The Effects of Salicylate on the Rectal Absorption of Phenylalanine and Some Peptides, and the Effects of These Peptides on the Rectal Absorption of Cefoxitin and Cefmetazole

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Abstract □ The disappearance of phenylalanine and phenylalanylglycine from a perfusate circulated across rat rectal tissue was enhanced significantly in the presence of salicylate or 5-methoxysalicylate at pH 4.5, 7.4, and 8.5. The disappearance of di-, tri-, and tetraphenylalanine from a perfusate at pH 7.4, although facilitated by the presence of salicylate and 5-methoxysalicylate, was also fairly substantial when no adjuvant was present. These peptide analogues of phenylalanine also enhanced the rectal absorption of cefoxitin and cefmetazole, two highly water soluble antibiotics. Phenylalanine and phenylalanylglycine, both poorly absorbed across the rectal membrane when administered alone, did not enhance the rectal absorption of either antibiotic.

Keyphrases □ Rectal absorption—phenylalanine and peptide analogues □ Adjuvant action—salicylate, 5-methoxysalicylate, and peptides □ Absorption study—*in situ* perfusion method, *in vivo* experiments using a microenema technique

Many peptide drugs have been developed for clinical therapy. However, their administration is generally limited to intramuscular, subcutaneous, or intravenous injection. Parenteral administration has inherent disadvantages for some patients such as small children, elderly persons, or those receiving frequent drug administration (*e.g.*, insulin).

Recently, there have been reports of attempts to develop new dosage forms which do not involve parenteral administration, *e.g.*, insulin suppositories (1-3). Our laboratory has been studying several novel nonsurfactant adjuvants that enhance rectal or small intestinal absorption of water-soluble drugs (4, 5) as well as high molecular weight drugs such as insulin, heparin (6, 7), and gastrin (8).

The rectal absorption of phenylalanine and di-, tri-, and tetraphenylalanine has been studied and the effects of adjuvants on their absorption have been examined and are reported here. In addition, these peptides were found to exert an enhancing effect of their own.

EXPERIMENTAL SECTION

Materials—Cefmetazole[1], cefoxitin[2], phenylalanine[3], and di-, tri-, and tetraphenylalanine[3] were used as obtained from the manufacturer. All other chemicals used were at least reagent grade.

Animals—Sprague-Dawley male rats, 224–250 g, were fasted for 16 h prior to the experiments. During the experiments, rats were anesthetized with sodium pentobarbital (60 mg/kg) and kept on a 38°C surface.

***In Situ* Perfusion of Rat Rectum**—The perfusion experiments were carried out by a method similar to one described previously (4). The rectum was exposed by an abdominal incision and a glass cannula was inserted in the distal direction and tied firmly to keep it in position. A second cannula was inserted through the anus and secured by ligation, thus exposing ~5 cm of the rectal compartment (rectum and lower colon) to the perfusate. The perfusate (5 mL) consisting of a phosphate buffer (0.067 M) was circulated at a rate of 1 mL/min at 38°C; 0.1 M HCl was employed to maintain the perfusate pH at 5.0. The ionic strength was maintained at 0.45 with sodium chloride.

***In Situ* Loop Technique of Rectal and Jejunal Compartments**—For this study, a 5-cm section of the rectal compartment, described above, or the upper jejunum were used by ligating at both ends. A 3.0-mL microenema prepared with a 0.067 M phosphate buffer solution was administered. At a designated time following administration, the rectal and jejunal loops were removed from the body and the amount of compound remaining was measured.

