	100.0 ± 0.0	72.3 ± 6.0
	L155,212	96.0 ± 4.0	98.0 ± 1.6	86.5 ± 0.2
	L155,502	100.0 ± 11.0	100.0 ± 0.0	90.0 ± 2.0
	Pentoprilat	100.0 ± 5.0	76.0 ± 0.0	73.0 ± 0.5
	Quinaprilat	104.0 ± 11.0	85.0 ± 2.2	90.5 ± 2.5
Phosphoryl	Ramiprilat	105.0 ± 10.0	76.0 ± 0.6	107.0 ± 5.0
	Spiraprilat	95.2 ± 5.0	77.0 ± 0.8	72.0 ± 0.7
	Ceranopril	100.0 ± 0.0	100.0 ± 0.0	87.0 ± 6.0
	Fosinoprilat	100.0 ± 0.0	98.0 ± 0.6	94.8 ± 2.5

The aminopeptidases were assayed with the appropriate substrate as described in Materials and Methods.

The results are the means ± SEM of three independent determinations with each inhibitor.

any of the aminopeptidases significantly (maximally 17%), except for thiorphan which caused 30% inhibition of AP-W. The inhibition of the three aminopeptidases by amastatin, bestatin and probestin was examined in more detail (Fig. 1). Amastatin was equipotent with all three aminopeptidases (i_{50} = 1.5–3.2 μ M). However, probestin was some 100–400-fold more potent an inhibitor of AP-N (i_{50} = 50 nM) than of either AP-A or AP-W (i_{50} = 19.9 and 5.0 μ M, respectively). In contrast, bestatin was an 11-fold more potent inhibitor of AP-W (i_{50} = 7.9 μ M) than AP-N (i_{50} = 89.1 μ M).

The effect on the activity of AP-A, -N and -W of specific inhibitors of angiotensin converting enzyme (at a final concentration of 0.1 mM) was also examined (Table 2). AP-A was not significantly inhibited (maximally 13%) by any of the inhibitors

tested. Only indolaprilat, pentoprilat, ramiprilat and spiraprilat caused partial inhibition (maximally 30%) of AP-N. None of the carboxylalkyl or phosphoryl inhibitors caused >28% inhibition of AP-W. However, the sulphydryl compounds, captopril, rentiapril, YS 980 and zofenoprilat, all caused significant (>54%) inhibition of AP-W. The effect of these sulphydryl compounds on the activity of AP-W was examined in more detail (Fig. 2). These results indicate that rentiapril (i_{50} = 1.6 μ M) was the most potent at inhibiting AP-W, with zofenoprilat (i_{50} = 7.0 μ M), YS 980 (i_{50} = 17.7 μ M) and captopril (i_{50} = 199 μ M) being increasingly less potent.

DISCUSSION

In the present study we have directly compared

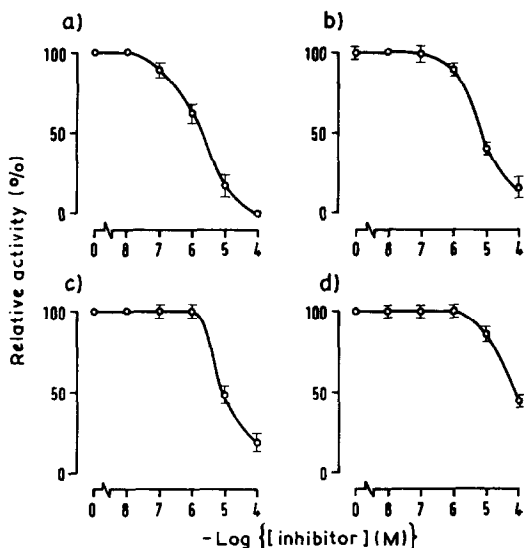


Fig. 2. Inhibition of AP-W by converting enzyme inhibitors. Pig kidney AP-W was assayed with 1 mM Asp-PheNH₂ as described in Materials and Methods. Each point is the mean of triplicate determinations. (a) Rentiapril; (b) zofenoprilat; (c) YS 980; (d) captopril.

