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In vitro study of intestinal absorption and metabolism of 8-L-arginine vasopressin and its analogues

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Summary

Factors influencing luminal metabolism and transepithelial transport of peptides were investigated in vitro using 8-L-arginine vasopressin (AVP) and analogues. Chromatographic (HPLC) systems were developed to resolve AVP and its metabolites in biological matrices. Metabolism of AVP by trypsin and carboxypeptidase-like activities present in the lumen of the ileum was rapid and resulted in formation of desglycinamide AVP, pressinoic acid (P) and pressinoic acid-proline (P-Pro). However, P and desamino-8-D-arginine vasopressin were metabolically stable under the same conditions. In Ussing chambers, AVP was found to be relatively refractory to degradation when well rinsed ileal mucosa was employed. Mucosal-to-serosal transport, measured as total radioactivity, is greater than serosal-to-mucosal transport. These differential rates of transport may be related to increased formation of metabolites in the mucosal versus the serosal bath. Metabolites which elute with retention times similar to P and more polar, unidentified metabolites, were found in the serosal bathing solution, but intact AVP could not be detected. These results suggest that even if luminal metabolism of AVP is abolished transepithelial transport of intact AVP is negligible.

Introduction

Oral administration of peptide drugs is often limited by their instability in the gastrointestinal environment and/or poor absorption from the gut. An understanding of factors that govern the metabolic stability and intestinal absorption of peptides is therefore an essential prerequisite to the design of orally active compounds.

This paper reports on the use of an in vitro methodology for evaluation of luminal stability, metabolism and transepithelial transport of 8-L-arginine vasopressin (AVP) and synthetic analogues of this nonapeptide. Metabolism by the luminal contents was examined by digestion of AVP and desamino-8-D-arginine vasopressin (DDAVP) in diluted intestinal juice. The major proteolytic enzymes present in the luminal contents include: endopeptidases – trypsin, chymotrypsin and elastase; and exopeptidases – mainly carboxypeptidases. Fig. 1 shows the structures of AVP and DDAVP with sites of potential en-

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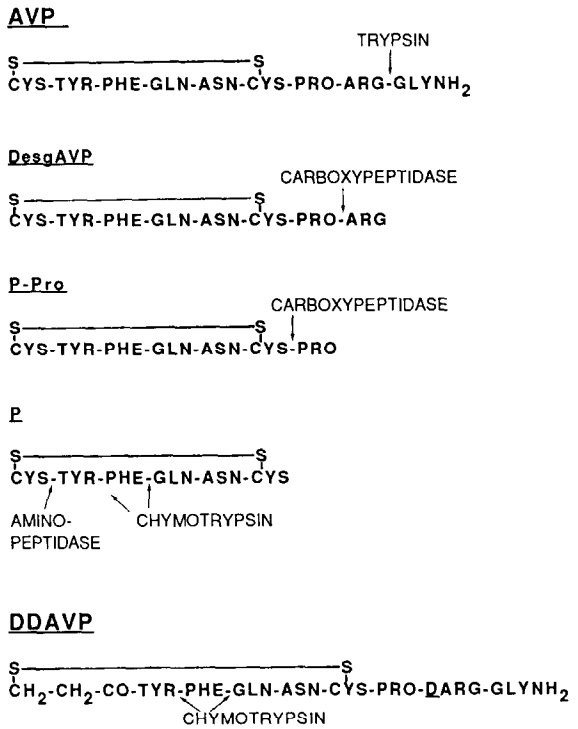

Fig. 1. Structures of AVP and DDAVP showing potential sites of attack by proteolytic enzymes and formation of metabolites.

zymatic attack marked. It should be noted that, if the cyclic part of AVP (i.e. pressinoic acid, P), is subject to metabolism, formation of a number of other metabolites can be expected. Metabolism and transintestinal transport of AVP by ileal mucosa were determined by employing the Ussing chamber technique which was previously used by Takaori et al. (1986) for studying the transport of oligopeptides. Vilhardt and Lundin (1986) have reported that AVP is transported through everted sacs of rat intestine at extremely low rates. Furthermore, AVP is orally active in vivo only at very high doses compared to parenterally effective doses (Saffran et al., 1979; Saffran, 1982). In contrast, DDAVP is known to induce considerable antidiuretic activity when given orally (Vavra et al., 1974; Vilhardt and Bie, 19893, 1984; Vilhardt et al., 1985), and demonstrates a higher transport rate than AVP (Vilhardt and Lundin, 1986).

This study was designed to elucidate in more detail factors responsible for the reported poor intestinal absorption of AVP. In particular, we have sought insight into the following questions: (1) Is AVP metabolized in the luminal environment and what are the sites of enzymatic degradation of this peptide in the luminal environment? (2) Does the cyclic component of the AVP molecule provide protection against enzymatic attack? (3) Is AVP metabolized by ileal mucosa? (4) Can AVP traverse the intestine intact, or is this peptide metabolized during transepithelial transport?

