[Chem. Pharm. Bull. 32(8)3187—3193(1984)]

Mechanism of the Enhancement of Rectal Permeability of Drugs by Nonsteroidal Anti-inflammatory Drugs[1)]

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(Received November 9, 1983)

The mechanisms of the enhancing effect of nonsteroidal anti-inflammatory drugs (NSAID) on rectal permeability were investigated. It was found that the interaction of NSAID with membrane components (proteins and lipids) plays an important role in the permeation process of marker drugs. Both the permeation of the drugs and the accumulation of NSAID were specifically decreased by pretreatment of the rectal membrane with $HgCl_2$ or papain. In liposomes prepared from rectal lipids, the permeability of the lipid layer was markedly increased by the presence of NSAID. Furthermore, NSAID induced a solvent drag effect in the permeation of marker drugs through the rectal membrane. It is suggested that the interactions of NSAID with the membrane components, as well as the solvent drag effect, are at least partly responsible for the enhanced permeability.

Keywords——nonsteroidal anti-inflammatory drug; papain; mercuric chloride; rectal lipid liposome; solvent drag effect; rectal permeability; sulfanilic acid; creatinine

In previous papers of this series,[2-6)] we showed that surfactants, disodium ethylenediaminetetraacetate (EDTA) and polyethylene glycol 400 (PEG) enhance the rectal permeability to nonabsorbable drugs, such as sulfaguanidine, sulfanilic acid, creatinine and high molecular drugs, and that the permeability enhancing effects are accompanied with histological changes and the release of proteins from the rectal mucosa. Recently, we reported[7)] that nonsteroidal anti-inflammatory drugs (NSAID), such as indomethacin (IM), phenylbutazone (PB), diclofenac sodium (DF) and aspirin (ASA), enhance the rectal permeability to nonabsorbable drugs. However, the observed permeability enhancement by NSAID could not be explained simply in terms of the solubilization of membrane components or histological changes. On the other hand, we found a good correlation between the rectal membrane permeability to nonabsorbable drugs and the amount of NSAID accumulated in the rectum. Yaginuma *et al.*[8)] and Nishihata *et al.*[9)] reported that some NSAID promote the absorption of drugs from the rectum, but the mechanism of the promoting effect has not been clarified yet.

It can be presumed that the accumulation of NSAID is due to the interaction between the drug and protein or lipid components in the rectal membrane. In this study, the interaction between NSAID and membrane components in the rectal tissue was examined in order to clarify the mechanism of the permeability enhancing effect. The possibility of a contribution of the solvent drag effect to the enhancement of rectal permeability by NSAID was also investigated.

Experimental

Materials——IM (Medicel Research Laboratory, Japan), PB (Sigma, U.S.A.), DF (Kodama Pharmaceutical

Co., Ltd., Japan), sulfanilic acid (SA), creatinine (CR) (marker drugs, Tokyo Kasei Co., Ltd., Japan), deuterium oxide (D_2O, purity 99.75%, E. Merck, West Germany) and dextran (m.w. 40000, Pharmacia Fine Chemicals, Sweden) were used as supplied. Egg phosphatidylcholine was prepared from egg yolks according to the method of Rhodes & Lea.[10] Other chemicals were of reagent grade.

Preparation of Drug Solution——The marker drug (SA: final concentration, 3 mg/ml) was dissolved in isotonic phosphate buffer solution (pH 7.4) and IM was added at the concentration of 5 mM. The drug solution without NSAID was used as the blank solution.

Binding Study of NSAID to Everted Rat Rectum——The binding of NSAID with rat rectum was studied by using the equilibrium dialysis method reported by Nishihata *et al.*[9]

Absorption and Exsorption Experiments——Absorption and exsorption were examined by the method described in our previous paper.[7] In the case of pretreatment with $HgCl_2$ or papain, 0.025 or 0.05 mM $HgCl_2$ or 0.1% papain in isotonic phosphate buffer at pH 7.4 was perfused at a flow rate of 20 ml/15 min for 3 min or 30 min, respectively, before the absorption and exsorption experiments.

