Enhancement of the Rectal Absorption of Sodium Ampicillin by *N*-Acylamino Acids in Rats

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Received July 16, 1986, from the *Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-Ku, Hiroshima 734, Japan.* Accepted for publication May 20, 1987.

Abstract □ The promoting efficacies of *N*-acyl derivatives of amino acids on the rectal absorption of sodium ampicillin were investigated using the rat rectal loop technique. *N*-Acyl derivatives with longer carbon chains in the acyl moiety showed a greater promoting potency. The promoting potencies of *N*-acyl derivatives of phenylglycine and phenylalanine were greater than those of glycine and alanine derivatives when compared at the same length of carbon chain in their acyl moieties. The promoting action of *N*-acylamino acids was not influenced by the presence of *N*-ethylmaleimide or ouabain. The promoting potencies of *N*-acylamino acids were depressed in the presence of calcium chloride in the rectal loop. The contribution of the calcium ion sequestration capacity of *N*-acylamino acids to their promoting efficacies is discussed.

Ampicillin has been used clinically for infectious diseases as an oral dosage form. However, its oral bioavailability is reported to be 30–50% in humans.[1] The low oral bioavailability is attributed to its poor lipid solubility.

Recently, the use of an adjuvant has raised much interest for improving bioavailability of poorly absorbed drugs, especially in rectal dosage forms. Salicylates,[2] enamine derivatives,[3] carboxylic acids derivatives,[4] bile salts,[5] and saponines[6] have been reported as absorption promoters for the rectal absorption of erratically absorbed drugs.

In a previous report, we demonstrated that *N*-acyl derivatives of collagen peptides, which were fractionated from the hydrolysates of collagen, markedly enhanced the rectal absorption of sodium ampicillin in rabbits and rats.[7] Through that study, the use of amino acids, instead of the peptide moiety, for promoting absorption was considered. The present paper describes the promoting effects of various *N*-acylamino acids on the rectal absorption of sodium ampicillin using the rat rectal loop technique.

Experimental Section

Materials—Sodium ampicillin (912 μg/mL) was a gift from Kyoto Pharmaceutical Industries, Ltd. (Kyoto, Japan) and was used without further purification. The following acid chlorides of reagent grade were purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan) for the synthesis of *N*-acyl derivatives of amino acids: butyryl (C4) chloride, caproyl (C6) chloride, octanoyl (C8) chloride, decanoyl (C10) chloride, and lauroyl (C12) chloride. The following reagent grade amino acids were used: glycine (G), DL-alanine (A), DL-phenylglycine (PG), and DL-phenylalanine (PA). All other reagents were of reagent grade.

Synthesis of *N*-Acylamino Acids—*N*-acylamino acids were synthesized according to the method of Jungermann et al.[8] An amino acid (0.024 mol) was dissolved in 20 mL of 10% sodium hydroxide aqueous solution, and mixed with 180 mL of acetone in a 500-mL beaker equipped with a pH electrode. The temperature of the mixture was maintained below 5 °C by placing the beaker in ice water during the following procedures. Fatty acid chloride (0.02 mol) was dissolved in an approximately equal volume of acetone and added in a dropwise manner to the mixture which was continuously stirred. The pH of the mixture was maintained in a range from 9 to 12 by addition of 10% sodium hydroxide aqueous solution. After addition of the fatty acid chloride solution was completed, the mixture was stirred continuously for >1 h at a temperature of <5 °C. Then, the solvent was evaporated to dryness under reduced pressure. The residue was mixed with an organic solvent or a mixture of organic solvents, listed in Table I, and precipitation was obtained. Recrystallization was repeated two or three times. The *N*-acylamino acids thus obtained are listed in Table I with their abbreviations, yields, and melting points.

