Metabolism of aspartame by human and pig intestinal microvillar peptidases

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The artificial sweetener aspartame (*N*-L-α-aspartyl-L-phenylalanine-1-methyl ester; Nutrasweet), its decomposition product αAsp-Phe and the related peptide αAsp-PheNH$_2$ were rapidly hydrolysed by microvillar membranes prepared from human duodenum, jejunum and ileum, and from pig duodenum and kidney. The metabolism of aspartame by the human and pig intestinal microvillar membrane preparations was inhibited significantly (> 78 %) by amastatin or 1,10-phenanthroline, and partially (> 38 %) by actinonin or bestatin, and was activated 2.9–4.5-fold by $CaCl_2$. The inhibition by amastatin and 1,10-phenanthroline, and the activation by $CaCl_2$ are characteristic of the cell-surface ectoenzyme aminopeptidase A (EC 3.4.11.7) and a purified preparation of this enzyme hydrolysed aspartame with a K_m of 0.25 mM and a $V_{max.}$ of 126 μmol/min per mg. A purified preparation of aminopeptidase W (EC 3.4.11.16) also hydrolysed aspartame but with a K_m of 4.96 mM and a $V_{max.}$ of 110 μmol/min per mg. However, rentiapril, an inhibitor of aminopeptidase W, caused only slight inhibition (maximally 19 %) of the hydrolysis of aspartame by the microvillar membrane preparations. Similar patterns of inhibition and kinetic parameters were observed for αAsp-Phe and αAsp-PheNH$_2$. Two other decomposition products of aspartame, βAsp-PheMe and cyclo-Asp-Phe, were essentially resistant to hydrolysis by both the human and pig intestinal microvillar membrane preparations and the purified preparations of aminopeptidases A and W. Although the relatively selective inhibitor of aminopeptidase N (EC 3.4.11.2), actinonin, partially inhibited the metabolism of aspartame, αAsp-Phe and αAsp-PheNH$_2$ by the human and pig intestinal microvillar membrane preparations, these peptides were not hydrolysed by a purified preparation of aminopeptidase N. Membrane dipeptidase (EC 3.4.13.19) only hydrolysed the unblocked dipeptide, αAsp-Phe, but the selective inhibitor of this enzyme, cilastatin, did not block the metabolism of αAsp-Phe by the microvillar membrane preparations.

INTRODUCTION

Aspartame (*N*-L-α-aspartyl-L-phenylalanine-1-methyl ester; αAsp-PheMe; Nutrasweet) is an artificial sweetener that is some 150–200 times sweeter than sucrose (reviewed in Stegink and Filer, 1984). Aspartame is used widely as an artificial sweetener in a range of food products, e.g. soft drinks, cereals, dairy products, chewing gum, gelatin, and as a tabletop sweetener. In dry powder form aspartame is relatively stable; however, on exposure to moisture, elevated temperatures or extremes of pH the esterified dipeptide is converted non-enzymically into a variety of decomposition products (Homler, 1984; Lipton et al., 1991). The ester bond can be hydrolysed, forming the dipeptide αAsp-Phe and methanol. Alternatively, methanol may be eliminated by the cyclization of aspartame to form its dioxopiperazine (3-carboxylmethyl-6-benzyl-2,5-dioxopiperazine; cyclo-Asp-Phe). Aspartame can also be converted into βAsp-PheMe and subsequently βAsp-Phe. None of these decomposition products is sweet.

The intestinal absorption and metabolism of aspartame and its decomposition products have been the subject of numerous studies (Oppermann et al., 1973; Oppermann, 1984; Tobey and Heizer, 1986; Burgert et al., 1991; Lipton et al., 1991). The major point to emerge from these studies is that in all species examined, including humans and pigs, aspartame is broken down in the gastrointestinal tract to its constituent amino acids which are then absorbed into the bloodstream. However, the enzyme(s) actually responsible for the hydrolysis of aspartame has yet to be unequivocably identified. The microvillar or brush-border membrane of intestinal epithelial cells is rich in a variety of cell-surface peptidases which present a substantial barrier to the entry of intact peptides into these cells (reviewed in Hooper, 1993). The majority of these peptidases are also present in the microvillar membrane of the kidney proximal tubules, and this membrane has thus been used as a model system to examine the metabolism of biologically active peptides by the family of cell-surface peptidases working in concert (see for example Stephenson and Kenny, 1987; Kenny and Hooper, 1991).