Accumulation of IM in the Rectal Membrane——The accumulation of IM in the rectal membrane was determined according to the method described in our previous paper.[7]

Drug Partitioning into Chloroform Containing Lipids——Drug partitioning between water and chloroform containing egg phosphatidylcholine was determined according to the method of Furusawa *et al.*[11]

Preparation of Liposomes and Transfer Rate Experiments——Two kinds of liposomes were prepared, one from a mixture of egg phosphatidylcholine (80 μmol), cholesterol (20 μmol) and dicetylphosphate (5 μmol), and the other from total lipid extracts of the rectal tissue (180 mg) obtained by the method of Folch *et al.*[12] Briefly, the lipid mixture or the total lipid extracts dissolved in chloroform were pipetted into 50 ml round-bottomed flasks. The chloroform was removed under a vacuum using a rotary evaporator. Then 5 ml of SA solution (25 mg/ml) was added to the thin, dry lipid film. Mechanical shaking with a vortex mixer for 10 min caused complete dispersion of the lipids. The suspension was then sonicated (Ohtake 5202 CPZ, Japan) under N_2 for 2.5 min on ice. A Sephadex G-25 column was used to separate the liposomal fraction from free SA and the liposomal suspension was immediately used for transfer experiments.

The overall transfer rate of SA across the liposomal membrane was determined by the modified dynamic dialysis method of Meyer & Guttman.[13] Five ml of liposomal suspension containing SA was introduced into a Visking cellulose tube (36/32) which was placed in 50 ml of external solution, isotonic phosphate buffered saline, in the presence or absence of NSAID. The temperature of the solution was maintained at 37 °C. The release rate of SA from the Visking dialysis sac was very rapid, so the rate of appearance in the external medium should be determined by the transport rate across the lipid bilayers of the liposomes. A semilogarithmic plot of the percentage of the drug remaining in the dialysis bag against time gave a straight line and the release rate constants were calculated from the slopes of these plots.

Solvent Drag Effect Experiments——The solvent drag effect was examined by the method of Karino *et al.*[14] with a slight modification. Silicon tubing was cannulated into both ends of the rectum and anus. The volume flow was determined by using dextran (m.w. 40000) instead of inulin. The effect of NSAID was investigated at the concentration of 5 mM IM or 10 mM DF.

Analytical Methods——The analytical methods for SA, CR, and NSAID were described in our previous paper.[7] D_2O was determined infrared spectrometrically by the method of Karino *et al.*,[14] which is based on that of Thornton *et al.*[15] The coloring method of Roe *et al.*[16] was used for dextran.

Results and Discussion

The Interaction with Proteins

In order to clarify the mechanism of NSAID-induced permeability enhancement, the interactions between NSAID and membrane components were investigated. First, the interaction with protein components was examined by chemical modification of the rectal membrane with $HgCl_2$ or papain. It is well known[17-20] that papain hydrolyzes the membrane surface-protein particles without any disruption of the unit membrane, and that $HgCl_2$ irreversibly modifies sulfhydryl groups of proteins within the membrane. In the present study, the rectum was pretreated with $HgCl_2$ or papain and then the experiments were carried out.

Table I shows the effect of $HgCl_2$ treatment on the binding of NSAID to the everted rat rectum. The amount of binding was in the order of IM > DF > PB, which is similar to that of their accumulation in the rectum.[7] The bindings of NSAID were reduced by pretreatment of the mucosal surface with 0.05 mM $HgCl_2$ for 3 min. This suggests that protein and sulfhydryl groups in the mucosal membrane are involved in the binding of NSAID.

TABLE I. Effect of Pretreatment with $HgCl_2$ on NSAID Binding to Rat Rectum

NSAID	Percent binding	
	Untreated	$HgCl_2$-treated[a]
Indomethacin	16.13±1.98	10.47±1.94[b]
Phenylbutazone	2.15±0.04	1.70±0.11[b]
Diclofenac sodium	10.71±0.22	8.06±0.77[b]

NSAID concentration was 5 mM.
a) Rectal mucosa was pretreated with 50 μM $HgCl_2$ for 3 min. Results are each the mean of six experiments ± S.E.M. Statistical significance: *b*) $p<0.05$.