Calcium Ion Sequestration Capacity of *N*-Acylamino Acid—Each *N*-acylamino acid was dissolved in 0.05 M Tris-HCl buffer (pH 7.9) to make a concentration of 10 mM. To 9 mL of *N*-acylamino acid solution, 5 mL of calcium ion solution (200 ppm) and 2 mL of ionic strength adjuster (ISA-Ca; TOA Electronics, Ltd., Tokyo, Japan) were added. The mixture was left at 37 °C for 10 min, then the concentration of unsequestrated calcium ion was measured at 37 °C using a calcium ion selective electrode (CA-135; TOA Electronics, Ltd.) connected to digital ion meter (IM-20E; TOA Electronics, Ltd.) according to the manual of TOA Electronics, Ltd. The amount of sequestrated calcium ion was calculated from differences between the initial and final unsequestrated concentrations of calcium ions.

Animal Studies—Male Wistar rats weighing 200–250 g were used. Rats were fasted for 16 h prior to experiments, but water was given freely. The rats were anesthetized by intraperitoneal injection of pentobarbital (Nembutal, sodium solution; Abbott Laboratories) at a dose of 30 mg/kg, and held supine on a surface kept at 37 °C to prevent decrease of body temperature during experiments.

Intravenous Administration—Sodium ampicillin was dissolved in 0.9% sodium chloride aqueous solution and administered to the rat tail vein at a dose of 7.5, 15, 30, or 60 mg/mL/kg. After drug administration, blood was withdrawn from a jugular vein with a heparinized syringe at appropriate time intervals for the analysis of ampicillin.

Absorption Study—The rectal loop method was used in the same manner as described in a previous report.[7] The test solution introduced into the rectal loop was prepared by dissolving sodium ampicillin (60 mg/mL) and various amounts of *N*-acylamino acids in a 0.05 M Tris-HCl buffer (pH 7.9), and the resulting solution was adjusted to pH 7.9 by adding a suitable amount of NaOH (1 M) if necessary. The osmotic pressure of the test solution was also adjusted to 560 mosM/kg H_2O by adding a suitable amount of sodium chloride (OSMOTRON-10; ORION Riken Co., Ltd., Tokyo, Japan). The test solution was introduced into the rectal loop at a rate of 1 mL/kg after the rectal temperature of the rat was confirmed to be >36 °C. Blood was collected at appropriate time intervals from a jugular vein for the analysis of ampicillin.

Influence of Osmotic Pressure on the Absorption-Promoting Effect of *N*-Acylamino Acids—The influence of the osmotic pressure of the dosing solution on the absorption-promoting effect of *N*-acylamino acids was examined using the in situ recirculating perfusion method. The osmolarities of the solution containing sodium ampicillin (5 mg/mL) and *N*-acylamino acid (5 mM) were adjusted to 140, 280, or 560 mosM/kg H_2O by adding a suitable amount of sodium chloride. The flow rate of the perfusate was 3 mL/min. During recirculation, blood was collected at appropriate time intervals for the determination of ampicillin.

Influence of *N*-Ethylmaleimide or Ouabain on the Absorption-Promoting Effect of *N*-Acylamino Acids—The influence of *N*-ethylmaleimide or ouabain on the absorption-promoting effect of *N*-acylamino acids was examined using the in situ recirculating perfusion method. The osmolarities of the solutions containing sodium ampicillin (5 mM), *N*-acylamino acid (5 mM), and *N*-ethylmaleimide (5 mM) or ouabain (1 mM) were adjusted to 280 mosM/kg

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Table I—*N*-Acyl Derivatives of Amino Acids

Compound	Molecular Weight	Recrystalizing Solvent	Yield, %	Melting Point, °C
N-Octanoylalanine (C8-A)	215.30	Acetone:hexane	73.5	100.5–101.5
N-Octanoylglycine (C8-G)	201.27	Acetone:hexane	72.0	108.5–109.5
N-Decanoylalanine (C10-A)	243.35	Acetone:hexane	67.6	97.0– 98.0
N-Decanoylglycine (C10-G)	229.32	Acetone:hexane	72.0	117.0–118.0
N-Lauroylalanine (C12-A)	271.40	Acetone:heptane	49.1	109.0–110.0
N-Lauroylglycine (C12-G)	257.37	Acetone	45.9	121.0–122.5
N-Palmitoylalanine (C16-A)	327.51	Acetone	63.6	116.5–117.5
N-Butyrylphenylalanine (C4-PA)	235.28	Toluene	72.2	96.0– 97.0
N-Butyrylphenylglycine (C4-PG)	220.24	Toluene	60.8	114.3–145.3
N-Caproylphenylalanine (C6-PA)	263.33	Toluene:hexane	64.7	110.5–111.2
N-Octanoylphenylalanine (C8-PA)	291.37	Hexane	77.1	99.0– 99.5
N-Octanoylphenylglycine (C8-PG)	276.34	Cyclohexane	75.2	100.0–104.0
N-Decanoylphenylalanine (C10-PA)	319.42	Cyclohexane	74.6	101.5–102.5
N-Lauroylphenylalanine (C12-PA)	347.48	Hexane	77.8	96.0– 98.0
N-Lauroylphenylglycine (C12-PG)	332.45	Cyclohexane	64.5	110.5–112.0