From the known substrate specificities of these cell-surface peptidases four of them could potentially play a role in the metabolism of aspartame and its decomposition products. These are: aminopeptidase A (AP-A; EC 3.4.11.7) which preferentially hydrolyses peptides with aspartic acid or glutamic acid at the N-terminus; aminopeptidase N (AP-N; EC 3.4.11.2) which has a broad specificity, hydrolysing N-terminal neutral and basic residues, although aspartic acid and glutamic acid are hydrolysed slowly; aminopeptidase W (AP-W; EC 3.4.11.16) which hydrolyses short peptides and exhibits maximal rates towards dipeptides in which the P_1' residue is aromatic; and membrane dipeptidase (MDP; EC 3.4.13.19) which hydrolyses only dipeptides but has a broad specificity including substrates in which the C-terminal residue has the D-configuration (McDonald and Barrett, 1986; Kenny and Hooper, 1991). In the present study we have used selective inhibitors to assess the contribution of each of these four peptidases in human and pig intestinal and pig kidney microvillar membrane preparations to the *in vitro* metabolism of aspartame, its decomposition products βAsp-PheMe, αAsp-Phe and cyclo-Asp-Phe, and the related peptide

Abbreviations used: AP-A, aminopeptidase A; AP-N, aminopeptidase N; AP-W, aminopeptidase W; MDP, membrane dipeptidase.
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αAsp-PheNH$_2$. We have also examined the ability of purified preparations of AP-A, AP-N, AP-W and MDP to hydrolyse aspartame and its analogues.

EXPERIMENTAL

Materials

Pig kidneys and intestines were kindly provided by ASDA Farmstores, Lofthousegate, West Yorkshire, U.K. Post-mortem human kidney and intestine were obtained from the General Infirmary at Leeds and St. James's University Hospital, Leeds, U.K. αAsp-PheMe, βAsp-PheMe, αAsp-PheNH$_2$, αAsp-Phe, actinonin, amastatin and bestatin were purchased from Sigma Chemical Co., Poole, Dorset, U.K. Rentiapril (SA 446) was a gift from Santen Pharmaceutical Co., Osaka, Japan. Cilastatin (MK0791) was a gift from Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A. The dioxopiperazine derivative of aspartame, cyclo-Asp-Phe, was produced by incubation of αAsp-PheMe (1 mM) in 0.1 M Tris/HCl, pH 8.0, for 24 h at 100 °C. The conversion was verified by reverse-phase h.p.l.c. All other materials were from sources previously noted.

Peptide assays

AP-A activity was routinely assayed with α-Glu-*p*-nitroaniline (5.0 mM) as substrate in 0.1 M Tris/HCl/1 mM $CaCl_2$, pH 7.4, at 37 °C in the absence or presence of 0.1 mM amastatin. AP-N was routinely assayed with L-Ala-4-methyl-7-coumarylamide (0.2 mM) as substrate in 0.1 M Tris/HCl, pH 7.4, at 37 °C in the absence or presence of 0.1 mM actinonin. AP-W was routinely assayed with αAsp-PheNH$_2$ (1 mM) as substrate in 0.1 M Tris/HCl, pH 7.0, at 37 °C in the absence or presence of 0.1 mM rentiapril. MDP was routinely assayed with Gly-D-Phe (1 mM) as substrate in 0.1 M Tris/HCl, pH 8.0, at 37 °C in the absence or presence of 0.1 mM cilastatin. Assays with aspartame, βAsp-PheMe, αAsp-Phe, αAsp-PheNH$_2$ and cyclo-Asp-Phe (all at 1 mM) were carried out in 0.1 M Tris/HCl, pH 7.0. After incubation at 37 °C, the samples were heated at 100 °C for 4 min and then centrifuged at 15000 *g*. When aspartame or βAsp-PheMe were used as substrate 5 μl of 0.5 M sodium acetate, pH 4.0, was added before heating at 100 °C in order to prevent the formation of the dioxopiperazine. The hydrolysates were then monitored by reverse-phase h.p.l.c. on a μBondapak C_{18} column with a u.v. (214 nm) detector at a flow rate of 1.5 ml/min and a 15 min gradient of 4.5–30% (v/v) acetonitrile in 0.08% (v/v) H_3PO_4 at pH 2.5, followed by 5 min elution at the final conditions. The retention times of the peptides and their products were: aspartame and βAsp-PheMe (retention time 12.5 min), cyclo-Asp-Phe (11.0 min), PheMe (9.6 min), αAsp-Phe (9.0 min), αAsp-PheNH$_2$ (7.3 min), Phe (6.6 min) and PheNH$_2$ (5.7 min). Quantification of the substrates and products was by calibration from standard curves. Enzyme samples and inhibitors were pre-incubated for 15 min at 4 °C. For inhibitor and kinetic studies a maximum of 20% substrate breakdown was observed.

