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Characterization of enhanced intestinal permeability; electrophysiological study on the effects of diclofenac and ethylenediaminetetraacetic acid

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The mechanism of the enhancing effects of diclofenac sodium and disodium ethylenediaminetetraacetate (EDTA) on intestinal permeability to sulphanilic acid were investigated in the rat isolated jejunum by an electrophysiological technique. The results suggested that diclofenac sodium increased the mucosal-to-serosal flux rate of sulphanilic acid by enhancing both transcellular and paracellular permeabilities while the effect of EDTA was located only in the paracellular pathway.

Pharmaceutical adjuvants that increase the permeability of gastrointestinal membrane to drugs, i.e. absorption promoters, have been widely investigated (Murakami et al 1981; Nishihata et al 1982, 1983). It has been shown that non-steroidal anti-inflammatory drugs (NSAID), as well as some surfactants, enhance rectal permeability to sulphanilic acid (Nakanishi et al 1983, 1984a). The binding of the promoters to the membrane components has been proposed as an explanation of their activity (Nakanishi et al 1984b), as has the enhancement of the solvent drag effect (Nakanishi et al 1984b) and the reduction in the length of glycocalyx filaments on the microvilli of epithelial cells (Sithigorngul et al 1983). Freel et al (1983) suggested that a dihydroxy bile salt alters the integrity of the tight-junctional complex between the epithelial cells to increase the paracellular permeability of rabbit colon.

We have used diclofenac sodium (DF) and disodium ethylenediaminetetraacetate (EDTA) as promoters and their effects on jejunal permeability to sulphanilic acid were examined in rats. Sulphanilic acid was selected as the marker because it is completely ionized at the physiological pH (7·4) and is easy to use for electrophysiological study, it has high polarity and belongs to the so-called poorly absorbable drugs (which is a favourable property for a marker of enhancement of intestinal permeability) and the assay procedure is well-documented and sensitive. To characterize the enhancing mechanism of the promoters, we used the electrophysiological in-vitro techniques developed by Ussing & Zerahn (1951) and estimated the alteration of paracellular and transcellular permeabilities.

Methods

Studies were made using sheets of rat jejunum mounted between two Lucite half chambers (Kimura et al 1982). The area of the opening was 1·0 cm². After preincubation with normal Ringer solution for 25 min, the mucosal solution was replaced by Ringer solution containing sulphanilic acid and a promoter at an initial concentration of 10 mM, the osmolarity and sodium concentration were always adjusted to the same as normal Ringer solution. Then, except for the open-circuit experiments (in Table 1), the transmural potential difference was clamped to the arbitrary values (−20 mV ~ + 30 mV) by applying electric fields externally (Yamashita et al 1984), and this voltage-clamp condition was maintained unchanged throughout the experiment. The chambers and contents were maintained at 37 °C and the Ringer solution was adjusted to pH 7·4 at 37 °C before the experiment. Mucosal-to-serosal flux rates of sulphanilic acid were determined by measuring the increments of serosal concentration as a function of time. The concentration was estimated spectrophotometrically according to Kimura et al (1981). All reagents were of reagent grade.

Results

The addition of DF or EDTA to the mucosal solution induced an increase in the mucosal-to-serosal flux rate of sulphanilic acid as shown in Table 1. These results were in agreement with previous reports (Tidball 1964; Nakanishi et al 1984a), indicating that this is an appropriate technique for examining the effect of the two promoters.

According to Schultz & Zalusky (1964), the transmembrane flux of ionized molecules via the paracellular shunt-pathway (J_d) depends on the potential difference across the membrane (V_t) and may be expressed as:

$$J_d = {}_oJ_d \cdot \exp(-zFV_t/2RT) \quad (1)$$

where the subscript o refers to the short-circuit condition, and z, F, R and T have their usual meanings. On the other hand, the flux through the transcellular pathway (J_m) is thought to be independent of V_t, and the total flux (J_t) is represented as:

$$J_t = J_m + J_d = J_m + {}_oJ_d \cdot \xi \quad (2)$$

where $\xi = \exp(-zFV_t/2RT)$.