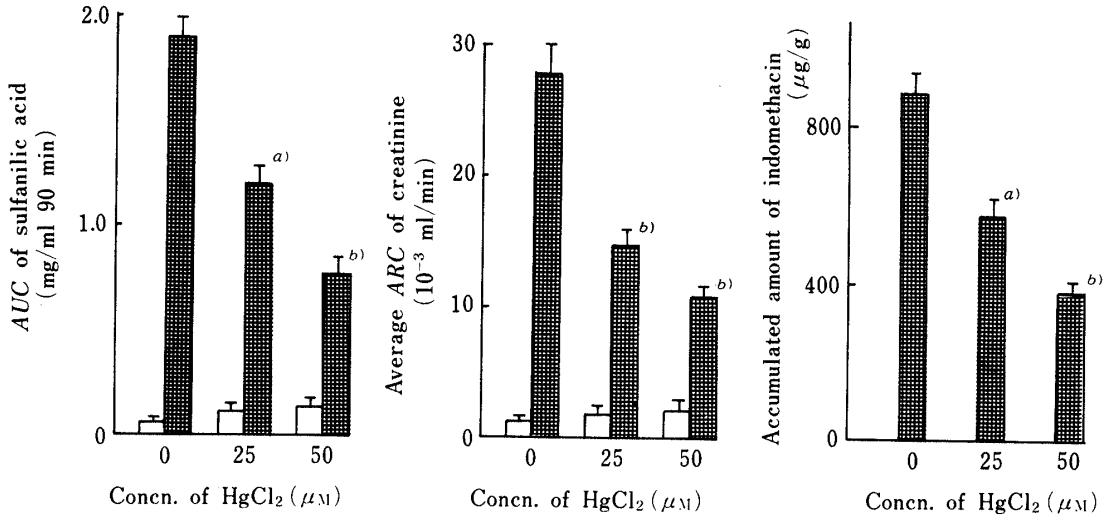

Fig. 1. Effect of $HgCl_2$ Pretreatment on the Rectal Absorption of Sulfanilic Acid, Average Apparent Rectal Clearance of Creatinine in the Presence of Indomethacin (5 mM) and Indomethacin Accumulation

□, phosphate buffer alone; ▩, phosphate buffer containing indomethacin. Results are each the mean ± S.E.M. of at least three experiments. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$.

Furthermore, the effect of chemical modification of the rectal membrane on the permeability enhancing effect of NSAID was investigated *in situ*. Figure 1 shows the effect of pretreatment with $HgCl_2$ on the permeability increase induced by IM. The results clearly show that the absorption of SA (*AUC*), the exsorption of CR (average apparent rectal clearance, *ARC*) and the accumulation of IM were reduced to approximately a half by the pretreatment with 0.05 mM $HgCl_2$. To confirm that $HgCl_2$ itself caused the permeability change, blank solution (without IM) was perfused after $HgCl_2$ pretreatment and then the membrane permeability was measured. $HgCl_2$ treatment affected neither the *AUC* of SA nor the average *ARC* of CR (Fig. 1). Consequently, it seems that the reduction of the IM-induced permeability enhancement by $HgCl_2$ treatment is in parallel with the decrease of IM accumulation in the membrane. The protein components of the membrane, particularly sulfhydryl groups, were affected by $HgCl_2$, and so presumably a decrease of the interaction between IM and these membrane components was responsible for the decreased accumulation of IM leading to the decreased permeability enhancing effect.

Figure 2 shows the effect of pretreatment of the rectum with 0.1% papain solution for 30 min on the permeability enhancement by IM. As is evident from the figure, the *AUC*