Membrane and enzyme preparations

All operations were carried out at 4 °C. Microvillar membranes were prepared from pig kidney cortex by the method of Booth and Kenny (1974) and from human and pig intestine by the method of Kessler et al. (1978). AP-W, AP-N, MDP and AP-A were purified from pig kidney cortex as described previously (Gee and Kenny, 1985; Bowes and Kenny, 1987; Littlewood et al., 1989; R. J. Hesp, J. Smith and N. M. Hooper, unpublished work). All four enzyme preparations were apparently homogeneous as assessed by SDS/PAGE. AP-A had a specific activity of 39.1 μmol of *p*-nitroanilide/min per mg of protein, AP-N a specific activity of 41.2 μmol of 4-methyl-7-coumarylamide/min per mg of protein, AP-W a specific activity of 15.0 μmol of PheNH$_2$/min per mg of protein and MDP a specific activity of 56.8 μmol of D-Phe/min per mg of protein.

RESULTS

Metabolism of aspartame and analogues by human and pig microvillar membranes

Aspartame, αAsp-Phe and αAsp-PheNH$_2$ were all rapidly metabolized by microvillar membrane preparations prepared from human duodenum, jejunum and ileum and from pig duodenum and kidney (Table 1). With aspartame negligible production (< 5 nmol) of αAsp-Phe or cyclo-Asp-Phe was observed with any of the membrane preparations. No hydrolysis of cyclo-Asp-Phe or of βAsp-PheMe was observed with any of the five microvillar membrane preparations even after incubation for 18 h at 37 °C, except in the case of βAsp-PheMe with the human ileal microvillar membranes (Table 1).

Effect of inhibitors on the metabolism of aspartame and analogues by human and pig microvillar membranes

The metabolism of aspartame by microvillar membranes prepared from human duodenum, jejunum and ileum, and pig duodenum, was inhibited substantially (> 75%) by amastatin, 1,10-phenanthroline, or a combination of 1,10-phenanthroline and rentiapril (Table 2). Actinonin and bestatin caused significant inhibition (35–69%) of the metabolism of aspartame by the membrane preparations. Rentiapril inhibited the breakdown by a maximum of 19% in the intestinal microvillar membranes, but caused 38% inhibition with the kidney microvillar membranes. Cilastatin had no detectable inhibitory effect on the metabolism of aspartame by any of the microvillar membrane preparations (Table 2). Amastatin caused substantial (> 54%) inhibition of the metabolism of the de-esterified dipeptide αAsp-Phe by all the membrane preparations, except for the human duodenal membranes (33% inhibition) (Table 3). Actinonin and cilastatin did not significantly inhibit (maximally 15%) the metabolism of αAsp-Phe by any of the membrane preparations (Table 3). Bestatin and rentiapril did not significantly inhibit the metabolism of αAsp-Phe by the human intestinal microvillar membranes (maximally 28%); however, they both caused significant inhibition (> 40%) of the metabolism of αAsp-Phe by the pig intestinal and kidney microvillar membranes (Table 3). The metabolism of aspartame and αAsp-Phe by the five microvillar membrane preparations was stimulated 2.2–8.3-fold in the presence of 5 mM $CaCl_2$ (Tables 2 and 3). The effect of inhibitors on the metabolism of αAsp-PheNH$_2$ by human jejunal, pig duodenal and pig kidney microvillar membranes was similar to that observed for aspartame (results not shown).