H_2O with sodium chloride.

Influence of Calcium Chloride on the Absorption-Promoting Effect of *N*-Acylamino Acids—This experiment was performed using the rat rectal loop method. The osmotic pressure of the dosing solution containing sodium ampicillin (30 mg/mL), *N*-acylamino acid (15 mM), and calcium chloride (15 mM) was adjusted to 280 mosM/kg H_2O with sodium chloride.

Analytical Method—The concentration of C12-A in the rectal tissue samples was determined by gas chromatography using a gas chromatograph (GC-6A; Shimadzu Ind. Co., Ltd., Kyoto, Japan) equipped with a FID detector at a column oven temperature of 190 °C. Silicon OV-17 (wt 2%, Chromosorb W. AW, 60–80 mesh; Nishio Ind. Co., Ltd., Japan) was used for the column. The flow rate of nitrogen gas was 23 mL/min.

Rectal tissue samples (1 g wet weight) were homogenized with a mixture consisting of acetone containing C10-A as an internal standard, 1 M HCl, and water (40:1:6 v/v/v), and extracted with 8 mL of benzene. After centrifugation for 10 min at 3 000 rpm, 7 mL of the benzene layer was transferred to another test tube and evaporated to dryness under reduced pressure. The residues were methylated with a diazomethane:ether solution. After allowing the samples to stand at room temperature for 30 min, the ether layer was evaporated under reduced pressure. The residue was then dissolved in 50 μL of methanol. One microliter of the solution was injected onto the column. The retention times of C10-A and C12-A were 8.0 and 17.2 min, respectively.

The concentration of ampicillin in whole blood was determined by microbioassay with *Bacillus subtilis* ATCC 6633 as a test organism. Whole blood was diluted with deionized water more than sixfold to minimize the effect of plasma protein binding on the antibacterial activity of ampicillin. The assay limit was 0.075 μg/mL in whole blood.

Results and Discussion

Influence of *N*-Acylamino Acids on the Rectal Absorption of Sodium Ampicillin in Rats—The effects of the concentration of *N*-acylamino acids on the rectal absorption of sodium ampicillin from aqueous solution (pH 7.9) were investigated at a dose of 60 mg of sodium ampicillin/kg, because ampicillin could not be detected microbiologically in the whole blood when sodium ampicillin was administered alone at a dose of <50 mg/kg. The luminal pH of rat rectum was reported as ~pH 7.4 by Crommelin et al.[9] However, in the present study, the pH of the dosing solution was adjusted to 7.9 on the basis of findings that the pH of the rectal fluid determined with a micro pH electrode ranged from 7.9 to 8.0, and that the pH of the rectal solution converged to 7.9–8.0 within 30 min when 100 μL of pH 7.4 or pH 8.2 Tris-HCl buffer (280 mosM/kg H_2O) was introduced into the rat rectal lumen. As an example, blood levels of ampicillin after rectal administration together with various concentrations of C12-A and C10-PA are shown in Figures 1A and 1B, respectively. Only a trace amount of ampicillin was detected in the whole blood without any adjuvants (peak blood level: 0.3 ± 0.1 μg/mL). However, a marked increase in the blood level of ampicillin was observed in the presence of C12-A or C10-PA, depending on the concentration of the coadministered *N*-acylamino acid. A peak blood level of ampicillin was obtained within 10–15 min after administration. No significant difference was observed in the disposition kinetics of ampicillin from blood between rectal and intravenous administrations. The existence of the higher concentration of *N*-acylamino acids in the rectal loop resulted in a higher blood level of ampicillin.