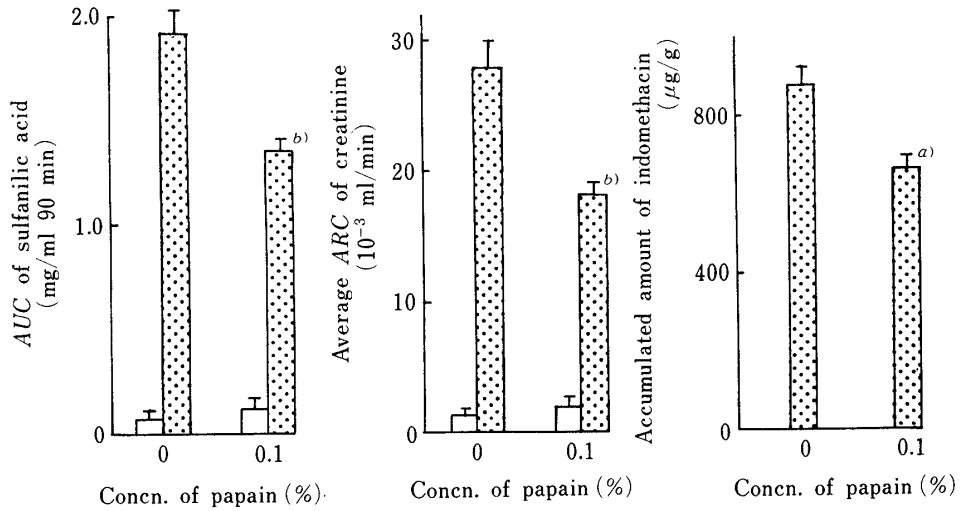

Fig. 2. Effect of Papain Pretreatment on the Rectal Absorption of Sulfanilic Acid, Average Apparent Rectal Clearance of Creatinine in the Presence of Indomethacin (5 mM) and Indomethacin Accumulation

□, phosphate buffer alone; ⊡, phosphate buffer containing indomethacin.
Results are each the mean ± S.E.M. of at least three experiments.
Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$.

increase of SA, the average *ARC* increase of CR and the accumulation of IM were reduced by the pretreatment with papain. As in the case of $HgCl_2$, the *AUC* of SA and the average *ARC* of CR after papain pretreatment without NSAID were not significantly different from the values of phosphate buffer (Fig. 2). The values of the reduction of the *AUC* of SA, the average *ARC* of CR and the accumulation of IM caused by the pretreatment with papain are about 1/1.7 times smaller than those caused by the pretreatment with $HgCl_2$. This may be because papain digestion is limited to the surface protein of the membrane.[20] Thus, the reduction of the interaction between IM and surface protein of the membrane was presumably responsible for the decreased accumulation of IM and the decreased permeability to marker drugs. These results suggest that the interaction of NSAID with protein and sulfhydryl groups in the membrane is involved in the permeability increase induced by NSAID.

The Interaction with Lipid

(a) Effect of Lecithin on the Transfer of NSAID——To elucidate the mechanism of the permeability enhancement by NSAID, the interaction with lipids was examined. Furusawa *et al.*[11] suggested the interaction of the ionized form of acidic drugs, such as salicylic acid, sulfisoxazole and *p*-aminobenzoic acid, with the membrane phospholipids. A similar technique was employed to investigate the interaction of NSAID with egg lecithin, a typical phospholipid. The effect of lecithin (10 mg/ml) on the transfer of NSAID (0.1 mM) from the pH 7.4 aqueous phase to the chloroform phase is shown in Fig. 3. IM and DF, negatively charged at this pH, showed increases in transfer to chloroform containing lecithin. However, the transfer of PB, which has a bulky heterocyclic molecule with a negative charge,[21] seemed not to be influenced by the addition of lecithin. This phenomenon suggests that an interaction between membrane phospholipids and IM or DF might occur during the transfer of the NSAID through the rectum.

(b) Permeation of Marker Drugs across Liposomal Membranes in the Presence or Absence of NSAID——It may be considered in view of the strong interaction of the ionized form of NSAID with membrane phospholipids that the permeability of the lipid bilayer is influenced by them. Thus, the effect of NSAID on the permeability of lipid membranes was