Distribution of cell-surface peptidase activities in human and pig microvillar membranes

AP-A and AP-N were present in all five of the membrane preparations examined (Table 4), although the activity of AP-N in pig kidney microvillar membranes was 4-fold higher than in any of the intestinal preparations. AP-W was relatively (2–4-fold) more abundant in the pig microvillar membrane preparations than in the human membranes, with its activity decreasing on descending the human intestine, with no activity

Table 1 Metabolism of aspartame and analogues by human and pig microvillar membranes

Microvillar membranes (human duodenum, 5.2 μg of protein; human jejunum, 6.0 μg; human ileum, 4.3 μg; pig duodenum, 4.9 μg; pig kidney, 4.2 μg) were incubated with the various substrates as described in the Experimental section. Results are the means of duplicate incubations and the figures refer to the production of PheMe in the case of aspartame and βAsp-PheMe, Phe in the case of αAsp-Phe, and $PheNH_2$ in the case of αAsp-$PheNH_2$.

	Specific activity (nmol/min per mg of protein)				
Membranes	Aspartame	αAsp-Phe	Cyclo-Asp-Phe	βAsp-PheMe	αAsp-$PheNH_2$
Human duodenal	33.0	12.2	0.0	0.0	11.0
Human jejunal	80.1	26.8	0.0	0.0	18.7
Human ileal	56.1	27.2	0.0	11.9	47.8
Pig duodenal	58.7	38.0	0.0	0.0	44.6
Pig kidney	60.6	55.2	0.0	0.0	64.0

Table 2 Effect of inhibitors on the metabolism of aspartame by human and pig microvillar membranes

Microvillar membranes (human duodenum, 5.2 μg of protein; human jejunum, 6.0 μg; human ileum, 4.3 μg; pig duodenum, 4.9 μg; pig kidney, 4.2 μg) were incubated in the absence and presence of the indicated inhibitors as detailed in the Experimental section. The results are the means of duplicate determinations and refer to the production of PheMe. The inhibitors were used at the same concentration as in Table 5.

	Relative activity (%)				
Inhibitor	Human duodenum	Human jejunum	Human ileum	Pig duodenum	Pig kidney
None	100.0	100.0	100.0	100.0	100.0
Actinonin	42.4	44.0	40.2	40.8	64.9
Amastatin	20.9	21.8	15.8	0.0	0.0
Bestatin	62.2	60.1	56.9	52.8	31.3
Cilastatin	102.1	102.5	115.8	114.5	103.0
Rentiapril	83.4	96.0	107.9	81.5	62.5
1,10-Phenanthroline	17.6	20.4	3.7	15.6	48.7
Rentiapril and 1,10-phenanthroline	15.5	23.3	0.0	0.0	6.6
$CaCl_2$	358.4	287.6	334.5	451.0	310.2

Table 3 Effect of inhibitors on the metabolism of αAsp-Phe by human and pig microvillar membranes

Microvillar membranes (human duodenum, 5.2 μg of protein; human jejunum, 6.0 μg; human ileum, 4.3 μg; pig duodenum, 4.9 μg; pig kidney, 4.2 μg) were incubated in the absence and presence of the indicated inhibitors as detailed in the Experimental section. The results are the means of duplicate determinations and refer to the production of Phe. The inhibitors were used at the same concentration as in Table 5.