Materials and Methods

Chemicals

8-L-arginine vasopressin (AVP), carboxypeptidase B (EC 3.4.17.2), carboxypeptidase Y (EC 3.4.17.4) and trypsin (EC 3.4.21.4), were purchased from Sigma Chemical Co. (St. Louis, MO). Desglycinamide arginine-L-vasopressin (DesgAVP), pressinoic acid (P) and desamino-8-L-arginine vasopressin (DLAVP) were obtained from Peninsula Labs., Inc. (Belmont, CA). Desamino-8-D-arginine vasopressin (DDAVP) was provided by the Department of Peptide Chemistry, (Smith Kline & French Labs., King of Prussia, PA, U.S.A.). Radiolabelled AVP[phenylalanyl-3,4,5,^{3}H], (70 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Pressinoic acid-Pro (P-Pro) was obtained by digestion of 5 μg of DesgAVP with 2.2 units of carboxypeptidase B in a total volume of 1 ml, at 37 °C for 0.5 h. Digestion was stopped by boiling for 5 min and the solution was then centrifuged for 5 min at 14,000 rpm. P-Pro was then purified by HPLC using mobile phase 2 (see below). All others chemicals were reagent grade or equivalent and were obtained from local sources.

Preparations of intestinal juice

Segments of ileum obtained from New Zealand white rabbits (2–4 kg) were opened along the mesenteric border. Luminal contents of a 25 cm segment of ileum were suspended in 10 ml of 0.9% NaCl containing 0.1 mM $CaCl_2$ and centrifuged at 5000 rpm for 10 min. The supernatant was stored in 0.2 ml portions at −20 °C.

Incubation procedure

Thawed and thoroughly mixed luminal contents (0.2 ml) were diluted (1 : 100) with Ringer (pH 7.4 when gassed with 5% CO_2/O_2) containing in mM: Na^+ 141, K^+ 5, Ca^{2+} 1.2, Mg^{2+} 1.2, Cl^- 122, HCO_3^- 25, HPO_4^{-2} 1.6, $H_2PO_4^-$ 0.4. Aliquots of this solution were placed in a water bath at 37 °C for 10 min. Arginine vasopressin (40 or 80 μg/ml) was added and the incubation was continued. At various times, digestion was stopped by boiling samples for 5 min. Samples were centrifuged for 2 min at 14,000 rpm and analyzed by HPLC. Digestion of AVP by trypsin and carboxypeptidase Y was performed by incubating 10 μg of AVP with 0.005 BAEE units of trypsin or 1.1 units carboxypeptidase Y in a total volume of 1 ml. At various times digestion was stopped by boiling samples for 5 min. Samples were centrifuged for 2 min at 14,000 rpm and analyzed by HPLC. In inhibitor studies, diluted intestinal juice was preincubated with *p*-aminobenzamidine (10 μM) or sodium benzylsuccinate (25 mM) for 20 min at 37 °C, followed by addition of AVP (40 μg/ml) and radiolabelled AVP (0.4–0.8 μCi). After 30 min of incubation the reaction was stopped as described above and HPLC analysis was performed.

Transepithelial transport studies

Ileal mucosa was stripped of underlying muscle as described previously (Smith and Field, 1980), rinsed 4 times with Ringer solution, mounted in Ussing chambers (1.13 cm^2 exposed surface area) and bathed on both tissue surfaces with 10 ml Ringer containing 10 mM glucose in the serosal bath and 10 mM mannitol in the mucosal bath. Solutions were circulated by gas lift with 5% CO_2 in O_2 and maintained at 37 °C by water-jacketed reservoirs. Tissues were allowed to equilibrate for 30–45 min. Viability of the tissue was assessed by measurements of electrical parameters: transepithelial potential difference, short-circuit current and conductance (Smith and Field, 1980). Metabolism and transport of AVP were measured in aliquots taken at 2 min, 1 h, 2 h and 3 h after introduction of [^{3}H]AVP (50 μM, 30 μCi) to the mucosal or serosal bathing solutions. Aliquots were diluted 1 : 1 with water, boiled for 5 min, centrifuged for 5 min at 14,000 rpm and analyzed by HPLC.

HPLC analysis

Identification of AVP and metabolites was conducted employing reversed-phase HPLC using a Shimadzu isocratic chromatographic system consisting of a model LC-6A pump, auto injector model SIL-6A, model SPD-6AV UV-VIS detector (220 nm), chromatopac model C-R3A data station, with a mobile phase consisting of a mixture of (1) 61% (v/v) of 25 mM CH_3COONH_4 with 39% of methanol or (2) 18% (v/v) CH_3CN in 0.1% TFA (v/v) at a flow rate of 1 ml/min through a Spheri-5, RP-18 column (5 μm, 4.6 mm × 22 cm), Brownlee Labs (Woburn, MA).