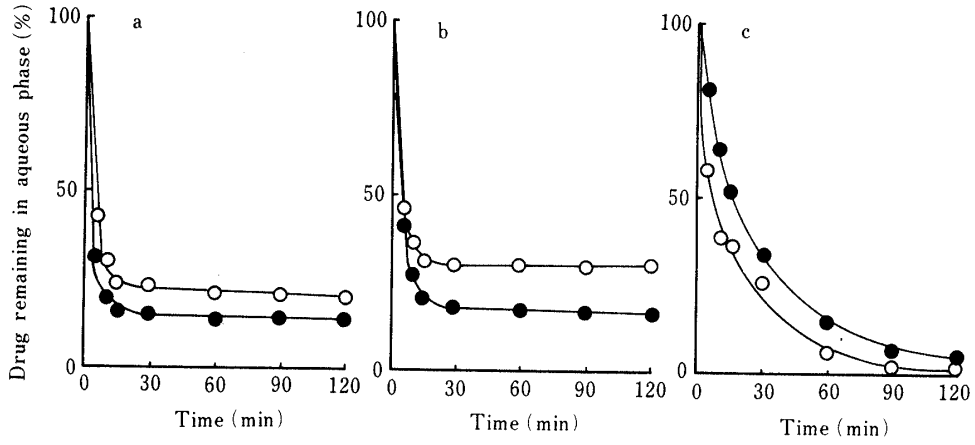

Fig. 3. Effect of Egg Lecithin on the Transfer of Indomethacin (a), Diclofenac Sodium (b), and Phenylbutazone (c) from Water into Chloroform

Concentrations of NSAID were 0.1 mM.
○, without lecithin; ●, with lecithin (10 mg/ml).

TABLE II. Effect of Indomethacin and Phenylbutazone on the Overall Release Rate of Sulfanilic Acid from Egg Lecithin Liposomes and Rectal Lipid Liposomes

NSAID	Release rate constant ($\times 10^{-3}$ min^{-1})[a]	
	Egg lecithin liposomes	Rectal lipid liposomes
None	0.650±0.016	0.727±0.011
5 mM Indomethacin	1.134±0.021[b]	1.854±0.050
10 mM Phenylbutazone	0.934±0.085[b]	—

[b] (None vs. 5 mM Indomethacin); [b] (None vs. 10 mM Phenylbutazone); [b] (5 mM Indomethacin vs. 10 mM Phenylbutazone); [b] (None vs. Rectal lipid liposomes); [c] (5 mM Indomethacin vs. Rectal lipid liposomes)

a) Results are each the mean of five experiments with S.E.M.
b) $p<0.05$. *c*) $p<0.01$.

examined by measuring the release rate of SA from egg lecithin liposomes or rectal lipid liposomes. Figure 4 shows the release of SA from the rectal lipid liposomes in the presence or absence of NSAID. Table II summarizes the release rate constants of SA from these liposomes. As can be seen from Table II, the release rate constants from rectal lipid liposomes are slightly faster than those from egg lecithin liposomes. This difference may be due to some of the lipid components, such as glycolipids and free fatty acids, not present in the egg lecithin liposomes.[22] For both liposomes, the presence of NSAID significantly increased the release rate constants of SA. Thus, it was found that NSAID interacted not only with protein components but also with lipid components (lipid bilayers) in the rectal mucosa and it can be considered that these interactions induced the permeability enhancement.

Induction of Solvent Drag Effect by NSAID

Several studies[14,23-25] have shown that water volume flow (water net flux) influences drug intestinal absorption. Karino *et al.*[14] discussed the solvent drag effect in drug intestinal absorption on the basis of the relationship between the absorption clearance of the drugs (CL_{drug}) and D_2O (CL_{D_2O}). According to their method, we investigated the contribution of the solvent drag effect to the enhanced rectal permeability induced by NSAID. Thus, from the correlation between CL_{drug} for SA and CL_{D_2O}, the sieving coefficients were calculated in the

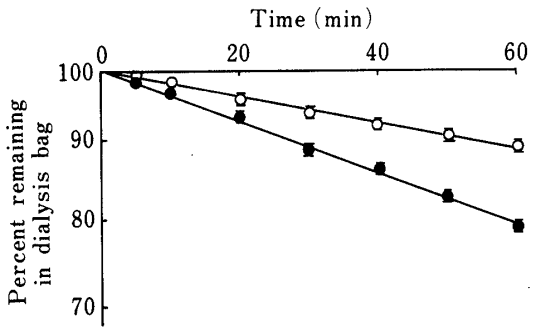

Fig. 4. Effect of Indomethacin on the Overall Release Rate of Sulfanilic Acid from Rectal Lipid Liposomes

○, phosphate buffer alone; ●, phosphate buffer containing indomethacin (5 mM).
Results are each the mean ± S.E. of six experiments.

