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The promotion of drug rectal absorption by water absorption

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The promotion of the rectal absorption of antipyrine by sodium taurocholate (TC-Na) or sodium ethylenediaminetetraacetate (EDTA-Na) has been examined by in-situ recirculating perfusion in the rat. These promoters significantly increased water influx, efflux and antipyrine absorption clearance (CL_{AP}). Ouabain treatment significantly reduced the increase in both rectal absorption of drug and water flux. Water absorption dependent on active sodium transport may thus possibly promote the rectal absorption of poorly absorbable drugs.

How bile salts promote intestinal absorption of poorly absorbable drugs has received much attention (Kakemi et al 1970; Kimura et al 1972; Feldman et al 1973). Although the direct effects of the salts on luminal membranes have been described, the detailed mechanism is still unknown. Sodium ethylenediaminetetraacetate (EDTA-Na) has been shown to change the intestinal membrane permeability of phenol red by chelation with calcium and magnesium ions in the region of the paracellular tight junction (Cassidy & Tidball 1967).

Binder et al (1978) and Goerg et al (1980) reported that deoxycholate induces an increase in the secretion of sodium and net flux of water in rat colon. Fix et al (1983) indicated that active sodium transport is an integral component in the promotion of rectal absorption of gentamicin by salicylate or ethylene (dinitrilo)tetraacetate, since ouabain, a metabolic inhibitor specifically blocking the membrane sodium potassium pump, reduces the promoting effects. A physiological model in which water transport is induced by active sodium transport has already been presented by Curran (1965). We found solvent drag to be involved in intestinal and rectal absorption of antipyrine (Karino et al 1982a, b; Hirasawa et al 1985). We separated the net flux of water into influx from the lumen to the blood and efflux, in the opposite direction, and found that water influx can be a measure of solvent drag (Karino et al 1982a).

In this paper, we examine the relation between sodium transport-dependent water influx and antipyrine absorption promoted by sodium taurocholate (TC-Na) and EDTA-Na in rat rectum. The effects of ouabain treatment on the promoting capacity were also examined.

Materials and methods

Materials. Deuterium oxide (D_2O, purity 99·75%) was obtained from E. Merck (Darmstadt, Germany). Fluorescein isothiocyanate dextran (FITC-dextran, mol. wt 39 000), TC-Na and ouabain were purchased from Sigma Chemical Co. (St Louis, MO, USA). EDTA-Na was obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). All other drugs and reagents were the same as described by Karino et al (1982b).

Absorption experiments. Male Wistar rats (200–280 g) which were fasted overnight were anaesthetized by an intraperitoneal injection of ethyl carbamate (1·1 mg kg^{-1}). The proximal ends of the rectum (about 3 cm length) and anus were cannulated, and the contents in the lumen washed out through the cannulae with 0·9% NaCl (saline) (20–30 ml) at 37 °C. Saline remaining in the lumen was expelled with air. The solution perfused was a 50 mM phosphate buffer (Na_2HPO_4 + KH_2PO_4, pH 6·5) containing 2 mM antipyrine, 5 mg/100 ml FITC-dextran as a non-absorbable volume marker and 1 g/100 ml TC-Na or EDTA-Na as a promoter. To examine the effects of the TC-Na concentration on antipyrine absorption, 2 g/100 ml TC-Na was also used. Finally, 4 ml of D_2O was added to 100 ml of the buffer solution, 15 ml of which, after being warmed to 37 °C was perfused by recirculation at a rate of 2·5 ml min^{-1}. In ouabain treatment, isotonic phosphate buffer containing 15 mM ouabain was first perfused for 30 min and further, the perfusion of the solution containing the drug, a promoter and ouabain was continued. The osmotic pressure of the perfused solution was adjusted to an isotonic pressure (280 mOsm) by adding NaCl (Vogel Osmometer, type OM-801, Germany). Body temperature was maintained by a heat lamp.

Ten min (lag time) after the start of the perfusion, a 2 ml sample of the perfusate was taken and a further 2 ml was taken 50 min later. After centrifugation of the samples at 3000 rev min^{-1} for 10 min, the concentrations of antipyrine and D_2O in the supernatant were determined according to Karino et al (1982b). The FITC-dextran concentration was determined fluorometrically at 495 nm (excitation) and 515 nm (emission) (HITACHI MPF-4, Japan).