Measurement of tritiated compounds

When HPLC was performed with [^{3}H]AVP 1 ml fractions of the eluent were collected and ^{3}H was determined by liquid scintillation spectrometry. The radioactivity was measured in a Packard, Tri-Carb 4640 counter using Ready-Solv MP (Beckman Instruments, Inc., Fullerton, CA) scintillation cocktail. Counts per minute were converted to dpm using the external standard channels ratio.

Results

Metabolism of AVP in luminal juice

An HPLC separation of AVP and metabolite standards added to Ringer (previously incubated with ileum in the Ussing chamber for 30 min) or intestinal juice is shown in Fig. 2. Employing mobile phase 1, P and P-Pro could not be completely resolved in this system (Fig. 2A). Employing mobile phase 2 complete resolution of AVP and metabolites was achieved, but AVP could not be resolved from tissue or intestinal juice interference (Fig. 2B). We used mobile phase 1 in the majority of our studies, expressing the concentration of P and P-Pro as a sum. Whenever the distinction between P and P-Pro was desired, mobile phase 2 was employed. Retention times of DDAVP and DLAVP using mobile phase 1 were 34.3 min and 25.8 min, respectively.

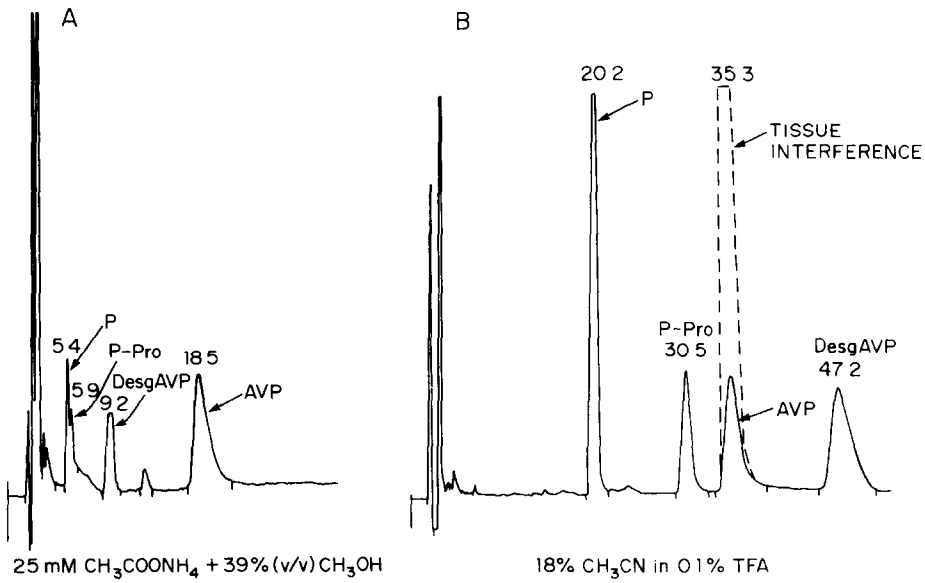

Fig. 2. HPLC separation of AVP, DesgAVP, P and P-Pro in mucosal bathing solution. A: mobile phase 1; concentrations of standards: AVP, 1 μg/ml; DesgAVP, P and P-Pro, 0.25 μg/ml; B: mobile phase 2, concentrations of standards: 1 μg/ml. Sample vol. 200 μl.

Fig. 3 shows representative results of digestion of AVP by intestinal juice. AVP was rapidly metabolized to DesgAVP and small amounts of P and P-Pro. Under the same conditions no metabolism of pressinoic acid or DDAVP was observed (data not shown). The cyclic hexapeptide P was stable when the concentration of intestinal juice was increased 10-fold. In contrast, the DDAVP enantiomer, DLAVP, was rapidly metabolized as measured by disappearance of this

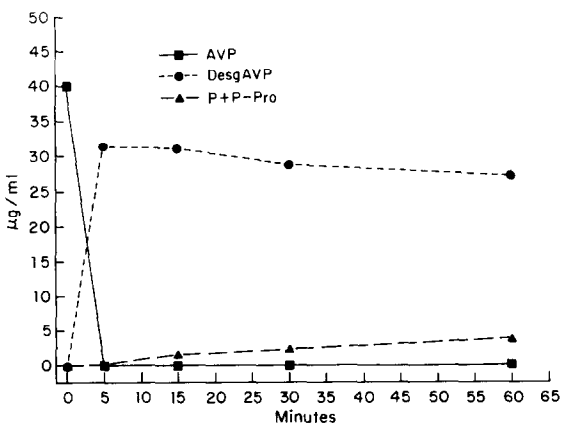

Fig. 3. Metabolism of AVP in rabbit intestinal juice. AVP (80 μg/ml) was incubated with an equal volume (0.4 ml) of intestinal juice at 37 °C. Samples were then diluted 1 : 1 with water and boiled for 5 min. After 5 min of centrifugation at 14,000 rpm, 50 μl samples were analyzed by HPLC employing mobile phase 1.