In Figures 2 and 3, the values of peak blood level and area under the concentration curve (AUC) of ampicillin after rectal administration are plotted as a function of the concentration of coadministered *N*-acylamino acid, respectively. In the case of *N*-acylalanine (Figures 2A and 3A), a greater effect was observed in C16-A and C12-A. The most potent effect of the adjuvants was observed in the dosing solution containing 15 mM or more of the adjuvants. In the case of *N*-acylphenylalanine (Figures 2B and 3B), C12-PA and C10-PA demonstrated a greater effect. Thus, adjuvants with a longer carbon chain in the acyl moiety showed a greater promoting effect on the rectal absorption of sodium ampicillin. In addition, *N*-acylphenylalanine appeared to have a greater promoting effect than alanine derivatives when compared at the same carbon chain length in the acyl moiety, indicating that the kind of amino acid moiety, as well as acyl moiety,

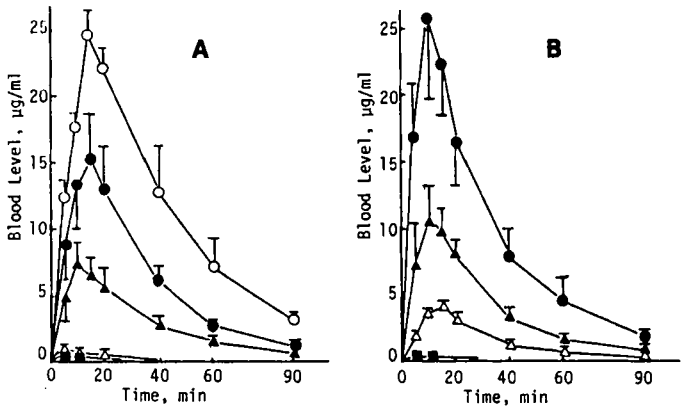

Figure 1—*Enhanced rectal absorption of sodium ampicillin in the presence of C12-A* (A) *or C10-PA* (B) *in rats (loop). Dose: sodium ampicillin, 60 mg/mL/kg. Concentration of N-acylamino acids:* (■) *0 mM;* (△) *2 mM;* (▲) *5 mM;* (●) *10 mM;* (○) *15 mM. The error bars represent SD, n = 3–4.*

also has an important role in the absorption-promoting potencies.

However, as shown in Figure 4A, no significant difference in the absorption-promoting potency was observed between *N*-acylalanine and *N*-acylglycine derivatives having the same carbon chain length in the acyl moiety. Similar results were also found between *N*-acylphenylalanine and *N*-acylphenylglycine (Figure 4B).

The rectal bioavailability of ampicillin, enhanced by *N*-acylamino acids, is summarized in Table II. The AUC values of ampicillin following intravenous administration of various doses showed some discrepancies from linear kinetics, especially at the higher dose (Figure 5). Thus, the rectal bioavailability of ampicillin enhanced by *N*-acylamino acids was estimated by comparing the AUC value between rectal and intravenous administrations as follows. The AUC values after intravenous administration were plotted against the dose of sodium ampicillin (Figure 5). Values of AUC obtained after rectal administration were applied to the dose–AUC curve of the intravenous administration. Thus, the absorbed amount after rectal administration (dose corresponding to the intravenous administration) was estimated. The bioavailability was calculated by dividing the estimated absorbed amount by the rectally administered dose (60 mg/kg).

Influence of Osmotic Pressure on the Absorption-Promoting Effect—As demonstrated in our previous report, the absorption-promoting potency of the *N*-acyl derivative of collagen peptide was influenced by the osmotic pressure in the dosing solution to the rectum.[7] Therefore, a similar investigation was carried out using the in situ rat rectal recirculation technique. The osmolarities of the purfusate used were 140, 280, and 560 mosM/kg H_2O. The rectal absorption of ampicillin without any adjuvants was not found under these conditions.