***In Vitro* Rectal Everted Sac Studies**—In these studies about 4 cm of the rectum, including the lower colon, were removed from the body and everted. Following the addition of 0.5 mL of buffer solution into the everted sac, the sac was ligated at both ends. The sac was then immersed in 5 mL of a buffer solution containing the test compounds and maintained for 1 h at 37°C while bubbling a mixture of 95% O_2 and 5% CO_2 through the solution. The concentration of the test compound inside the everted sac was measured, and the permeation percent was calculated from the ratio of the test compound in the inside solution to the initial concentration in the outside solution, *i.e.*:

$$\frac{\text{(concentration inside after 1 h)} \times 100}{\text{initial concentration outside}} = \%\ \text{permeation}$$

***In Vivo* Absorption Studies**—A microenema (1.0 mL/kg) was administered into the anus through a polyethylene tube inserted at a depth of 1 cm. The anus was ligated with thread to prevent leakage of the drug solution. After administration, blood samples were collected from the jugular vein as a function of time.

Assay Method—Cefoxitin and cefmetazole concentrations in the plasma or in the perfusate were measured by HPLC (9). Phenylalanine and its peptides were assayed by HPLC under the following conditions: RP-18 column material with a column length of 15 cm (4.6 mm i.d.); the mobile phase for phenylalanine (I) consisted of methanol and 0.05 M citrate phosphate buffer at pH 3.4 (15:85); the mobile phase for diphenylalanine (II) consisted of methanol-acetonitrile-0.05 M ammonium acetate (28:12:60); the mobile phase for triphenylalanine (III) consisted of methanol-acetonitrile-0.05 M ammonium acetate (25:20:55); and the mobile phase for tetraphenylalanine (IV) consisted of methanol-acetonitrile-0.05 M ammonium acetate (25:25:50). The flow rate was 1.0 mL/min, and a spectrophotometric detector at 254 nm was used.

RESULTS AND DISCUSSION

The absorption of amino acids from the upper digestive tract usually occurs by active transport. In fact, absorption of L-phenylalanine (I) from the jejunum using the *in situ* loop technique was significantly greater than that from the rectal compartment. In the absence of sodium salicylate, 10 ± 1% of a 15-mg/mL dose of phenylalanine disappeared from a rectal loop compared with 79 ± 6% from a jejunal loop after 60 min. In the presence of 60 mg/mL of sodium salicylate, 62 ± 9% of the phenylalanine was lost from the rectal loop while 84 ± 5% was lost from the jejunal loop. As shown in Table I, there was no absorption of phenylalanine from the rectal perfusate at pH 4.8 and only slight absorption at pH 7.4 and 8.5 in the absence of salicylate.

In previous papers (4-6), it has been reported that salicylate and 5-methoxysalicylate enhanced the absorption of many kinds of water-soluble drugs from the rat rectum. The present study indicates that the rectal absorption of amino acids is also facilitated by the presence of salicylate and 5-methoxysalicylate. The presence of 1.0% sodium salicylate or sodium 5-methoxysalicylate in the rat rectal perfusate caused a significant disappearance of I from the perfusate (Table I).

As described above, studies using the *in situ* loop technique also showed that coadministration of 60-mg sodium salicylate/kg significantly enhanced the absorption of I from the rectal compartment and slightly facilitated the

[1] Sankyo Co., Tokyo, Japan.
[2] Merck, Rahway, N.J.
[3] Sigma Chemical Co., St. Louis, Mo.

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Table I—Disappearance of Phenylalanine or Its Peptides from the Rat Rectal Perfusate at 30 min after Circulation [a]

Compound	pH in Perfusate	Amount Lost from Perfusate with Additives, %		
		0.4% Sodium Chloride	1% Sodium Salicylate	1% Sodium 5-Methoxysalicylate
Phenylalanine	4.8	<1.0 (n = 6)	12.4 ± 0.8[b] (n = 6)	—
	7.4	4.8 ± 0.7 (n = 10)	18.6 ± 0.6[b] (n = 10)	28.3 ± 2.0[b] (n = 10)
	8.5	2.8 ± 0.5 (n = 10)	23.1 ± 1.0[b] (n = 10)	—
Diphenylalanine	7.4	23.5 ± 2.2 (n = 6)	39.5 ± 1.7[b] (n = 6)	43.6 ± 2.5[b] (n = 6)
Triphenylalanine	7.4	14.8 ± 1.3 (n = 6)	39.4 ± 3.0[b] (n = 6)	36.8 ± 2.8[b] (n = 6)
Tetraphenylalanine	7.4	20.4 ± 3.2 (n = 4)	44.6 ± 3.2[b] (n = 4)	39.7 ± 4.4[b] (n = 4)

