EFFECT OF SURFACTANTS ON THE ABSORPTION OF *p*-AMINO-BENZOIC ACID FROM THE RAT INTESTINE*

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Effect of surfactants on the absorption of *p*-aminobenzoic acid (PABA) and *p*-acetamidobenzoic acid (Ac-PABA) from the rat intestine was investigated using the *in situ* recirculation technique. The surfactants tested were polyoxyethylene-(20)-sorbitol monooleate (Tween 80), polyoxyethylene-(9-10)-*p*-*t*-octylphenol (Triton X-100), sodium dodecylsulfate (SLS) and cetyltrimethylammonium bromide (CTAB). The micellar interaction of PABA and Ac-PABA with these surfactants were almost negligible except CTAB. The absorption of Ac-PABA from the small intestine was increased by these four surfactants. The order of magnitude of the absorption enhancing effect was as follows: Triton X-100 > SLS ≥ CTAB >> Tween 80. The absorption of PABA and Ac-PABA from the large intestine was also increased by 20 mM SLS. In addition, the exsorption of PABA to the small intestinal lumen was greatly increased by the addition of SLS to the perfusate. In spite of such a general increase in the membrane permeability, the absorption of PABA from the small intestine was significantly inhibited by these four surfactants. The extent of inhibition was dependent on the concentration of surfactants and the exposure time of intestine to surfactants. SLS greatly accelerated the release of protein from the small intestine and an inverse correlation was found between the amount of released protein and the absorption of PABA in the presence of SLS. The specific inhibitory effect of surfactants may be attributed to the solubilization and the following release of protein, which is responsible for the absorption of PABA.

Keywords—*p*-aminobenzoic acid; *p*-acetamidobenzoic acid; intestinal absorption; surfactant; polysorbate 80; Triton X-100; sodium dodecylsulfate, cetyltrimethylammonium bromide; protein solubilization; rat

We have investigated the absorption mechanism of *p*-aminobenzoic acid (PABA) from the rat intestine comparing with that of *p*-acetamidobenzoic acid (Ac-PABA).¹⁻³⁾ Our previous study demonstrated the anomalous characteristics of the absorption of PABA from the rat small intestine and the contribution of protein within the brush border membrane to the absorption process of PABA.³⁾

Partial and selective solubilization of membrane protein can be obtained by using various surfactants.^{4,5)} It seems of interest, therefore, to investigate the effect of surfactants on the absorption of PABA for the purpose of elucidating its absorption mechanism.

Surfactants are one of the most important group of adjuvants in pharmaceutical preparations and their effect on the absorption of various drugs

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has been studied extensively.⁶⁻¹³⁾ Surfactants can influence several critical determinants of the rate and extent of drug absorption—*viz.*, drug solubility and dissolution rate, gastric emptying and membrane permeation. These complications prevent formulation of conclusions concerning the general nature of the influence of surfactants on the absorption of drugs from the gastrointestinal tract.

This work is attempted to elucidate some characteristics of the surfactant effect on the drug absorption, especially on the membrane permeation process, and to get further information about the absorption mechanism of PABA.

MATERIALS AND METHODS

Materials—The surfactants tested were polyoxyethylene-(20)-sorbitol monooleate (Tween 80), polyoxyethylene-(9-10)-*p*-*t*-octylphenol (Triton X-100), sodium dodecylsulfate (SLS) and cetyltrimethylammonium bromide (CTAB). *p*-Aminobenzoic acid, *p*-acetamidobenzoic acid and these four surfactants were obtained from Nakarai Chemicals Co. (Japan). All other reagents used in these experiments were of the finest grade available.

Preparation of Drug Solution—Drugs (final concentration; 0.1 mM) were dissolved in the isotonic buffer solution ($\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$, pH 6.5) and the surfactants were added at concentrations above their critical micellar concentrations (CMC).

Absorption Experiments—Male Wistar rats (160–230 g) were used in all experiments. Absorption of drugs from the small intestine was measured by the *in situ* recirculation technique as described previously.^{2,3)} The bile duct was ligated. Thirty ml of drug solution were recirculated through the whole small intestine at the rate of 5 ml/min. Absorption of drugs from the large intestine was also measured by the recirculation of drug solution (30 ml) from the proximal end of the colon to the anus.