	Relative activity (%)				
Inhibitor	Human duodenum	Human jejunum	Human ileum	Pig duodenum	Pig kidney
None	100.0	100.0	100.0	100.0	100.0
Actinonin	84.9	93.4	86.7	89.6	99.1
Amastatin	66.7	31.7	46.0	34.2	32.2
Bestatin	111.7	89.6	71.6	60.5	40.7
Cilastatin	99.0	100.6	88.8	123.2	92.2
Rentiapril	98.9	99.8	89.1	59.6	59.1
1,10-Phenanthroline	59.8	74.7	54.1	71.2	74.2
Rentiapril and 1,10-phenanthroline	62.5	68.9	37.2	18.3	28.2
$CaCl_2$	457.1	477.2	464.5	830.4	224.8

Table 4 Activities of cell-surface peptidases in human and pig microvillar membrane preparations

Microvillar membranes were prepared and the peptidases assayed in the absence and presence of the appropriate inhibitor as detailed in the Experimental section. The results are the means of duplicate determinations. Figures in parentheses are activities (%) relative to that in pig kidney microvillar membranes for each peptidase.

	Specific activity (nmol/min per mg of protein)			
Membranes	AP-A	AP-N	AP-W	MDP
Human duodenal	47.1 (50)	59.2 (24)	6.0 (25)	27.1 (9)
Human jejunal	51.2 (54)	61.3 (25)	4.5 (18)	48.1 (17)
Human ileal	100.0 (106)	49.4 (20)	0.0 (0)	93.7 (33)
Pig duodenal	89.8 (95)	67.8 (28)	10.9 (45)	0.0 (0)
Pig kidney	94.2 (100)	244.1 (100)	24.4 (100)	286.9 (100)

detectable in the ileum (Table 4). In contrast the activity of MDP increased from the duodenum through to the ileum in human, with no activity detected in the pig duodenal microvillar membrane preparation (Table 4).

Effect of inhibitors on the activities of the cell-surface peptidases

Among the cell-surface aminopeptidases actinonin is relatively specific at inhibiting AP-N (Tieku and Hooper, 1992), although there is slight (17%) inhibition of AP-A (Table 5). In contrast, amastatin inhibits all three aminopeptidases, while bestatin inhibits AP-W and to a lesser extent AP-N and MDP (Table 5). Of a large range of inhibitors examined (Tieku and Hooper, 1992) only the sulphydryl-converting enzyme inhibitors, e.g. rentiapril, were found to selectively inhibit AP-W and not AP-A or AP-N (Table 5). The only compound which inhibits AP-A (and AP-N), but is virtually ineffective against AP-W, is the metal-chelating agent, 1,10-phenanthroline (Table 5). None of these compounds causes >30% inhibition of MDP, which is selectively inhibited by cilastatin (Table 5) (Campbell et al., 1984). Of the four cell-surface peptidases studied only AP-A was activated in the presence of 5 mM $CaCl_2$ (Table 5).

Hydrolysis of aspartame and analogues by purified pig kidney peptidases

Both aspartame and αAsp-$PheNH_2$ were rapidly hydrolysed by AP-A and AP-W, but were virtually resistant to hydrolysis by

Table 5 Effect of inhibitors on the activities of the cell-surface peptidases

Purified samples of AP-A, AP-N, AP-W and MDP were assayed as described in the Experimental section. Results are the means of duplicate determinations with each inhibitor.

Inhibitor	Concentration (mM)	Relative activity (%) AP-A	AP-N	AP-W	MDP
None	–	100.0	100.0	100.0	100.0
Actinonin	0.1	83.5	9.0*	104.0*	87.0
Amastatin	0.1	0.0	0.0*	0.0*	82.0
Bestatin	0.1	86.6	48.0*	11.0*	71.0
Cilastatin	0.1	103.9	103.0*	83.0*	0.0
Rentiapril	0.1	107.0	90.0*	0.0*	93.0
1,10-Phenanthroline	0.5	0.0	12.0	93.0	70.0
$CaCl_2$	5.0	260.0	101.0	103.0	88.0

* From Tieku and Hooper (1992).