[a] Uncertainties are expressed as *SEM*. [b] $p < 0.001$ compared with the control not containing adjuvant (using a Student's t test).

absorption of I from the jejunum. The significant enhancement of the rectal absorption of peptides with nonsurfactant adjuvants has had limited examination, except for reports by Nishihata *et al.* (4-7, 10-14) on facilitated rectal and intestinal absorption of insulin in the presence of adjuvants.

To further study facilitated peptide absorption, II, III, and IV were examined. As seen in Table I, the disappearance of these peptides from rat rectal perfusate at pH 7.4 was significantly facilitated by the presence of salicylate and 5-methoxysalicylate. This result indicates that rectal administration can offer an effective alternative route for peptide drug delivery.

Matthews (15) has reported that uptake of dipeptides from the small intestine is more rapid than that of comparable equimolar concentrations of the constituent amino acids. The present study shows that absorption of II is faster than that of I even from the rectum. Shoof *et al.* (16) have reported that the uptake of II into the brush-border membrane prepared from the small intestine occurred significantly, and hydrolysis of II occurs at a point on the cytosol side of a diffusion barrier located in the membrane. It has been demonstrated (17) that salicylate at pH 7.4 was taken up into the brush-border membrane vesicles prepared from the rat rectal compartment, including the rectum and lower colon. These findings may explain why II is substantially absorbed from the perfusate even when no adjuvant is present. That is, rectal absorption of II by itself occurs *via* the microvillus membrane in the rectal compartment as well as in the small intestine.

In the study using the *in vitro* rat rectal everted sac technique described above, the percents of the compounds permeating the everted sac membrane after 1 h were 12.4 ± 3.5, 29.6 ± 4.3, 18.3 ± 4.6, and 16.7 ± 3.1% for L-phenylalanine, II, III, and IV, respectively. When the outside solution initially contained 1% sodium salicylate along with 500 μg/mL of the test compound (I, II, III, or IV), the percents of drug permeating the sac increased to 34.6 ± 6.2 ($p < 0.001$ compared with the solution of the drug without salicylate, n = 4), 41.7 ± 6.4 ($p < 0.01$, n = 3), 36.7 ± 5.8 ($p < 0.001$, n = 4), and 30.6 ± 5.4% ($p < 0.001$, n = 3), for I, II, III, and IV, respectively. These results are an additional indication that the losses of phenylalanine, II, III, and IV from the perfusion studies described earlier involved permeation through the rectal membrane.

Nishihata and Higuchi (17) reported that the enhancing action of salicylate on the absorption of cefoxitin and I into the brush-border membrane vesicles appeared to involve the simultaneous uptake of salicylate into the vesicles. This observation raised the question as to whether II, also significantly absorbed by itself, may act as an adjuvant. Subsequent experiments indicated that the presence of 500 μg of II/mL, in a perfusate containing 500 μg of cefoxitin at pH 7.4, did in fact facilitate the disappearance of cefoxitin, raising the drug bioavailability to 27 ± 2% ($p < 0.001$, Student's t test, n = 6), compared with 4 ± 1% (n = 6) in the absence of II.