Exsorption Experiments—The exsorption of PABA to the gut lumen was studied by the method described in the report from this laboratory.⁷⁾

PABA, 20 μmol /0.2 ml in pH 6.5 buffer solution, was administered intravenously to the rat from the femoral vein and the small intestine was perfused with pH 6.5 buffer solution at the rate of 5 ml/min. Ten ml each of the perfusate was collected continuously and the amounts of PABA and protein in the perfusate were determined.

Analytical Methods—PABA and Ac-PABA were determined spectrophotometrically by the method described previously.¹⁾ The protein concentration was determined by the method of Lowry *et al.* with bovine serum albumin as the standard.¹⁴⁾

RESULTS AND DISCUSSION

Table I summarizes the effect of four surfactants, Tween 80, Triton X-100, SLS and CTAB, on the absorption of PABA and Ac-PABA from the rat small intestine. There was a marked difference between PABA and Ac-PABA with regard to the effect of these surfactants. The absorption of PABA was significantly reduced by the addition of these surfactants. The extent of this reduction was not only dependent on the concentration of the surfactant but also on the exposure time of intestine to the surfactant. For instance, while Tween 80 did not affect the absorption of PABA in 20 min, a significant reduction occurred in 60 min. The absorption of PABA in 20 min was reduced by 20 mM SLS, but not by 10 mM SLS. In 60 min, however, the absorption of PABA was reduced to a similar extent by 10 mM and 20 mM SLS. The maximum reduction in the absorption of PABA in 20 min was observed in the case of 20 mM CTAB. In this case, as described later, the effect of micellar complex formation with PABA may be partly operative.

In contrast to PABA, the absorption of Ac-PABA from the rat small intestine was increased by these four surfactants. The absorption enhancing effects of these surfactants, except Tween 80, have remained almost unchanged above their lower concentrations tested. The magnitude of the enhancing effect increased in the order, Tween 80 << CTAB $\leq$ SLS < Triton X-100. The addition of Triton X-100 showed a seven-fold increase in the absorption of Ac-PABA compared to that of con-

TABLE I. *Effect of Surfactants on the Absorption of PABA and Ac-PABA from the Small Intestine*

Additives	Concentration of additives	Time (min)	% absorbed	
			PABA	Ac-PABA
None		20	46.4±1.8 (6)	2.5±0.8 (4)
		60	79.8±2.2 (6)	10.8±1.2 (4)
Tween 80	1% (w/v)	20	48.4±4.4 (4)	2.7±0.8 (4)
		60	74.0±0.9 (4)	18.3±0.4 (4)
	5% (w/v)	20	43.2±5.0 (4)	5.5±1.2 (4)
		60	69.9±1.0 (4)	—
Triton X-100	1% (w/v)	20	41.3±2.3 (4)	18.4±3.0 (4)
	5% (w/v)	20	33.2±2.2 (4)	16.7±2.0 (3)
SLS	10 mM	20	44.0±4.3 (4)	13.4±2.6 (4)
		60	68.4±3.4 (4)	—
	20 mM	20	40.1±2.5 (4)	13.9±1.8 (3)
		60	65.3±2.2 (8)	—
CTAB	10 mM	20	42.5±2.6 (4)	11.9±2.4 (4)
	20 mM	20	26.0±1.1 (4)	11.8±1.4 (4)

Initial concentration of drugs = 0.1 mM.

Results are expressed as the mean ± S.D. with the number of experiments in parentheses.

trol experiment. Another non-ionic surfactant, Tween 80, showed a much less effect and the absorption of Ac-PABA in 20 min was not affected by the addition of 1% Tween 80. However, the effect of Tween 80 seems to depend on the exposure time, since Tween 80 at this concentration significantly increased the 60 min-absorption of Ac-PABA.