either AP-N or MDP (Table 6). In contrast, the unblocked dipeptide αAsp-Phe was hydrolysed by AP-A, AP-W and MDP, and no hydrolysis was observed with AP-N (Table 6). The decomposition products of aspartame, cyclo-Asp-Phe and βAsp-PheMe, were resistant to hydrolysis by all four peptidases even after incubation for 18 h at 37 °C (Table 6). The kinetic parameters of the hydrolysis of aspartame, αAsp-Phe and αAsp-$PheNH_2$ by AP-A, AP-W and MDP were determined (Table 7). With AP-A the presence of the free charged C-terminal carboxyl group on αAsp-Phe lowered the observed $V_{max.}$ value 4-fold as compared with aspartame and αAsp-$PheNH_2$, although the K_m values for all three peptides were similar. The K_m values observed with AP-W for all three peptides were an order of magnitude higher than those observed with AP-A, although the $V_{max.}$ values were similar.

DISCUSSION

Upon incubation with human or pig intestinal microvillar membranes aspartame was rapidly hydrolysed (Table 1), the initial detectable product being PheMe, with a slower increase in the rate of appearance of Phe (results not shown). With the pig kidney microvillar membranes the PheMe produced was rapidly de-esterified to Phe (results not shown), probably as a result of the high level of esterase activity in these membranes (see for example Grima et al., 1991). In contrast, the decomposition products of aspartame, cyclo-Asp-Phe and βAsp-PheMe, were not metabolized by any of the microvillar membrane preparations (Table 1), except for the slight metabolism of βAsp-PheMe by the human ileal microvillar membranes. Thus these latter two compounds would either be absorbed into the bloodstream intact or excreted directly from the body. These results are in agreement with the findings of Lipton et al. (1991), who demonstrated that cyclo-Asp-Phe and βAsp-Phe were resistant to hydrolysis by homogenates of rat intestinal mucosal cells.

The metabolism of aspartame by human and pig intestinal microvillar membranes was inhibited substantially by the relatively non-selective aminopeptidase inhibitor, amastatin, and the metal chelator, 1,10-phenanthroline (Table 2), and is probably due to the inhibition of AP-A. Although no selective inhibitor of AP-A currently exists, this enzyme is markedly activated in the presence of Ca^{2+} ions (Table 5) (Danielsen et al., 1980), and the metabolism of aspartame was found to be significantly enhanced

Table 6 Hydrolysis of aspartame and analogues by purified pig kidney peptidases

The purified peptidases were incubated with the indicated substrate, and the products separated and quantified as detailed in the Experimental section. The results are the means of triplicate determinations with each substrate. In the presence of the appropriate inhibitor no hydrolysis was observed.

Peptidase	Specific activity (μmol/min per mg protein) αAsp-PheMe	αAsp-Phe	Cyclo-Asp-Phe	βAsp-PheMe	αAsp-$PheNH_2$
AP-A	101.4	17.4	0.0	0.0	77.9
AP-N	0.1	0.0	0.0	0.0	0.0
AP-W	15.4	13.0	0.0	0.0	16.0
MDP	0.0	8.2	0.0	0.0	0.0

Table 7 Kinetic parameters of the hydrolysis of aspartame and analogues by purified pig kidney cell-surface peptidases

The purified peptidases were incubated with the indicated substrate, and the products separated and quantified as detailed in the Experimental section. K_m and $V_{max.}$ values were determined from Eadie–Hofstee plots and are the mean of triplicate determinations in each case. For calculation of the $k_{cat.}$ molecular-mass values of 143000 for AP-A, 130000 for AP-W and 45000 for MDP were used.

Enzyme	Substrate	K_m (mM)	$V_{max.}$ ($\mu mol \cdot min^{-1} \cdot mg^{-1}$)	$k_{cat.}$ (min^{-1})	$k_{cat.}/K_m$ ($min^{-1} \cdot \mu M^{-1}$)
AP-A	Aspartame	0.25 ± 0.08	125.5 ± 14.5	17947	71.78
	αAsp-Phe	0.67 ± 0.18	28.4 ± 1.4	4061	6.06
	αAsp-$PheNH_2$	0.33 ± 0.17	98.9 ± 8.0	14143	42.85
AP-W	Aspartame	4.96 ± 1.40	109.6 ± 46.2	14248	2.87
	αAsp-Phe	3.11 ± 0.83	53.8 ± 22.1	6994	2.25
	αAsp-$PheNH_2$	7.48 ± 2.76	106.1 ± 21.1	13793	1.84
MDP	αAsp-Phe	0.95 ± 1.06	15.8 ± 7.6	711	0.75