The adjuvant effects of III and IV were also studied. The disappearance of cefoxitin from rat rectal perfusate was slightly facilitated by the presence of III (16 ± 3% at a dose of 500 μg of III/mL) or IV (15 ± 2%, n = 6 at a dose of 200 μg of IV/mL). This result appears to indicate that the adjuvant effect of the peptides II, III, or IV on rectal drug absorption is contingent upon the simultaneous absorption of the peptides themselves. Their uptake into the microvillus membrane may alter the nature of the membrane barrier. The

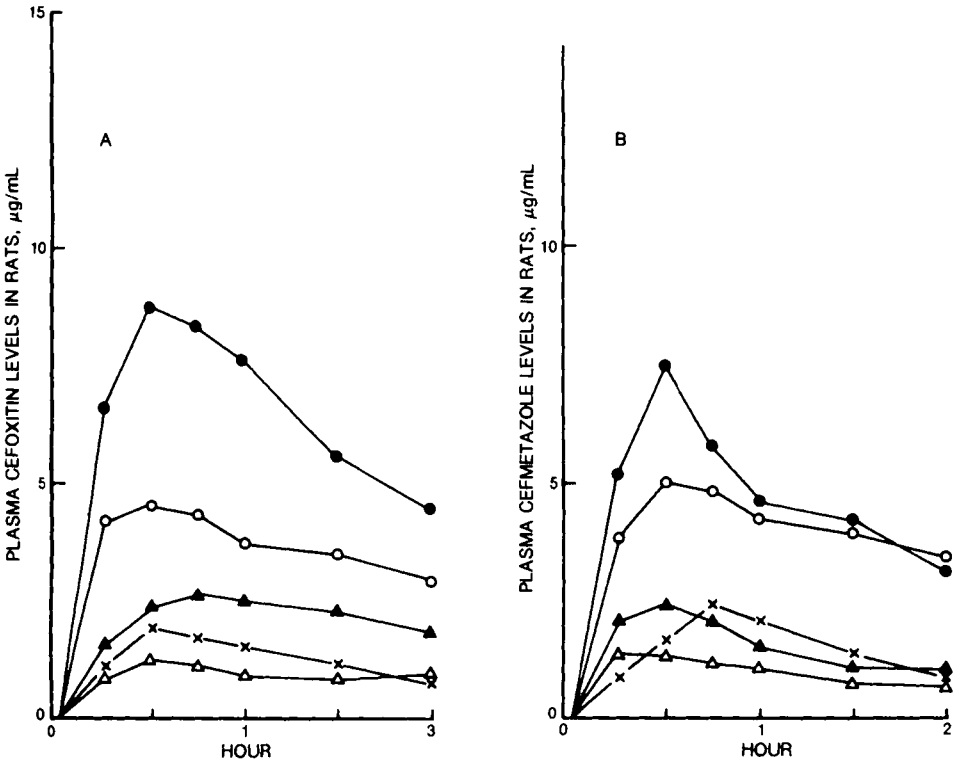

Figure 1 - *(A) Plasma cefoxitin and (B) plasma cefmetazole levels after rectal administration of a microenema containing either 20 mg of cefoxitin/kg or 30 mg of cefmetazole/kg and one of the following: 1 mg of II/mL/kg (△); 27 mg of NaCl/mL/kg (×); 5 mg of II and 27 mg of NaCl/mL/kg (●); 5 mg of II/mL/kg (▲); 1 mg of II and 27 mg of NaCl/mL/kg (○). Rectal administration of cefoxitin or cefmetazole in a microenema without II and/or NaCl produced a plasma cefoxitin level of <0.4 μg/mL and a plasma cefmetazole level of <0.2 μg/mL at peak heights.*

enhancing effect of II was further studied in *in vivo* experiments using a microenema technique. Plasma cefoxitin levels in the rat after rectal administration of a microenema containing 20 mg of cefoxitin and 1–5 mg of II increased significantly over drug levels obtained in the absence of II (Fig. 1). The higher the concentration of II in the microenema, the higher the plasma cefoxitin level. Increasing the ionic strength with NaCl resulted in an even greater increase in the plasma drug levels. These results seem to indicate that rectal absorption of cefoxitin by itself, and enhanced by II, shows some Na^+ dependency. As shown in Fig. 1, rectal absorption of cefmetazole was also enhanced by the presence of II in the microenema.

As shown above and in earlier reports (5, 8), the promotion of the absorption of these antibiotics appears to require the simultaneous absorption of the adjuvant, which alters the nature of the membrane barrier, increasing its permeability.

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