In the present experimental condition, two possible ways come to mind by which the rate of drug transfer from solution across biological membrane may be altered. First, a change in the physical-chemical properties of the drug due to the presence of a surfactant, an intraluminal effect, could reduce or enhance the rate of transport and, second, an alteration in the permeability of the membrane could also influence the transfer rate. With regard to the first mechanism, PABA and Ac-PABA would have little tendency to be trapped into the surfactant micelle, since they have low partition coefficients to organic solvents at pH 6.5.³⁾ Preliminarily, the interaction of PABA and Ac-PABA with surfactants were examined by the equilibrium dialysis method using Visking cellulose tubings (8/32). Both PABA and Ac-PABA showed no detectable interaction with non-ionic

Tween 80 and Triton X-100. Anionic surfactant, SLS, was found to have a little interaction with PABA and Ac-PABA. However, the fraction of the micellar complex with SLS was so small that the loss of their thermodynamic activities in the medium due to the micellar complex formation was almost negligible. Cationic surfactant, CTAB, was found to have some interaction with PABA and Ac-PABA. This interaction would be electrostatic one since the dominant ionic species of PABA and Ac-PABA are the anion at pH 6.5. Therefore, changes in the absorption of PABA and Ac-PABA would be expected to reflect the action of the surfactant on the intestinal epithelium and not depend on the physico-chemical interaction of surfactants with drugs except for CTAB.

Various surfactants were shown to promote the absorption of poorly absorbable drugs, such as cephaloridine,⁹⁾ cephalothin,⁹⁾ and phenolsulfonphthalein,¹³⁾ due to an increase in the membrane permeability. The observed enhancement of the absorption of Ac-PABA can be explained in the same manner by an increase in the membrane permeability in the presence of surfactants. On the other hand, in spite of a general increase in the membrane permeability, the absorption of PABA

was reduced by the addition of surfactants. There have been relatively sparse reports demonstrating such an inhibitory effect of surfactants on the absorption of drugs, which have no significant micellar interaction.^{8,11)}

To clarify the mechanism of this inhibitory effect, the absorption of PABA was further investigated in the presence of SLS. Table II shows the time course of the absorption of PABA and the release of protein from the rat small intestine. The inhibitory effect of 20 mM SLS on the absorption of PABA gradually increased as the exposure time increased. Furthermore, SLS accelerated the release of protein from the small intestine and the amount of released protein was markedly increased compared to that of control experiment. This acceleration indicates the direct action of SLS on the intestinal membrane. The percent inhibition of the absorption of PABA in 20 min by 10 mM SLS was 5.2% (statistically not significant) and the amount of released protein was 15.8 ± 3.3 mg. The inhibition percent by 20 mM SLS was 13.6% and the amount of released protein was 30.4 ± 5.4 mg. There was a good correlation between the percent inhibition and the amount of released protein.

In another experiment, effect of SLS on the membrane permeability was investigated by using the drug exsorption technique.⁷⁾ As shown in Fig. 1, the rate of exsorption to the gut lumen of the intravenously administered PABA was greatly increased by the addition of 20 mM SLS to the perfusate. In addition, the rate of protein release into the perfusate was simultaneously increased. It is difficult to compare directly the rate of protein release

in the exsorption experiment (Fig. 1) with the cumulative amount of released protein in the absorption experiment (Table II) because of the difference in the experimental conditions. The enhanced exsorption of PABA indicates the apparent increase in the permeability to PABA from blood to lumen by the addition of SLS.

We have reported a marked difference in the ab-

FIG. 1. Effect of SLS on the Exsorption of PABA to the Small Intestinal Lumen (a) and the Release of Intestinal Protein (b)

PABA, 20 μ mol/0.2 ml, was administered intravenously to the rat from the femoral vein and the small intestine was perfused with pH 6.5 buffer solution at the rate of 5 ml/min.

○: perfused with pH 6.5 buffer alone, ●: perfused with pH 6.5 buffer containing 20 mM SLS, ◐: perfused with pH 6.5 buffer alone after 20 mM SLS treatment.