No difference was observed in the absorption-promoting potencies of *N*-acylamino acids among three different osmolarities of the perfusate. Nishihata et al.[10] reported that the addition of sodium chloride to the microenema containing sodium ampicillin and sodium salicylate increased the plasma ampicillin concentration after rectal administration compared with absorption from microenemas in which sodium ampicillin was administered only with sodium salicylate. Discrepancies between their results and ours in the effect of sodium chloride may be attributable to the differences in adjuvant, pH, and the concentration of sodium chloride employed. Furthermore, the discrepancies between *N*-acylamino acids and *N*-acyl derivatives of collagen peptide in the influence of the osmolarity on the absorption-promoting actions may be derived from the difference of physicochemical properties between amino acid and peptide moieties.

Effect of *N*-Ethylmaleimide or Ouabain on the Adjuvant Effect—The structural integrity of the cell membrane and the membrane of intracellular organelles depends on an appropriate glutathione status.[11] The presence of a sulfhydryl group within the brush border membrane is considered important to the active transport of drugs such as aminocephalosporins.[12] For example, Kimura et al.[12] reported that the mucosal-to-serosal flux of cefadroxil was suppressed by 50% in the presence of 1 mM *N*-ethylmaleimide, a sulfhydryl-blocking agent, in rat jejunum and ileum. Nishihata et al.[13] reported that the enhanced cefmetazole absorption caused by the coadministration of either diethylethoxymethylene malonate or diethyl maleate was suppressed by treatment with dimercaprol in an in vitro rectal everted sac, suggesting the participation of sulfhydryl groups in the absorption-promoting action of such adjuvants.

In the present study, an attempt was made to examine the possibility of the participation of a sulfhydryl group in the absorption-promoting action of C12-A, as an example, using the in situ recirculating perfusion method. However, the

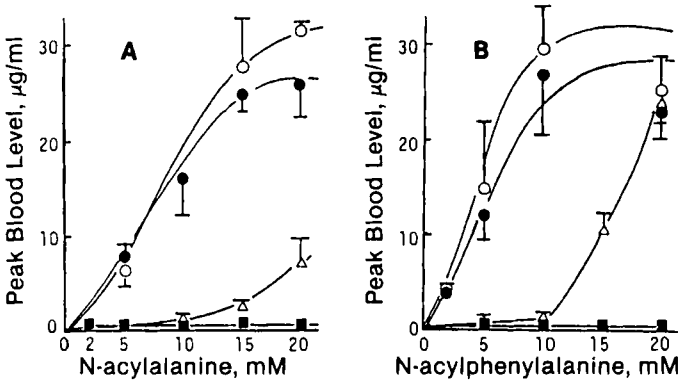

Figure 2—*Effect of the concentration of N-acyl derivatives of alanine* (A) *or phenylalanine* (B) *on the peak blood level of ampicillin after rectal administration (loop). Dose: sodium ampicillin, 60 mg/mL/kg. Key:* (■) *C8-A;* (△) *C10-A;* (●) *C12-A;* (○) *C16-A in Figure* 2A, *and* (■) *C4-PA and C6-PA;* (△) *C8-PA;* (●) *C10-PA;* (○) *C12-PA in Figure* 2B. *The error bars represent the SD, n = 3–4.*

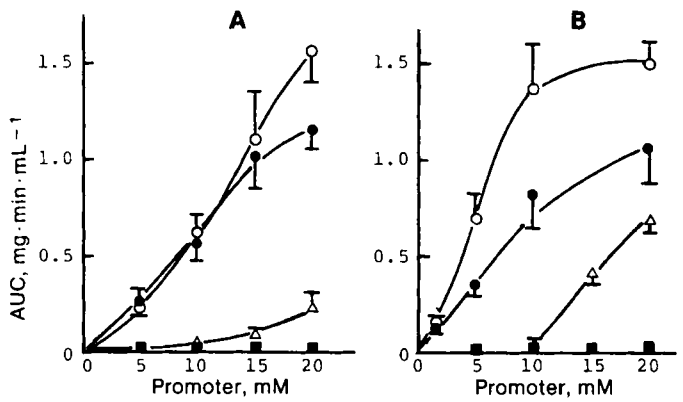

Figure 3—*Effect of the concentration of N-acyl derivatives of alanine* (A) *or phenylalanine* (B) *on the AUC value of ampicillin after rectal administration (loop). Dose: sodium ampicillin, 60 mg/mL/kg. Key:* (■) *C8-A;* (△) *C10-A;* (●) *C12-A;* (○) *C16-A in Figure* 3A, *and* (■) *C4-PA and C6-PA;* (△) *C8-PA;* (●) *C10-PA;* (○) *C12-PA in Figure* 3B. *The error bars represent the SD, n = 3–4.*