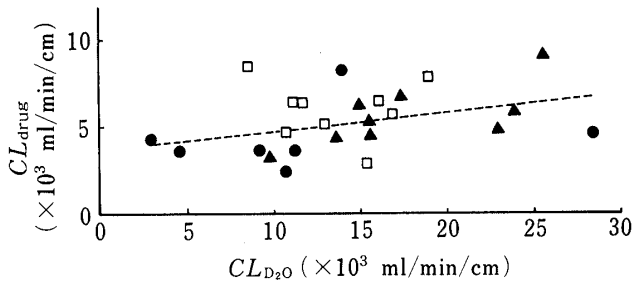

Fig. 5. Relationship between Absorption Clearance of Sulfanilic Acid (CL_{drug}) and D_2O (CL_{D_2O})

●, hypotonic; □, isotonic; ▲, hypertonic perfused solution.
The line was obtained by linear regression analysis.
$Y=0.0915X+3.9008$
$r=0.3191$, $p>0.10$

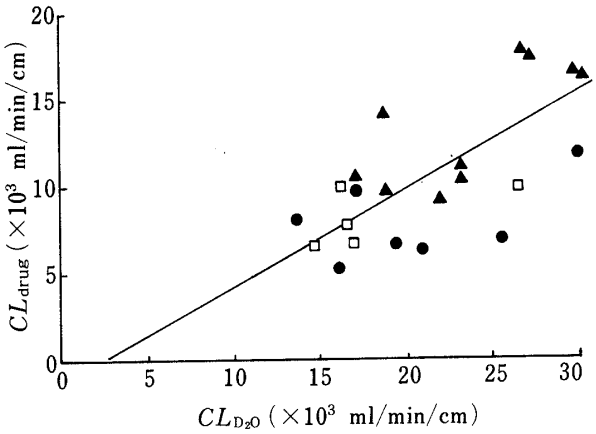

Fig. 6. Relationship between Absorption Clearance of Sulfanilic Acid (CL_{drug}) and D_2O (CL_{D_2O}) in the Presence of Indomethacin

●, hypotonic; □, isotonic; ▲, hypertonic perfused solution.
The line was obtained by linear regression analysis.
$Y=0.5650X-1.8153$
$r=0.8585$, $p<0.001$

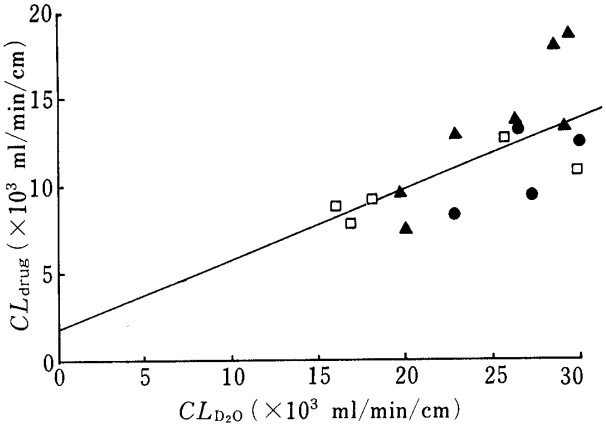

Fig. 7. Relationship between Absorption Clearance of Sulfanilic Acid (CL_{drug}) and D_2O (CL_{D_2O}) in the Presence of Diclofenac Sodium

●, hypotonic; □, isotonic; ▲, hypertonic perfused solution.
The line was obtained by linear regression analysis.
$Y=0.4148X+1.4668$
$r=0.5449$, $p<0.02$

presence or absence of NSAID.

The relationships between CL_{drug} for SA and CL_{D_2O} in the presence or absence of NSAID are depicted in Fig. 5 (in the absence of NSAID, control), Fig. 6 (in the presence of 5 mM IM) and Fig. 7 (in the presence of 10 mM DF). As can be seen in Fig. 5, in the absence of NSAID (control), no significant correlation between CL_{drug} for SA and CL_{D_2O} was obtained, indicating that these is no solvent drag effect (correlation coefficient = 0.3191 ($p>0.1$)). On the other hand, the correlation between CL_{drug} for SA and CL_{D_2O} was significant in the presence of NSAID, suggesting the occurrence of significant solvent drag effects (correlation coefficient = 0.8585 ($p<0.001$) for IM, and 0.5449 ($p<0.02$) for DF). The slopes of the regression lines of CL_{drug} *versus* CL_{D_2O}, *i.e.* the sieving coefficients for SA, were 0.5650 and 0.4148, and the reflection coefficients were 0.4350 and 0.5852 in the presence of IM and DF, respectively.