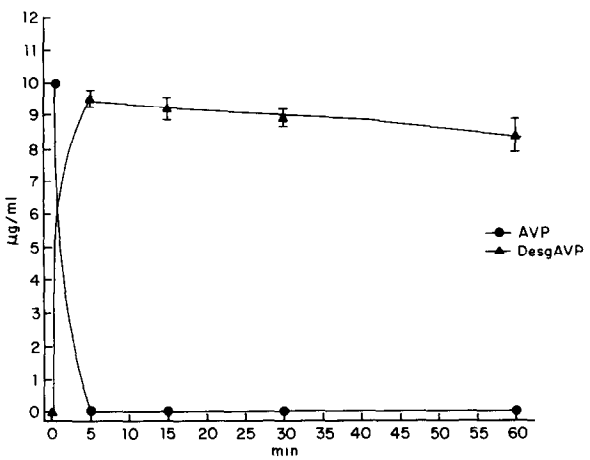

Fig. 4. Digestion of AVP by trypsin. AVP (10 μg) was incubated at 37 °C with trypsin (0.005 BAEE units) in a total volume of 1 ml. At various times the reaction was stopped by boiling for 5 min. Samples were then diluted and analyzed by HPLC using mobile phase 1. Results are means ± S.E.M. ($n = 4$).

compound. These results suggest that trypsin and carboxypeptidase activities are, as predicted, the major contributors to AVP metabolism under the conditions of our experiments. Susceptibility of AVP to digestion by trypsin or carboxypeptidase was investigated and the results are summarized in Figs. 4 and 5. Degradation of AVP by trypsin (cleavage of Arg-GlyNH_2 peptide bond), was accompanied by formation of DesgAVP as a major metabolite (Fig. 4). Incubation of AVP with carboxypeptidase Y resulted in formation of DesgAVP, P and P-Pro (Fig. 5). During the course of incubation the concentration of DesgAVP, which is an intermediate product, initially increased and then decreased while the concentration of P/P-Pro increased.

The mechanism of proteolytic degradation of AVP was studied by monitoring the metabolism of AVP in the presence (10 μM) or absence of *p*-aminobenzamidine, a competitive inhibitor of trypsin. Because the inhibitor could not be chromatographically separated from AVP and its metabolites and caused an interference with the UV detection, radiolabelled AVP was employed in these studies. Fig. 6 demonstrates that under the conditions employed, benzamidine blocks tryptic action on AVP thereby reducing metabolism by

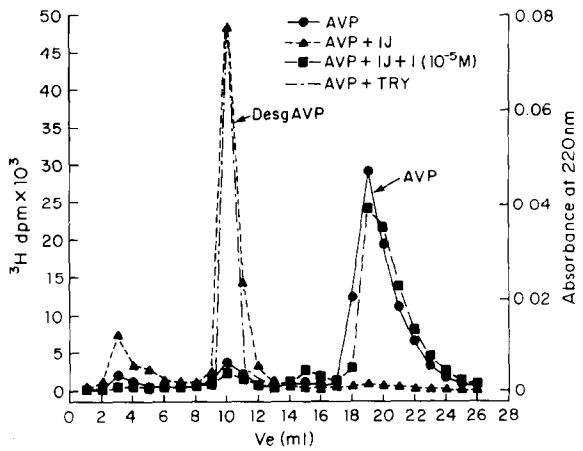

Fig. 6. Metabolism of AVP in rabbit intestinal juice in the absence or presence of the trypsin inhibitor *p*-aminobenzamidine. 0.4 ml of [^{3}H]AVP (40 μg/ml, 0.8 μCi) was incubated for 30 min at 37 °C with equal amounts of water (●———●) or intestinal juice ▲------▲). Intestinal juice was first preincubated with *p*-aminobenzamidine (10^{-5} M) for 20 min, followed by incubation with [^{3}H]AVP (as above) for 30 min (■— — —■). Samples were then diluted and analyzed as described in Fig. 3 using mobile phase 1. HPLC fractions (1 ml) were collected and assayed for radioactivity as described in Materials and Methods. Digestion of AVP (0.4 ml, 40 μg/ml) by 0.5 ml of trypsin (0.005 BAEE units) was performed for 30 min and 50 μl samples were prepared for HPLC analysis as described in Fig. 3 and monitored by UV (- — - — -).