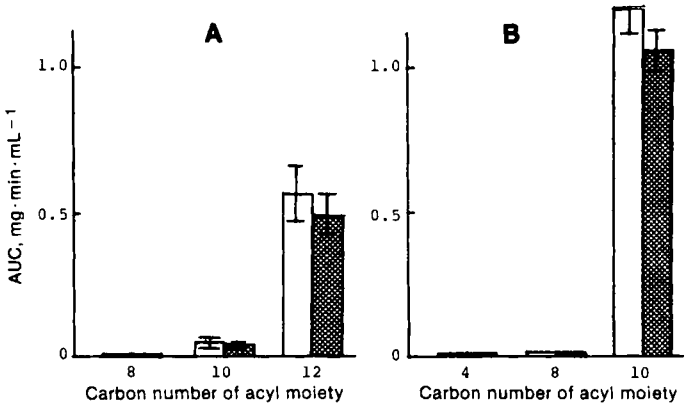

Figure 4—*A comparison of the promoting effect on the rectal absorption of sodium ampicillin between N-acylalanine and N-acylglycine* (A) *and between N-acylphenylalanine and N-acylphenylglycine* (B). *Dose: sodium ampicillin 60 mg/mL/kg. Concentration of N-acylamino acid: 10 mM. Key:* (▭) *N-acylalanine;* (▤) *N-acylglycine in Figure* 4A; (▭) *N-acylphenylalanine;* (▤) *N-acylphenylglycine in Figure* 4B. *The error bars represent the SD, n = 3–4.*

Table II—Enhanced Rectal Absorption of Sodium Ampicillin by Various *N*-Acyl Derivatives of Amino Acids in Rats[a]

Promoter	Concentration, mM	AUC, $\mu g \cdot min \cdot mL^{-1}$	Amount, mg[b]	EBA, %[c]
Control		8.0 ± 6.6	0.3 ± 0.3	0.5 ± 0.4
C10-A	5	7.6 ± 3.1	0.3 ± 0.2	0.5 ± 0.3
	10	50.6 ± 21.3	2.0 ± 0.8	3.3 ± 1.4[d]
	15	82.8 ± 28.3	3.2 ± 1.1	5.4 ± 1.8[d]
	20	248.6 ± 82.4	9.7 ± 3.2	16.1 ± 5.3[d]
C12-A	5	262.6 ± 69.3	10.1 ± 2.6	16.8 ± 4.3[d]
	10	564.0 ± 97.9	19.5 ± 2.6	32.4 ± 4.3[d]
	15	1171.0 ± 118.2	33.9 ± 2.4	56.5 ± 4.0[d]
	20	1159.0 ± 144.3	33.0 ± 4.0	55.1 ± 6.6[d]
C16-A	5	230.3 ± 64.7	8.9 ± 2.4	14.8 ± 4.0[d]
	10	587.6 ± 100.4	20.1 ± 2.5	33.5 ± 4.2[d]
	15	1535.0 ± 247.7	41.3 ± 8.3	68.7 ± 13.8[d]
	20	1565.9 ± 147.9	42.1 ± 2.9	70.1 ± 4.8[d]
C4-PA	20	7.7 ± 4.2	0.3 ± 0.2	0.5 ± 0.3
C6-PA	20	8.0 ± 6.6	0.3 ± 0.3	0.5 ± 0.4
C8-PA	5	4.9 ± 3.2	0.3 ± 0.2	0.5 ± 0.3
	10	12.2 ± 7.0	0.6 ± 0.3	1.0 ± 0.6
	20	711.7 ± 74.1	23.3 ± 1.9	38.8 ± 3.1[d]
C10-PA	2	127.6 ± 16.7	5.3 ± 0.7	8.8 ± 1.1[d]
	5	352.2 ± 63.0	13.3 ± 2.1	22.2 ± 3.4[d]
	10	1211.2 ± 118.6	34.6 ± 2.5	57.7 ± 4.1[d]
	20	1182.9 ± 138.9	34.1 ± 2.8	56.9 ± 4.7[d]
C12-PA	5	720.4 ± 121.6	23.9 ± 3.5	39.8 ± 5.8[d]
	10	1365.4 ± 266.6	37.8 ± 5.4	63.0 ± 9.0[d]
	20	1239.2 ± 106.2	35.3 ± 2.1	58.2 ± 3.3[d]