Thus, in the presence of NSAID, the membrane reflection for SA was decreased and a solvent drag effect was induced. Consequently, the solvent drag effect may contribute to the

enhancement of permeability in the presence of NSAID.

The experimental results obtained in this study can be summarized as follows. 1) The permeation of marker drugs and the accumulation of NSAID were specifically inhibited by pretreatment of the rectal membrane with $HgCl_2$ or papain. The interaction of NSAID with proteins and sulfhydryl groups within the rectal membrane is therefore involved in the increased permeability to marker drugs; 2) NSAID interacted with lecithin, and the lipid bilayer (liposome) permeability to SA was markedly increased in the presence of NSAID; 3) A solvent drag effect for SA was induced and the rectal membrane reflection was decreased in the presence of NSAID. Thus, we may conclude that the enhanced permeability of the rectal membrane induced by NSAID is caused by the interaction of NSAID with both protein and lipid components in the rectal mucosa, as well as by solvent drag effects.

References and Notes

1) Parts of this work were presented at the 103rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1983.
2) K. Nakanishi, S. Miyazaki, M. Masada, and T. Nadai, *Yakugaku Zasshi*, **102**, 1133 (1982).
3) K. Nakanishi, M. Masada, and T. Nadai, *Chem. Pharm. Bull.*, **31**, 3255 (1983).
4) K. Nakanishi, M. Masada, and T. Nadai, *Chem. Pharm. Bull.*, **31**, 4161 (1983).
5) K. Nakanishi, H. Saitoh, S. Yamashita, M. Masada, and T. Nadai, *J. Pharm. Dyn.*, **6**, s-96 (1983).
6) K. Nakanishi, M. Masada, and T. Nadai, *Chem. Pharm. Bull.*, **32**, 1628 (1984).
7) K. Nakanishi, M. Masada, and T. Nadai, *Chem. Pharm. Bull.*, **32**, 1956 (1984).
8) H. Yaginuma, T. Nakata, H. Toya, T. Murakami, M. Yamazaki, and A. Kamada, *Chem. Pharm. Bull.*, **29**, 2974 (1981).
9) T. Nishihata, J. H. Rytting, and T. Higuchi, *J. Pharm. Sci.*, **71**, 865 (1982).
10) D. N. Rhodes and C. H. Lea, *Biochem. J.*, **65**, 526 (1957).
11) S. Furusawa, K. Okumura, and H. Sezaki, *J. Pharm. Pharmacol.*, **24**, 272 (1972).
12) J. Folch, M. Lees, and G. H. Sloane Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
13) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **59**, 33 (1970).
14) A. Karino, M. Hayashi, T. Horie, S. Awazu, H. Minami, and M. Hanano, *J. Pharm. Dyn.*, **5**, 410 (1982).
15) V. Thornton and F. E. Condon, *Anal. Chem.*, **22**, 690 (1950).
16) J. H. Roe, *J. Biol. Chem.*, **208**, 889 (1954).
17) G. G. Forstner, *Biochem. J.*, **121**, 781 (1971).
18) D. Louvard, S. Maroux, Ch. Vannier, and P. Desnuelle, *Biochim. Biophys. Acta*, **375**, 236 (1975).
19) I. Bihler and R. Cybulsky, *Biochim. Biophys. Acta*, **298**, 429 (1973).
20) M. Yasuhara, H. Kobayashi, Y. Kurosaki, T. Kimura, S. Muranishi, and H. Sezaki, *J. Pharm. Dyn.*, **2**, 177 (1979).
21) S. Miyazaki, T. Yamahira, Y. Morimoto, and T. Nadai, *Int. J. Pharm.*, **8**, 303 (1981).
22) T. Kimura, M. Yoshikawa, M. Yasuhara, and H. Sezaki, *J. Pharm. Pharmacol.*, **32**, 394 (1980).
23) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch Pharmacol.*, **281**, 175 (1974).
24) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch Pharmacol.*, **281**, 197 (1974).
25) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.*, **23**, 1856 (1975).