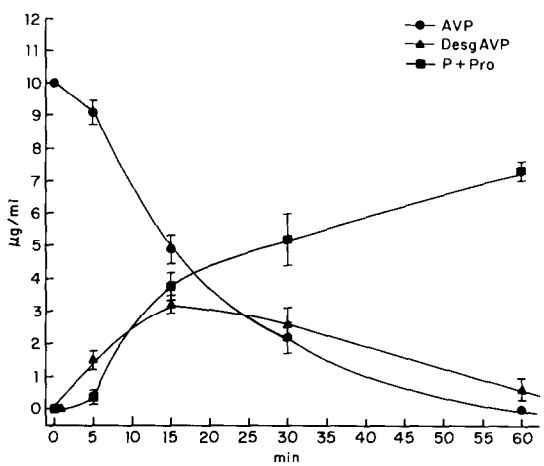

Fig. 5. Digestion of AVP by carboxypeptidase Y. AVP (10 μg) was incubated at 37 °C with carboxypeptidase Y (1.1 units) in a total volume of 1 ml. At various times the reaction was stopped by boiling for 5 min. Samples were then diluted and analyzed by HPLC as described in Fig. 3 using mobile phase 1. Results are means ± S.E.M. (n = 4).

intestinal juice. Incubation of AVP with intestinal juice resulted in formation of DesgAVP as a major metabolite. Trypsin digestion was performed as a control, and formation of DesgAVP was observed (monitored by UV at 220 nm). In contrast, preincubation of intestinal juice with the trypsin inhibitor markedly reduced the metabolism of AVP.

DesgAVP may also be formed as a results of carboxypeptidase cleavage at the Arg-GlyNH_2 bond. In order to distinguish if primary trypsin cleavage is essential to carboxypeptidase activity, similar experiments were performed in the presence or absence of the carboxypeptidase inhibitor, sodium benzylsuccinate (25 mM) (Fig. 7). As in the experiments employing trypsin, AVP was stable, while DesgAVP was the major metabolite after incubation with intestinal juice, even when the carboxypeptidase inhibitor was included. As a control, cleavage of AVP by carboxypeptidase Y

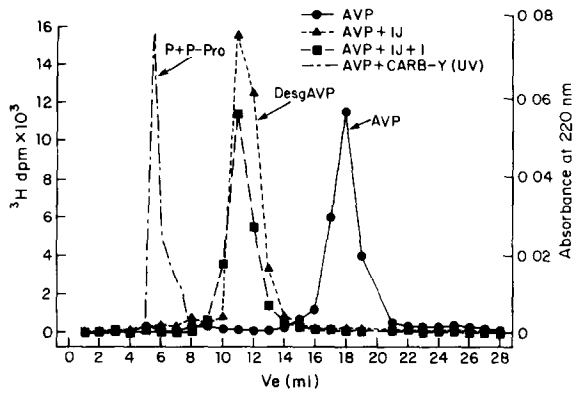

Fig. 7. Metabolism of AVP in rabbit intestinal juice in the presence or absence of the carboxypeptidase inhibitor, sodium benzylsuccinate. 0.5 ml of AVP (40 μg/ml, 0.4 μCi) was incubated for 30 min at 37 °C with: an equal volume of water (●———●); or intestinal juice (▲------▲). Intestinal juice was preincubated with 25 mM sodium benzylsuccinate, followed by incubation with [^{3}H]AVP (■— — —■). Samples were diluted and analyzed as described in Fig. 3. HPLC fraction (1 ml) were collected and assayed for radioactivity as described in Materials and Methods using mobile phase 1. Digestion of AVP (40 μg/ml) by carboxypeptidase Y solution (1.1 units) was performed for 30 min and 50 μl samples were prepared for HPLC analysis as described in Fig. 3 using mobile phase 1 and monitored by UV (- — · — -).

was studied (monitored by UV at 220 nm) resulting in formation of P and P-Pro as metabolites.

Metabolism of AVP by ileal mucosa

Fig. 8 illustrates the effect of incubation of AVP in Ussing chambers in which well-rinsed ileal mucosa were mounted. When AVP was incubated in the serosal bathing solution, metabolism was negligible, while some degradation occurred when AVP was incubated in the mucosal bathing solution. The results show that in contrast to rapid metabolism by luminal enzymes, AVP is relatively stable when exposed to mucosal brush border enzyme activities. Fig. 9 represents a typical HPLC chromatogram of mucosal bathing solution after 2 min or 3 h of incubation. The major metabolites formed under these conditions were P and P-Pro and small quantities of DesgAVP, but large quantities of AVP (ca. 80%) remained intact after 3 h incubation in contact with the washed mucosal surface.