[a] Each value is the mean ± SEM of 3–4 rats. [b] Amount of sodium ampicillin absorbed after rectal administration (see text for details). [c] Extent of bioavailability was calculated by dividing the absorbed amount by the dose (60 mg). [d] Significantly different from control ($p < 0.05$).

promoting action of C12-A on the rectal absorption of sodium ampicillin was not influenced by the concomitant presence of 5 mM *N*-methylmaleimide in the perfusate.

To further examine the role of an active transport system in the absorption-promoting actions of *N*-acylamino acids, the effect of ouabain on the adjuvant action was examined. Ouabain, an inhibitor of the sodium pump, is known to inhibit the active transport of drugs such as glucose.[14] However, the absorption-promoting effects of *N*-acylamino acids were not influenced by the presence of ouabain in the perfusate.

Therefore, in the case of *N*-acylamino acids, the participation of a sulfhydryl group in the membrane and of an active transport system in the absorption-promoting mechanism can be ruled out.

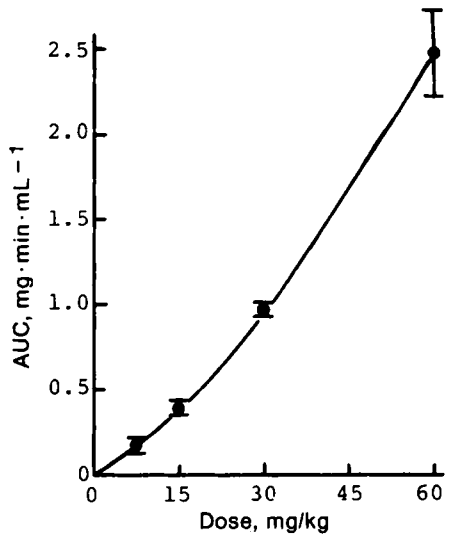

Figure 5—*Values of AUC after intravenous administration of sodium ampicillin at various doses in rats. The AUC values are 174.5 ± 28.2 $\mu g \cdot min \cdot mL^{-1}$ for the dose of 7.5 mg/kg, 385.4 ± 31.2 $\mu g \cdot min \cdot mL^{-1}$ for 15 mg/kg, 978.4 ± 74.6 $\mu g \cdot min \cdot mL^{-1}$ for 30 mg/kg, and 2467.6 ± 267.4 $\mu g \cdot min \cdot mL^{-1}$ for 60 mg/kg, respectively. Each value is the mean ± SD of four rats.*

Effect of Calcium Ion on the Adjuvant Effect—In a previous report, we suggested that compounds that are permeable through membranes by themselves and have calcium-ion sequestration capacity might facilitate the membrane permeation of erratically absorbed water-soluble drugs of low molecular weight.[5] Some adjuvants with promoting effects for the rectal absorption of ampicillin, such as enamine derivatives,[3] bile salts,[5] fatty acids,[15] saponines,[6] and *N*-acylcollagen peptide,[7] were considered to have such characteristics. Thus, the calcium-ion sequestration capacities of *N*-acylamino acids were determined in the present study and are shown in Table III. The calcium-ion sequestration capacities of *N*-acylamino acids increased with an increase of the carbon number in the acyl moieties.

The effect of calcium ion on the promoting action of *N*-acylamino acid on the rectal absorption of sodium ampicillin is shown in Figure 6. The coexistence of $CaCl_2$ depressed the promoting effect of C12-A. Similar depressions of the adjuvant effect in the presence of $CaCl_2$ were observed with other *N*-acylamino acids. No precipitation in the dosing solution was observed in the presence of $CaCl_2$. The depression of the promoting action of *N*-acylamino acids by the presence of calcium ions may be due to the masking of the calcium- or magnesium-ion sequestrating ability of *N*-acylamino acids.