(2.8–4.5-fold) in the presence of 5 mM $CaCl_2$ (Table 2). These results are substantiated by the observation that purified pig kidney AP-A was capable of hydrolysing aspartame with a K_m of 0.25 mM and a $V_{max.}$ of 126 μmol/min per mg. Although purified pig kidney AP-W can hydrolyse aspartame, the kinetic parameters are less favourable than with AP-A (Table 7) and the relatively selective inhibitor of this enzyme, rentiapril, only inhibited by a maximum of 19% the metabolism of aspartame by the intestinal microvillar membrane preparations (Table 2). The partial inhibition by bestatin of the metabolism of aspartame in the five microvillar membrane preparations (Table 2) is probably due to the inhibition of AP-W. Although the relatively selective inhibitor of AP-N, actinonin, partially blocked the metabolism of aspartame (Table 2), the esterified dipeptide was not hydrolysed to any significant extent by purified pig kidney AP-N; thus the inhibition by actinonin of the metabolism of aspartame may be due either to its partial inhibitory action on AP-A and/or to its inhibition of another uncharacterized activity.

The slight differences observed in the effect of inhibitors on the metabolism of aspartame in human duodenal, jejunal and ileal microvillar membranes can be accounted for by considering the distribution of the cell-surface peptidases in these tissues (Table 4). For example, rentiapril has a decreasing inhibitory effect on the metabolism of aspartame on moving from the duodenum through to the ileum (Table 2) which is paralleled by a decrease in the activity of AP-W in these tissues (Table 4). This is in contrast with the pig intestine where AP-W, as measured by an immunoradiometric assay using a monoclonal antibody, was found to increase on descending the intestine (Gee and Kenny, 1985).

On passage through the small intestine the methyl group on aspartame may be removed by esterase activities generating the free dipeptide αAsp-Phe (Oppermann, 1984). Thus we also examined both the effect of inhibitors on the metabolism of αAsp-Phe by human and pig intestinal microvillar membranes (Table 3) and the ability of the purified peptidases to hydrolyse this peptide (Tables 6 and 7). Although purified MDP hydrolysed αAsp-Phe, the kinetic parameters for this hydrolysis were rather unfavourable and the specific inhibitor of MDP, cilastatin, had a minimal (< 12%) inhibitory effect on the metabolism of this unblocked dipeptide by the intestinal microvillar membrane preparations. From the inhibitory data and the $CaCl_2$ activation data (Table 3) it would appear that, as with aspartame, AP-A is the major enzyme involved in the metabolism of αAsp-Phe by the human intestinal microvillar membranes.

αAsp-$PheNH_2$ is present at the C-terminal end of the gastrin family of peptides which includes cholecystokinin-8, and is released from these peptides by the action of another cell-surface peptidase angiotensin-converting enzyme (EC 3.4.15.1) (Dubreuil et al., 1990). Although purified preparations of both AP-A and AP-W were capable of hydrolysing αAsp-$PheNH_2$ with similar kinetic parameters to aspartame, from the inhibitor profile (results not shown) it appears that AP-A is the major peptidase involved in the hydrolysis of αAsp-$PheNH_2$ by the human and pig intestinal microvillar membranes.

In conclusion, the use of selective peptidase inhibitors and purified enzyme preparations have indicated that AP-A is the major activity in human and pig intestinal microvillar membranes involved in the hydrolysis of aspartame, its decomposition product, αAsp-Phe, and the related peptide αAsp-$PheNH_2$. A minor contribution to the hydrolysis of these three peptides is also made by AP-W. Two other decomposition products of aspartame, βAsp-PheMe and cyclo-Asp-Phe, are resistant to hydrolysis by both the intestinal microvillar membrane preparations and the purified peptidases.

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