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Effects of sodium salicylate and caprylate as adjuvants of drug absorption on isolated rat small intestinal epithelial cells

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Organic anions such as sodium salicylate (Nishihata et al., 1981, 1982, 1983) and sodium caprylate (Yata et al., 1983) have recently been reported to enhance the absorption of water-soluble and poorly absorbable drugs and polypeptides from gut lumen in vivo. It is quite evident that the action of such adjuvants on intestinal membranes plays an important role for the enhancement of drug absorption. The effects of these adjuvants on the physical structure, especially membrane dynamics, of intestinal membranes have not yet been investigated, despite the importance of membrane physical structure to solute transport. In a previous paper, we reported salicylic acid to be taken up by rat small intestinal epithelial cells and simultaneously to perturb the cell membrane. This apparently resulted in promoting drug absorption from the gut lumen (Kajii et al., 1985). Consequently, in the present study, to elucidate changes occurring in the membrane more clearly, the effects of the above adjuvants on rat small intestinal epithelial cells were investigated by the chemical modification of various membrane domains, using different types of fluorescent probes and measuring fluorescence polarization, by which the membrane perturbation can be sensitively detected.

Isolated rat small intestinal epithelial cells were obtained from male Wistar rats fasted overnight (180–230 g b. wt.) according to a slightly modified method of Kimmich (1970), using 40 cm segments of the small intestine from the proximal end of the jejunum. All experiments were performed on cell suspensions exceeding 80% viability. Epithelial cell viability was tested by trypan blue exclusion. The protein concentration of each cell suspension was determined by the method of Lowry et al., using bovine serum albumin as the standard (Lowry et al., 1951).

1,6-diphenyl-1,3,5-hexatriene (DPH), 2-(9-anthroyloxy)stearic acid (2-AS) and 12-(9-anthroyloxy)stearic acid (12-AS) were dissolved in tetrahydrofuran, each to a concentration of 1 mM. Eosin-5-maleimide (EM) and 8-anilino-1-naphthalene sulfonate (ANS) were dissolved in a buffer solution consisting of 120 mM NaCl, 3 mM K_2HPO_4, 1 mM $MgCl_2$ and 10 mM Tris(hydroxymethyl)aminomethane, pH 7.4. A small volume of ANS solution was added directly to each cell suspension in a cuvette to measure fluo-

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rescence. For preparation of the other fluorescent probes, a small volume of fluorescent probe solution was added to all the cell suspensions, followed by incubation for 15 min at 37°C. The cells labeled by the fluorescent probes were separated from the unlabeled fluorescent probes by washing the cells with buffer and centrifuging them at 1500 g for 10 min, using a Kubota KR/180FA centrifuge. This procedure was repeated 3 times at 4°C.

Fluorescence polarization measurements were carried out at 37°C, using a Hitachi fluorescence spectrophotometer 650-60. A small volume of concentrated salicylate or caprylate solution was added to the cell suspensions in the cuvettes. The emission light was passed through a 430 nm cut-off filter for DPH, 2-AS, 12-AS and ANS, and a 510 nm cut-off filter for EM. Fluorescence polarization was determined as previously described (Kajii et al., 1985).

Thirty mM salicylate, at which concentration water-soluble drug absorption in vivo was enhanced (Nishihata et al., 1982, 1983) caused changes in the fluorescence polarization of the fluorescent probe labeled cells (Fig. 1). Eighteen mM caprylate was found to cause changes in the fluorescence polarization (Fig. 2). DPH has been found to monitor the hydrophobic interior of plasma membranes (Shinitzky and Inbar, 1974). Both salicylate and caprylate brought about a decrease in DPH fluorescence polarization. 2-AS probably localizes in the bilayer closer to the aqueous interface and 12-AS probably detects the interior of a lipid layer (Bashford et al., 1976). The fluorescence polarization of both 2-AS and 12-AS underwent an increase although that of 2-AS by salicylate was not significant. The effect of such organic anions on ANS fluorescence polarization were not marked. The fluorescence polarization of EM, which binds covalently and preferentially to the sulfhydryl groups of proteins (Cherry, 1978), showed significant changes in the presence of salicylate and caprylate. Thus, use of such fluorescent probes provides indication of the perturbation of intestinal epithelial cell membrane lipids and proteins. Such perturbation was not found by the trypan blue exclusion test to have any effect on cell viability and thus may possibly be reversible. It should be emphasized that the action of