Table 3. Comparison of the potency of inhibitors on the activities of AP-A, -N and -W

Inhibitor	AP-A	<i>I</i> ₅₀ (μM) AP-N	AP-W
Actinonin	>100	1.99	NI
Amastatin	2.00	3.16	1.58
Bestatin	NI	89.1	7.9
Probestin	19.9	0.05	5.0
Captopril	NI	NI	199
Rentiapril	NI	NI	1.6
YS 980	NI	NI	17.7
Zofenoprilat	NI	NI	7.0

The *I*₅₀ value is the concentration of inhibitor giving 50% inhibition of enzyme activity under the assay conditions described in Materials and Methods.

NI, negligible inhibition observed at an inhibitor concentration of 0.1 mM.

the effect of several metallopeptidase inhibitors on the activities of the cell surface zinc aminopeptidases A, N and W in an attempt to identify compounds which could be used in metabolic studies to discriminate between these activities. During this work we also reassessed the inhibitory action of a number of previously characterized aminopeptidase inhibitors. Bestatin (Ubenimex) was originally identified as an inhibitor of the soluble aminopeptidase B and the cytosolic leucine aminopeptidase [24, 25]. Subsequently, it was shown to inhibit purified AP-N [26] and AP-W [18]. The results of the present study indicate that bestatin is a more potent inhibitor of AP-W than AP-N (*I*₅₀ values of 8 and 89 μM, respectively) (see Table 3). These

results are somewhat surprising as bestatin has long been considered a potent inhibitor of AP-N and has been used extensively to inhibit this activity in metabolic studies [27, 28]. It was originally noted, however, that bestatin inhibited monkey and canine kidney cell surface leucine aminopeptidase activity (probably AP-N) with an *I*₅₀ value in the range 32–162 μM [29]. Bestatin has been shown to display immunomodulating properties [29–31] and to act as an antitumour agent [32–34]. These chemotherapeutic actions are believed to be due, in part, to its inhibition of cell surface AP-N. However, in the light of the present results, these actions may either/also be due to inhibition of cell surface AP-W.

Probestin was isolated recently from *Streptomyces azureus* and shown to inhibit AP-N [35]. In the present study, we show that probestin also inhibits AP-A and AP-W with *I*₅₀ values in the micromolar range (see Table 3). Thus, probestin appears to be a relatively broad acting aminopeptidase inhibitor, although it is somewhat more potent against AP-N. Amastatin was originally identified as an inhibitor of AP-A [36], and has since been shown to inhibit also AP-N [26] and AP-W [18]. In this study, we have directly compared the inhibitory potency of amastatin with AP-A, -N and -W and obtained results in agreement with these earlier reports, indicating that amastatin is essentially equipotent against these three enzymes (see Table 3). Actinonin was originally identified as an inhibitor of AP-N which lacked activity towards AP-A [37], and it has also been shown not to inhibit AP-W [18]. These results have been confirmed in the present study (see Table 3). Thus, within the cell surface aminopeptidase family, actinonin can be considered as a selective inhibitor of AP-N.

Due to the key role played by angiotensin converting enzyme in the renin-angiotensin system, numerous inhibitors of this zinc peptidyl dipeptidase have been synthesized and some have been used successfully in the treatment of hypertension and congestive heart failure [38, 39]. These compounds can be divided into three classes depending on the group that coordinates to the active site zinc atom: (i) sulphhydryl, (ii) carboxylalkyl and (iii) phosphoryl. Recently, we observed that AP-P was sensitive to inhibition by some of these converting enzyme inhibitors [19]. In the present study we have extended these observations by assessing the effect of inhibitors of angiotensin converting enzyme on AP-A, -N and -W. None of the compounds examined caused significant inhibition of AP-A or AP-N. However, the sulphhydryl compounds rentiapril, zofenoprilat and YS 980 all inhibited AP-W with *I*₅₀ values in the micromolar range (1.6–17.7 μM). These sulphhydryl compounds are all analogues of captopril [38]. YS 980 is structurally identical to captopril apart from having a sulphur atom at position 4 of the prolyl ring. Zofenoprilat differs from captopril in possessing an aromatic ring attached to position 4 of the prolyl ring by a sulphur bridge, while rentiapril differs from YS 980 in having a hydroxylated aromatic ring attached to position 5 of the prolyl ring. The increased potencies towards AP-W of rentiapril and