Table III—Calcium-Ion Sequestration Capacity of *N*-Acylamino Acids[a]

N-Acyl Alanine	Bound Ca^{++}, g ion/M	*N*-Acyl Phenylalanine	Bound Ca^{++}, g ion/M
C8-A	0.03	C4-PA	0.04
C10-A	0.17	C6-PA	0.06
C12-A	0.42	C8-PA	0.24
C16-A	0.45	C10-PA	0.50
		C12-PA	0.50

[a] Determined at pH 7.9 and 37 °C.

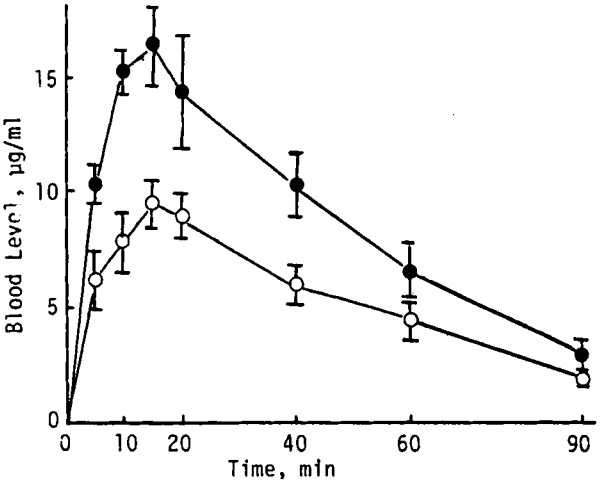

Figure 6—*Effect of calcium ion on the promoting effect of C12-A in rats (loop). Dose: sodium ampicillin, 30 mg/kg; osmolarity of the drug solution: 280 mosM/kg H_2O. Key: (●) C12-A (15 mM); (○) C12-A (15 mM) plus $CaCl_2$ (15 mM). The error bars represent the SD, n = 3–4.*

In this experiment, the effect of calcium ion on the rectal tissue uptake of C12-A itself was also investigated. The concentration of C12-A in the rectal membrane 15 min after rectal administration was 0.551 ± 0.055 μmol/g of tissue in the absence of calcium chloride, and 0.561 ± 0.064 μmol/g of tissue in the presence of 15 mM calcium chloride in the dosing solution. Thus, the rectal tissue uptake of C12-A itself was not influenced by the presence of calcium chloride, although the absorption-promoting potency of C12-A for sodium ampicillin was suppressed by coexisting calcium ion. This finding is in agreement with the case of enamine derivatives of amino acids as reported previously.[16]

It is well known that local changes in cytosolic calcium ion concentration in submicromolar ranges trigger many important cellular functions including shape change and motility.[17] Cassidy and Tidball reported that disodium ethylenediamine tetraacetate (EDTA), placed in the rat intestinal lumen, evoked a fivefold increase in membrane permeability to phenol red and that at the same time the mucosal contents of magnesium and calcium were decreased significantly.[18] They also showed, with electron microscopy, rounded swellings on the microvilli in the area of the junctional complexes between adjacent epithelial cells and widening of intercellular channels, particularly in the region of the intermediate junctions, in the presence of EDTA.

It has been proposed that EDTA and long-chain fatty acid anions bind calcium in the membrane and open the tight junction between the duodenal enterocytes.[19] Munch and Rasmussen also suggested that materials binding calcium ions to an appreciable degree make the tight junctions between luminal ends of the enterocytes more permeable to water than they ordinarily are.[20] An increase in the membrane permeability to sodium ampicillin evoked by *N*-acylamino acids may be correlated to the calcium-ion sequestration capacity of the *N*-acylamino acids. The relationships among the absorption-promoting effect of *N*-acylamino acids, the binding ability of *N*-acylamino acids to rectal tissue, and the calcium-ion sequestration capacity of *N*-acylamino acids require further investigation.

References and Notes

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