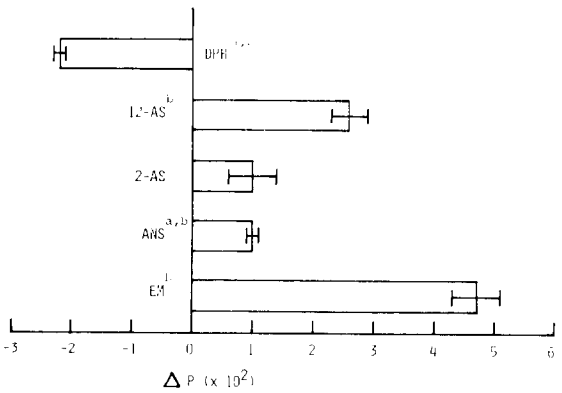

Fig. 1. Effect of salicylate on fluorescence polarization of rat small intestinal epithelial cells labeled by fluorescent probes. ΔP, (fluorescence polarization with 30 mM salicylate) − (fluorescence polarization without salicylate). Typical examples for each fluorescent probe are shown in the figure. Results are means ± S.E.M. The number of determinations was 10–15 for each probe. The cell viability (%) tested by trypan blue exclusion was 85.8 ± 0.9 (21) in the absence of salicylate and 83.5 ± 1.0 (21) in the presence of 30 mM salicylate. The viability is expressed as means ± S.E.M. with numbers of animals in parentheses. Cell concentrations were 0.96 ± 0.18 mg protein/ml (means ± S.E.M.). Excitation (Ex) and emission (Em) wavelengths for fluorescent probe, (Ex, Em in nm): DPH, (380, 455); 12-AS, (390, 460); 2-AS, (390, 452); ANS, (380, 480); EM, (525, 550). [a] The data from the previous work (Kajii et al., 1985). [b] Fluorescence polarization in the presence of 30 mM salicylate differed significantly ($P < 0.01$) from that in the absence of salicylate.

such adjuvants on cell membranes is not destructive.

Typical plasma membranes of small intestinal epithelial cells each possess a brush border and basolateral membrane whose functions and composition of these constituents such as membrane lipid and protein constituents are distinct from each other (Brasitus and Schachter, 1980). The heterogeneity of epithelial cell membranes and the existence of intracellular organellas also influence fluorescent probe distribution in cells. Thus, the action of the above adjuvants on brush border and/or basolateral membranes is further under investigation to elucidate more clearly the manner in which these adjuvants promote drug absorption (Kajii et al., 1986).

The present study has demonstrated that organic anions, known to be adjuvants, perturb

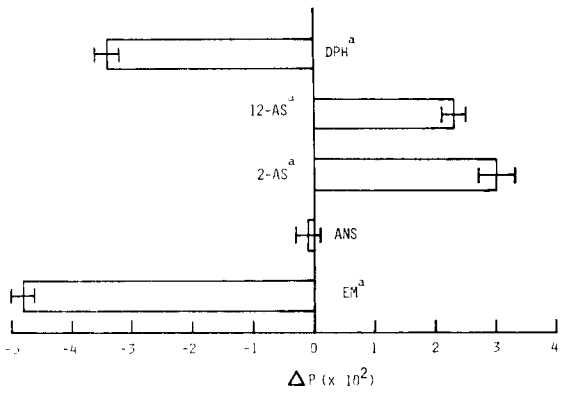

Fig. 2. Effect of caprylate on fluorescence polarization of rat small intestinal epithelial cells labeled by fluorescent probes ΔP, (fluorescence polarization with 18 mM caprylate) − (fluorescence polarization without caprylate). Typical examples for each fluorescent probe are shown in the figure. Results are means ± S.E.M. The number of determinations was 10–15 for each probe. Cell concentrations were 0.62 ± 0.20 mg protein/ml (means ± S.E.M.). The cell viability (%) tested by trypan blue exclusion was 86.1 ± 1.0 (25) in the absence of caprylate and 84.3 ± 1.2 (25) in the presence of 18 mM caprylate. The viability is expressed as means ± S.E.M. with number of animals in parentheses. (Ex, Em in nm): DPH, (380, 455); 12-AS, (364, 460); 2-AS, (364, 452); ANS, (380, 480); EM, (525, 550). [a] Fluorescence polarization in the presence of 18 mM caprylate differed significantly ($P < 0.01$) from that in the absence of caprylate.

the rat small intestinal epithelial cells, particularly membrane lipids and proteins without causing serious damage to the cells. Such membrane perturbation probably enhances drug absorption from the gut lumen.

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