Data analysis. Net flux of water was determined from the change in FITC-dextran concentration. Water influx and efflux, including D_2O diffusive permeability, and antipyrine absorption clearance (CL_{AP}) were calculated as described by Karino et al (1982a), i.e. water influx was calculated by the change in luminal D_2O concentra-

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Table 1. Promoting effects of sodium taurocholate (TC-Na) and sodium ethylenediaminetetraacetate (EDTA-Na) on water fluxes (influx, efflux and net flux) and absorption clearance of antipyrine (CL_{AP}), and inhibitory effects of ouabain on these promoting effects (n = no. of experiments).

	n	Influx ($\mu l\ min^{-1}$)	Efflux ($\mu l\ min^{-1}$)	Net flux ($\mu l\ min^{-1}$)	CL_{AP} ($\mu l\ min^{-1}$)
Promoting effects of TC-Na and EDTA-Na					
Control	17	19·3±1·9	24·7±1·7	−5·4±1·1	15·7±2·0
1% TC-Na	8	69·5±5·7[a]	73·1±6·0[a]	−3·6±0·7[b]	42·9±4·4[a]
2% TC-Na	9	74·1±7·5[a]	78·7±7·7[a]	−4·6±1·3[b]	44·7±2·7[a]
1% EDTA-Na	7	38·1±4·9[a]	42·4±5·9[a]	−4·3±1·5[b]	29·5±4·7[a]
Inhibitory effects of ouabain					
Control	13	17·0±2·2[b]	23·5±2·7[b]	−6·5±1·1[b]	11·8±1·8[b]
2% TC-Na	10	37·0±1·9[c,f]	38·4±2·0[c,f]	−1·4±0·7[e]	17·6±1·4[d,f]
1% EDTA-Na	6	19·0±2·8[e,f]	29·6±2·4[e,f]	−10·6±1·3[e]	16·0±4·0[e,f]

Values are the mean ± s.e. The inhibitory effects of ouabain were compared with both the control values under ouabain treatment and the promoting effects of the respective promoters.

[a] Significantly larger than control value ($P < 0·01$).

[b] Not significantly different from control value ($P > 0·05$).

[c] Significantly larger than the control value under ouabain treatment ($P < 0·01$).

[d] Significantly larger than the control value under ouabain treatment ($0·01 < P < 0·05$).

[e] Not significantly different from the control value under ouabain treatment ($P > 0·05$).

[f] Significantly less than the values enhanced by the respective promoting effects ($P < 0·01$).

tion, and efflux obtained as the difference between influx and net flux.

Results and discussion

Promoting effects of TC-Na and EDTA-Na. As shown in Table 1, 1% TC-Na, 2% TC-Na and 1% EDTA-Na increased the water influx, efflux, and CL_{AP}, to values significantly greater than those of the control. However, the net flux of water had small negative values showing net secretion from the blood to the lumen in all cases. No significant enhancing effects of TC-Na and EDTA-Na were observed. Thus the change in water transfer may be more accurately detectable in unidirectional water fluxes (influx and efflux), than in net flux. The increase in influx which mediates antipyrine absorption as solvent drag (Karino et al 1982a) was caused by EDTA-Na as well as TC-Na, used as a promoter by Hirasawa et al (1985).

Binder et al (1978) and Goerg et al (1980) reported positive values for the control net flux of water, in contrast to our results. However, when we used their buffer system (pH 8), containing bicarbonate instead of phosphate ions to elucidate the cause for this difference in results, the control net flux of water was 1·53 $\mu l\ min^{-1}$ (n = 9) and the enhancing effects of TC-Na were similar to those in Table 1. Thus, variance with the results of Binder et al (1978) and Goerg et al (1980) is considered to be due to differences in composition and pH of the perfused solutions.

Effects of ouabain on the promoting capacity of TC-Na and EDTA-Na. The inhibitory effects of ouabain are also listed in Table 1. Ouabain treatment has no significant effect on water influx in the control. Nor was there a significant change in CL_{AP} with the control, suggesting ouabain had no direct effects on antipyrine absorption. But the increase in both CL_{AP} and water influx by TC-Na and EDTA-Na was significantly reduced by ouabain. This indicates that promotion of the rectal absorption of antipyrine may be mediated by an increase in the water influx dependent on sodium transport. Increased solvent drag may be considered one possible promotion mechanism.

The values of CL_{AP} and water influx enhanced by TC-Na were reduced significantly, but not completely, by ouabain (15 mM). Both the CL_{AP} and water fluxes enhanced by EDTA-Na were reduced to control values by ouabain. The promoting effects of EDTA can be confirmed as being by a paracellular route (Cassidy & Tidball 1967), since EDTA is believed not to enter living cells (Nell et al 1976). The effects of TC-Na may be by both paracellular and transcellular routes since it can chelate metal ions and dissolve lipid and protein. Our results indicate that ouabain may have inhibitory effects on the promotion by TC-Na and EDTA-Na in the paracellular route.

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