zofenoprilat may be due to their large aromatic moieties mimicking the aromatic P₁' residue of the substrate. The order of potency of these sulphhydryl compounds is essentially the reverse of that seen with AP-P [19] where the order was YS 980 (I₅₀ = 19.5 μM), captopril (I₅₀ = 110 μM), zofenoprilat (I₅₀ = 440 μM) and rentiapril caused only 35% inhibition at a concentration of 1 mM. The slight inhibition of AP-W observed with thiorphan (Table 1) is probably also due to the zinc coordinating sulphhydryl group in this compound.

Even though the sulphhydryl converting enzyme inhibitors are 1000-fold less potent at inhibiting AP-W than angiotensin converting enzyme [19], an *in vitro* I₅₀ value in the micromolar range may be significant in causing inhibition of AP-W *in vivo*. For example, teprotide, the first effective converting enzyme inhibitor *in vivo*, displays an I₅₀ toward the enzyme of 1.0 μM [40]. In addition, the inhibitor of membrane dipeptidase (EC 3.4.13.11), cilastatin, which is coadministered with the β-lactam antibiotic imipenem to prevent its metabolism by dipeptidase in the kidney, displays an I₅₀ value of 0.1 μM *in vitro* [41]. In clinical use, numerous side effects have been noted with captopril, including coughing, skin rashes and taste disturbance [39, 42]. The cause of these adverse effects remains unclear, although similar side effects have been noted with penicillamine leading to speculation that the sulphhydryl group may be responsible. The results of the present study suggest that these effects may be due to additional inhibition of AP-W by the sulphhydryl converting enzyme inhibitors.

In conclusion, we have shown that both amastatin and probestin have broad inhibitory profiles acting on the three cell surface zinc aminopeptidases A, N and W. We also confirm that actinonin is a relatively selective inhibitor of AP-N, but that bestatin is a relatively poor inhibitor of this peptidase and is much more potent towards AP-W. This raises the question as to whether the chemotherapeutic properties of bestatin may involve inhibition of AP-W. The observation that the sulphhydryl converting enzyme inhibitors, rentiapril and zofenoprilat, are relatively potent and totally specific for AP-W over any of the other cell surface zinc aminopeptidases should enable the role of this enzyme in the metabolism of bioactive peptides under normal and disease situations to be assessed, and may explain some of the side effects noted with the clinical use of these compounds. However, no selective inhibitor of AP-A has been identified.

Acknowledgements—We thank Mrs J. Ingram for the purified samples of aminopeptidases N and W. S.T. is in receipt of a British Council Studentship.