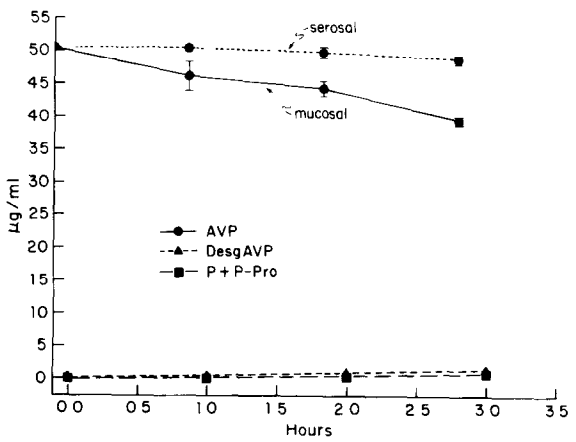

Fig. 8. Metabolism of AVP in the mucosal (●———●) or serosal (●------●) bathing solutions. [^{3}H]AVP (50 μM, 30 μCi) was added to the mucosal or serosal bathing solution. At appropriate incubation times, aliquots were removed, diluted, boiled, centrifuged and analyzed by HPLC using mobile phase 1. Results are means ± S.E.M. ($n = 3$).

Transepithelial transport studies

Fig. 10 presents the appearance of tritium in the opposite bathing solution for mucosal (m)-to-serosal (s) and s-to-m transport as a function of time. A substantial difference between m-to-s and s-to-m transport rate was observed in all experiments. After 3 h of incubation 1.5–3% of total radioactivity added to mucosal bath could be de-

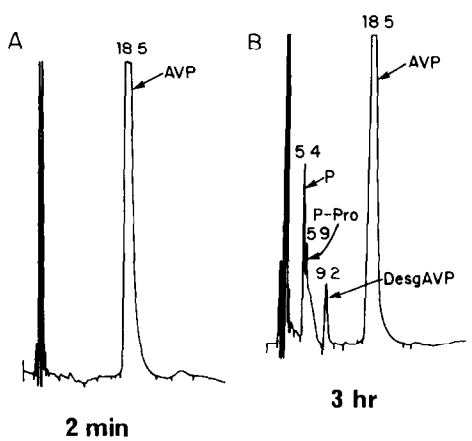

Fig. 9. HPLC analysis of mucosal bathing solutions 2 min or 3 h after addition of AVP. An aliquot of mucosal bathing solution was removed 2 min or 3 h after addition of AVP (50 μM, 30 μCi), diluted and prepared for HPLC as described in Fig. 3. using mobile phase 1.

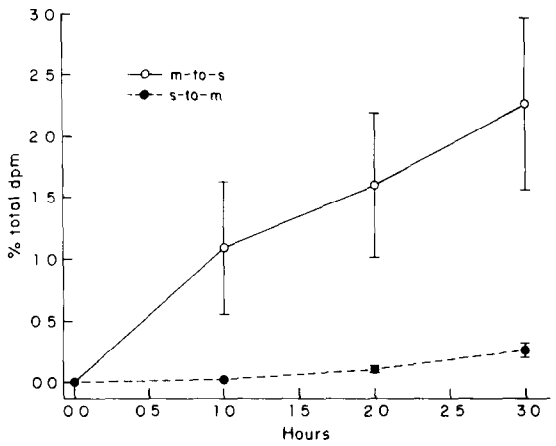

Fig. 10. Rate of appearance of ^3H, measured as percentage of total radiactivity added to the mucosal (○———○) or serosal (●------●) bathing solution. [^{3}H]AVP (50 μM, 30 μCi) was added to the mucosal or serosal bathing solution at $t = 0$ min. Radioactivity appearing in the opposite bathing solution was determined as described in Materials and Methods (mean ± S.E.M., $n = 6$).

tected in the serosal bath while only 0.1–0.3% of the total radioactivity appeared in the mucosal bath after serosal addition. Transepithelial conductance was constant over the time course of these experiments and did not significantly differ in tissues employed to examine m-to-s and s-to-m transport. However, as previously reported (Field et al., 1968), AVP increased short-circuit current transiently (data not shown).

Representative results for the HPLC analysis of concentrated serosal bathing solution after mucosal addition of AVP are shown in Fig. 11. Intact AVP was not detected in the serosal bathing solution and the major radioactivity peak obtained using mobile phase 1 (Fig. 11A) has a similar retention time to P and/or P-Pro. However, with prolonged transport time, a peak with shorter retention time was formed, suggesting formation of more polar metabolite(s). Using mobile phase 2 (Fig. 11B), three radioactivity peaks were detected. Peak 3 has a retention time similar to P, peak 2 has a retention time (3.54 min) similar to phenylalanine (radiolabeled amino acid residue of the AVP molecule) and peak 1 has a retention time very close to the solvent front. The identity of peak 1 has not been established.