TABLE II. Effect of SLS on the Absorption of PABA and the Release of Protein from the Small Intestine as a Function of Time

Time (min)	Absorption of PABA (%)		Cumulative amount of released protein (mg)	
	Control	+SLS	Control	+SLS
10	33.2 ± 2.6 (4)	28.8 ± 2.0 (3)	4.2 ± 0.8 (4)	25.3 ± 2.6 (3)
20	46.4 ± 1.8 (6)	40.1 ± 2.5 (4)	3.4 ± 0.7 (4)	30.4 ± 5.4 (4)
40	67.4 ± 5.9 (3)	56.7 ± 4.9 (4)	4.8 ± 1.6 (4)	41.9 ± 6.2 (4)
60	79.8 ± 2.2 (6)	65.3 ± 2.2 (8)	7.6 ± 1.2 (4)	57.7 ± 10.1 (8)

Initial concentrations of PABA and SLS were 0.1 and 20 mM, respectively.

Results are expressed as the mean $\pm$ S.D. with the number of experiments in parentheses.

sorption characteristics of PABA between small and large intestine.³⁾ As an additional clue to elucidate the alteration in the permeability of the membrane, the effect of SLS on the absorption of PABA and Ac-PABA from the large intestine was investigated. As is evident from Table III, SLS greatly enhanced the absorption of PABA to a similar extent as Ac-PABA in the large intestine. This means that the effect of SLS on the absorption of PABA in the large intestine was quite opposite to that in the small intestine. This different effect of SLS seems to be mainly caused by the difference in the absorption mechanism of PABA from these absorptive sites. Our previous study demonstrated that the absorption of PABA from the small intestine was specific and not consistent with the pH-partition theory, while that of Ac-PABA was consistent with the pH-partition theory.³⁾ The exsorption experiment from the small intestine and the absorption experiment from the large intestine suggest that SLS increases the membrane permeability similarly in both small and large intestine. The increase in the release of membrane components such as protein reflects the disruption of the membrane lipid-protein complex, resulting in the increase in the membrane permeability to drugs. In spite of such a general increase in the membrane permeability, the absorption of PABA from the small intestine was significantly inhibited in the presence of 20 mM SLS. The inhibitory effect of SLS may be attributed to the solubilization and the following release of membrane components which are responsible for the facilitated absorption of PABA from the small intestine. The observed acceleration of protein release in the presence of SLS supports this speculation.

TABLE III. *Effect of SLS on the Absorption of PABA and Ac-PABA from the Large Intestine*

Additives	% absorbed (20 min)	
	PABA	Ac-PABA
None	2.2 ± 1.5 (4)	1.0 ± 1.0 (3)
SLS (20 mM)	12.2 ± 3.3 (4)	8.4 ± 1.5 (4)

*Initial concentration of drugs = 0.1 mM.
Results are expressed as the mean ± S.D. with the number of experiments in parentheses.*

Feldman *et al.*¹⁰⁾ demonstrated that sodium taurodeoxycholate accelerates the release of total phosphorus, lipid phosphorus and protein from the everted rat small intestine at concentrations above the CMC. Furthermore, Feldman and Reinhard examined the effect of a series of anionic surfactants and showed a loss of protein from the everted rat intestine at surfactant concentrations above the CMC.¹²⁾ These studies indicate the direct action of surfactants on the intestinal membrane, resulting in an alteration in the membrane permeability.

Various surfactants are known to inhibit the intestinal absorption of inorganic electrolytes and water soluble nutrients, *i.e.* compounds considered to be transported by active and carrier-mediated processes.¹⁵⁻¹⁸⁾ The observed inhibitory effect of surfactants on the absorption of PABA may further support the specific absorption mechanism of PABA from the rat small intestine, which was suggested in our previous study.³⁾

In this study, the magnitude of each surfactant effect was found to be somewhat different from every other surfactant tested. This quantitative difference may be dependent on their solubilizing potency of biological membrane. For non-ionic surfactants there is an interesting correlation between structure and solubilizing potency. Studies with mitochondria, microsomes and bacterial membranes showed that almost all effective surfactants are in the 12.5—14.5 HLB, hydrophile-lipophile balance, range.⁵⁾ Those with higher HLB values (such as Tweens) failed in solubilization of membrane.⁵⁾ These previous findings are in good agreement with the present result that Triton X-100 (HLB; 13.5) is much more potent than Tween 80 (HLB; 15.0) for the effect on the permeability of the intestinal membrane. Further quantitative investigations would be able to elucidate the exact mechanism of action of surfactants in altering the membrane permeability to drugs.

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