REFERENCES

- Kenny AJ and Hooper NM, Peptidases involved in the metabolism of bioactive peptides. In: *Degradation of Bioactive Substances: Physiology and Pathophysiology* (Ed. Henrickson JH), pp. 47–79. CRC Press, Boca Raton, 1991.
- Carretero OA and Scicli AG, Zinc metallopeptidase inhibitors: a novel antihypertensive treatment. *Hypertension* **68**: 366–371, 1991.
- Mancia G, Angiotensin-converting enzyme inhibitors in the treatment of hypertension. *J Cardiovasc Pharmacol* **18** (Suppl 7): S1–S3, 1991.
- Letarte M, Vera S, Tran R, Addis JBL, Onizuka RJ, Quackenbush EJ, Jongeneel CV and McInnes RR, Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. *J Exp Med* **168**: 1247–1253, 1988.
- Look AT, Ashmun RA, Shapiro LH and Peiper SC, Human myeloid plasma membrane glycoprotein CD13 (gp 150) is identical to aminopeptidase N. *J Clin Invest* **83**: 1299–1307, 1989.
- Hegen M, Niedobitek G, Klein CE, Stein H and Fleischer B, The T cell triggering molecule Tp103 is associated with dipeptidyl aminopeptidase IV activity. *J Immunol* **144**: 2908–2914, 1990.
- Wu Q, Li L, Cooper MD, Pierres M and Gorvel JP, Aminopeptidase A activity of the murine β-lymphocyte differentiation antigen BP-1/6C3. *Proc Natl Acad Sci USA* **88**: 676–680, 1991.
- Delmas B, Gelfi J, L'Haridon R, Vogel LK, Sjostrom H, Noren O and Laude H, Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. *Nature* **357**: 417–420, 1992.
- Yeager CL, Ashmun RA, Williams RK, Cardellicchio CB, Shapiro LH, Look AT and Holmes KV, Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature* **357**: 420–422, 1992.
- Hooper NM, Hryszko J and Turner AJ, Purification and characterization of pig kidney aminopeptidase P. A glycosyl-phosphatidylinositol anchored ectoenzyme. *Biochem J* **267**: 509–515, 1990.
- Simmons WH and Orawski AT, Membrane-bound aminopeptidase P from bovine lung. Its purification, properties and degradation of bradykinin. *J Biol Chem* **267**: 4897–4903, 1992.
- McDonald JK and Barrett AJ, *Mammalian Proteases: a Glossary and Bibliography, Volume 2, Exopeptidases*. Academic Press, London, 1986.
- Matsas R, Stephenson SL, Hryszko J, Kenny AJ and Turner AJ, The metabolism of neuropeptides. Phase separation of synaptic membrane preparations with Triton X-114 reveals the presence of aminopeptidase N. *Biochem J* **231**: 445–449, 1985.
- Matsas R, Turner AJ and Kenny AJ, Endopeptidase -24.11 and aminopeptidase activity in brain synaptic membranes are jointly responsible for the hydrolysis of cholecystokinin octapeptide (CCK-8). *FEBS Lett* **175**: 124–128, 1984.
- Nau R, Schäfer G, Deacon CF, Cole T, Agoston DV and Conlon JM, Proteolytic inactivation of substance P and neurokinin A in the longitudinal muscle layer of guinea pig small intestine. *J Neurochem* **47**: 856–864, 1986.
- Nagatsu I, Nagatsu T, Yamamoto T, Glenner GG and Mehl JW, Purification of aminopeptidase A in human serum and degradation of angiotensin II by the purified enzyme. *Biochim Biophys Acta* **198**: 255–270, 1970.
- Gee NS and Kenny AJ, Proteins of the kidney microvillar membrane. The 130 kDa protein in pig kidney, recognised by monoclonal antibody GK5C1 is an ectoenzyme with aminopeptidase activity. *Biochem J* **230**: 753–764, 1985.
- Gee NS and Kenny AJ, Proteins of the kidney microvillar membrane. Enzymic and molecular properties of aminopeptidase W. *Biochem J* **246**: 97–102, 1987.
- Hooper NM, Hryszko J, Oppong SY and Turner AJ, Inhibition by converting enzyme inhibitors of pig kidney aminopeptidase P. *Hypertension* **19**: 281–285, 1992.
- Bowes MA and Kenny AJ, An immunohistochemical study of endopeptidase-24.11 and aminopeptidase N in lymphoid tissues. *Immunology* **60**: 247–253, 1987.