Discussion

These studies demonstrate that AVP is very unstable in the lumen contents of the small intestine and is virtually completely degraded within five minutes by a highly diluted preparation of rabbit intestinal contents. The results suggest that the predominant mechanism for metabolism of AVP by intestinal juice involves sequential clea-

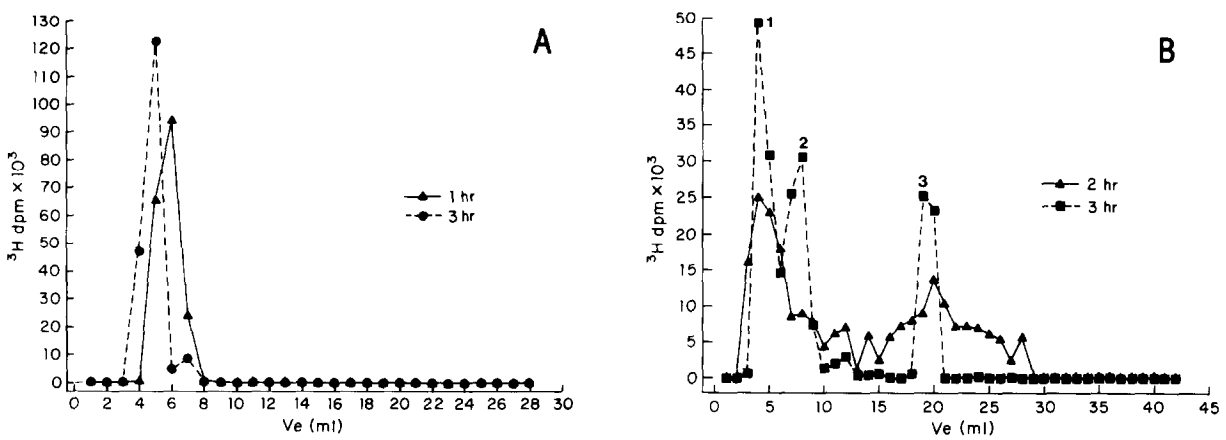

Fig. 11. HPLC analysis of the serosal bathing solution after mucosal addition of [^{3}H]AVP. [^{3}H]AVP (50 μM, 30 μCi) was added to mucosal bathing solution. 1 ml samples were collected after 1, 2 or 3 h, boiled for 5 min, centrifuged for 5 min at 14,000 rpm and dried under N_2. Samples were redissolved in 250 μl of water and 50 μl aliquots were analyzed by HPLC employing mobile phase 1 (A) or 2 (B).

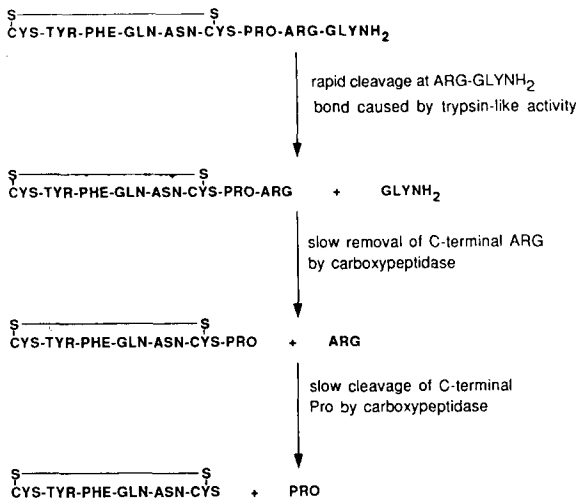

Fig. 12. Major metabolic pathway for AVP in the presence of intestinal juice.

vage of peptide bonds from the C terminus (Fig. 12). The initial rapid cleavage of the Arg-GlyNH_2 bond is attributed to trypsin activity resulting in the formation of DesgAVP. The presence of P-Pro and P (Fig. 3) indicates further cleavage of neighboring Pro-Arg and P-Pro peptide bonds respectively, by carboxypeptidase activities. Evidence for this metabolic pathway comes from trypsin digestion studies performed in the presence or absence of trypsin inhibitor. Trypsin digestion products and a major metabolite formed after incubation of AVP with intestinal juice coeluted with DesgAVP in the two different mobile phases employed in this study. The dramatic decrease in AVP metabolism in the presence of trypsin inhibitor strongly suggests that this enzyme is a major contributor to AVP metabolism. These results also indicate that under the experimental conditions employed, metabolic degradation of AVP stops at the cyclic part of the AVP molecule (P). Carboxypeptidase Y, which is a non-specific hydrolyzer of C-terminal amino acid residues, caused sequential cleavage of GlyNH_2, Arg and Pro tail residues, but did not cause breakdown of the cyclic fragment. This conclusion correlates with the results obtained from the stability studies employing P. The cyclic hexapeptide was refractory to metabolism even at a higher concentration of intestinal juice (1 : 10). Evidently, the conformation of the cyclic peptide results in resistance to cleavage not only by carboxypeptidases but also to primary cleavage by chymotryptic enzymes. The mechanism of enzymatic degradation of AVP in the intestine seems to be distinctively different from metabolism of this hormone in brain synaptic membranes where aminopeptidase predominates the proteolytic mechanism (Burbach and Lebouille, 1983). We have also found that P was refractory to digestion by aminopeptidase M (data not shown).