The mucosal-to-serosal flux rates of sulphanilic acid were measured under various externally applied potential differences and were plotted against the ξ (Fig. 1).

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Table 1. Increase of mucosal-to-serosal flux rate of sulphanilic acid by the addition of DF or EDTA to the mucosal solution in the open-circuit condition.

Adjuvant	Flux rate of sulphanilic acid (n mol cm^{-2} min^{-1})	Ratio
None	1·895 ± 0·065 (6)	1
DF	4·699 ± 0·168 (5)	2·5
EDTA	4·932 ± 0·180 (4)	2·6

Initial concentration of each agent in the mucosal solution was 10 mM. Sulphanilic acid flux rates are expressed as the mean ± s.e. with the number of experiments in parentheses.

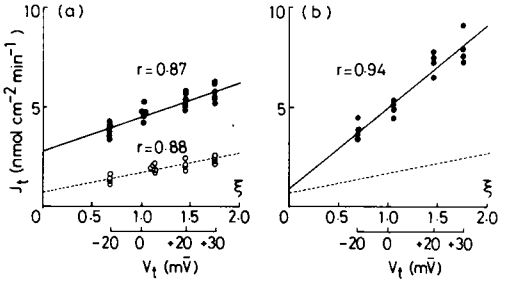

FIG. 1. Effects of transmural potential difference (V_t) on the flux rates (J_t) of SA in the presence of DF (a) or EDTA (b). The dotted line respresents the SA flux rate when no promoter was added. The intercept and the slope of each line are summarized in Table 2. The explanation of ξ, the abscissa, is described in detail in the text.

Table 2. The intercept and the slope of each line in Fig. 1.

Adjuvant	Intercept	Slope
None	0·75 (0·15)	0·91 (0·12)
10 mM DF	2·80 (0·28)*	1·67 (0·21)*
10 mM EDTA	0·95 (0·52)	4·06 (0·40)*

Results are shown with s.d. in parentheses.
* Significantly changed ($P < 0·01$) by the presence of the adjuvant (analysis of variance).

The intercept and the slope of each line are summarized in Table 2 and represent the flux through the transcellular route and the paracellular route in the short-circuit condition, respectively. It appears that sulphanilic acid penetrates the intestinal membrane by both pathways in the absence of promoters (dotted line in Fig. 1a, b). The addition of DF to the mucosal solution produced a 3·7-fold increase in the transcellular flux rate, while the paracellular flux was less, but significantly, affected (1·7-fold increase). On the other hand, EDTA induced the 4·5-fold selective increase in the paracellular flux only.

Discussion

DF and EDTA increased the mucosal-to-serosal flux rate of sulphanilic acid across the jejunal membrane by a similar amount. However, from the results of our voltage-clamp experiments, it is evident that the enhancing mechanisms of the promoters are different due to the difference of their affinity for the epithelial membrane. In previous reports (Nakanishi et al 1984a, b), it was shown that DF accumulates in the epithelium of rat rectum during the luminal perfusion and that the interaction of DF with membrane components (protein and lipids) increases the permeation of sulphanilic acid. So it may be reasonable to assume that the interaction of DF with the membrane components causes permeability changes in the cellular membranes of the intestinal epithelium.

EDTA has low lipophilicity and affinity for the mucosal membrane seems to be low. Yata et al (1983) propose that the effect of EDTA, and other chelating agents, on drug absorption depends on its chelating activity with calcium. Removal of endogenous calcium from the intestinal epithelial cell line by the formation of a calcium-EDTA complex is thought to loosen the barrier function, especially the intercellular tight-junctional pathway. This hypothesis is supported by our results, although details of the calcium location remain unsolved.

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