21. Booth AG and Kenny AJ, A rapid method for the preparation of microvilli from rabbit kidney. *Biochem J* **142**: 575–581, 1974.
22. Fulcher IS and Kenny AJ, Proteins of the kidney microvillar membrane. The amphipathic forms of endopeptidase purified from pig kidneys. *Biochem J* **211**: 743–753, 1983.
23. Hooper NM, Low MG and Turner AJ, Renal dipeptidase is one of the membrane proteins released by phosphatidylinositol-specific phospholipase C. *Biochem J* **244**: 465–469, 1987.
24. Suda H, Aoyagi T, Takeuchi T and Umezawa H, Inhibition of aminopeptidase B and leucine aminopeptidase by bestatin and its stereoisomer. *Arch Biochem Biophys* **177**: 196–200, 1976.
25. Umezawa H, Aoyagi T, Suda H, Hamada M and Takeuchi T, Bestatin, a new aminopeptidase B inhibitor produced by actinomycetes. *J Antibiot* **29**: 97–99, 1976.
26. Rich DH, Moon BJ and Harbeson S, Inhibition of aminopeptidases by amastatin and bestatin derivatives. Effect of inhibitor structure on slow-binding processes. *J Med Chem* **27**: 417–422, 1984.
27. Giros B, Gros C, Solhonne B and Schwartz J-C, Characterization of aminopeptidases responsible for inactivating endogenous (Met⁵) enkephalin in brain slices using peptidase inhibitors and anti-aminopeptidase M antibodies. *Mol Pharmacol* **29**: 281–287, 1986.
28. Palmieri FE, Bausback HH and Ward PE, Metabolism of vasoactive peptides by vascular endothelium and smooth muscle aminopeptidase M. *Biochem Pharmacol* **38**: 173–180, 1989.
29. Umezawa H, Ishizuka M, Aoyagi T and Takeuchi T, Enhancement of delayed-type hypersensitivity by bestatin, an inhibitor of aminopeptidase B and leucine aminopeptidase. *J Antibiot* **29**: 857–859, 1976.
30. Ishizuka M, Masuda T, Kanbayashi N, Fukasawa S, Takeuchi T, Aoyagi T and Umezawa H, Effect of bestatin on mouse immune system and experimental murine tumours. *J Antibiot* **33**: 642–652, 1980.
31. Ishizuka M, Sato J, Sugiyama Y, Takeuchi T and Umezawa H, Mitogenic effect of bestatin on lymphocytes. *J Antibiot* **33**: 653–662, 1980.
32. Tsuruo T, Naganuma K, Iida H, Yamori T, Tsukagoshi S and Sakurai Y, Inhibition of lymph node metastasis of P388 leukemia by bestatin in mice. *J Antibiot* **34**: 1206–1209, 1981.
33. Saiki I, Murata J, Watanabe K, Fujii H, Abe F and Azuma I, Inhibition of tumour cell invasion by Ubenimex (bestatin) *in vitro*. *Jpn J Cancer Res* **80**: 873–878, 1989.
34. Ota K, Review of ubenimex (bestatin) clinical research. *Biomed Pharmacol* **45**: 55–60, 1991.
35. Aoyagi T, Yoshida S, Nakamura Y, Shigihara Y, Hamada M and Takeuchi T, Probestin, a new inhibitor of aminopeptidase M, produced by *Streptomyces azureus* MH663-2F6. *J Antibiot* **43**: 143–148, 1990.
36. Aoyagi T, Tobe H, Kojima F, Hamada M, Takeuchi T and Umezawa H, Amastatin, an inhibitor of aminopeptidase A, produced by actinomycetes. *J Antibiot* **31**: 636–638, 1978.
37. Umezawa H, Aoyagi T, Tanaka T, Suda M, Okuyama A, Naganawa H, Hamada M and Takeuchi T, Production of actinonin, an inhibitor of aminopeptidase M, by actinomycetes. *J Antibiot* **38**: 1629–1630, 1985.
38. Cohen ML, Synthetic and fermentation-derived angiotensin-converting enzyme inhibitors. *Annu Rev Pharmacol Toxicol* **25**: 307–323, 1985.
39. Unger T, Gohlke P and Gruber M-G, Converting enzyme inhibitors. In: *Pharmacology of Anti-hypertensive Therapeutics* (Eds. Ganten D and Mulrow PJ), pp. 377–481. Springer, Berlin, 1990.
40. Thorsett ED and Wyvratt MJ, Inhibition of zinc peptidases that hydrolyse neuropeptides. In: *Neuropeptides and their Peptidases* (Ed. Turner AJ), pp. 229–292. Ellis Horwood, Chichester, U.K., 1987.
41. Kahan FM, Kropp H, Sundelof JG and Birnbaum J, Thienamycin: development of imipenem-cilastatin. *J Antimicrob Chemother* **12** (Suppl D): 1–35, 1983.
42. Edwards CRW and Padfield PL, Angiotensin-converting enzyme inhibitors: past, present and bright future. *Lancet* **i**: 30–34, 1985.