Stability studies of DDAVP under the same or more drastic conditions (1 : 10 dilution of intestinal juice) demonstrate that when the trypsin cleavage site is eliminated by substitution of L-Arg with D-Arg, (which is not a substrate for this protease), metabolism was not observed. However, DLAVP which is expected to be cleaved by trypsin was rapidly metabolized. These results support the conclusion that primary attack by trypsin is followed by further metabolism of DesgAVP by carboxypeptidases. Carboxypeptidases present in the lumen of the intestine are mainly carboxypeptidases A and B (Scheele et al., 1981). Carboxypeptidase A requires a free carbonyl group on the C-terminal residue, which must be of the L-configuration (Barman, 1969). Both AVP and DDAVP are in the form of amides. Carboxypeptidase B, which is specific for arginine and lysine residues, can cleave AVP only after trypsin cleavage. The stability of DDAVP confirms that without trypsin cleavage, carboxypeptidases do not act on AVP. The metabolic stability of DDAVP in contrast to AVP can also explain the difference between the oral bioavailabilities of AVP and DDAVP.

In contrast to the rapid metabolism of AVP in the intestinal lumen, AVP was found to be relatively stable in contact with well-rinsed ileal mucosa. Thorough rinsing of the tissue is necessary since even traces of intestinal contents caused rapid metabolism of AVP. Previously Takaori et al. (1986) reported that protease inhibitors were required to increase peptide stability in the mucosal bathing solution. Our studies have demonstrated that following 3 h of incubation, approximately 80% of AVP was still intact in the mucosal bathing solution and even more (> 90%)

was present when AVP was added to the serosal bath. However, formation of small amounts of DesgAVP, P and P-Pro were observed. Because of the extensive washing procedures employed, we attribute this to brush border carboxypeptidase activities.

Transport studies indicated that m-to-s transepithelial transport rate was much greater (about 10 times) than s-to-m rate. The higher rates of m-to-s compared to s-to-m transport in conjunction with differential rates of metabolism in the mucosal versus the serosal bathing solution suggest that there is a correlation between these two phenomena. Careful analysis of transport rate versus the amount of metabolite in any particular experiment further support this conclusion. Higher transport rates were observed in experiments with higher rates of metabolism.

Transport of intact AVP through the ileal mucosa was not observed even when the tissue was thoroughly washed prior to mounting in the chamber. This is in agreement with results reported by Vilhardt and Lundin (Vilhardt and Lundin, 1986) who determined the rate of transport of AVP, DDAVP and other AVP analogues through everted sacs of rat intestine. In contrast to DDAVP which was transported at a rate of almost 7% of the initial concentration per hour, only a very small fraction of AVP (~ 0.2%) traversed the intestinal wall. It should be noted however, that another potential factor influencing intestinal absorption is the anatomical site of the intestine. It was demonstrated recently (Lundin and Vilhardt, 1986), that the in vivo transport rate of DDAVP was highest in the ileocecal region, while absorption from the ileum itself was much lower. The presence of P (or a metabolite which coelutes with P in both mobile phases) suggests that this cyclic structure can partially survive cytosolic peptidase action, as cytosolic activity has been reported to be due to di- and tripeptidases. An alternative explanation for the results is that P formed as a metabolite in the mucosal bath is subsequently transported intact to the serosal bath. On the other hand, the presence of more polar, not clearly identified metabolites, suggests that endocytotic mechanisms may also take place: if this is the case, AVP would be subject to lysosomal proteases which could result in the formation of smaller, more polar fragments.

Regardless of the origin of the metabolites present in the serosal bath, it should be stressed that intact AVP is not transported through the ileal mucosa. Using the same technique Takaori et al. (1986) reported transport of intact renin inhibitor, a linear nonapeptide, through the rabbit jejunum. However, careful analysis of the data presented call this conclusion into question. Although the authors conclude intact peptide is transported, the chromatographic data presented in this paper leave this conclusion open to question.

Our results suggest that even if luminal metabolism of AVP is abolished, transepithelial transport of intact AVP is negligible. Thus a mechanism for the transport of AVP could not be elucidated. Detailed studies of the transport of DDAVP and other analoges of AVP which are more resistant to proteolysis using the same technique need to be conducted. Results from such studies will significantly contribute to an understanding of factors governing transepithelial transport of peptides facilitating oral delivery.

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