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# Promotion of Drug Rectal Absorption Related to Water Absorption

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Promotion of the rectal absorption of antipyrine by sodium caprate (CA-Na) and diethyl maleate (DEM) was examined by *in situ* recirculating perfusion in the rat and compared with the results obtained previously with sodium ethylenediaminetetraacetate (EDTA-Na), a paracellular promoter. Both CA-Na and DEM significantly increased antipyrine absorption clearance ($CL_{AP}$) and water influx, in the same way as EDTA-Na. The increased levels of both $CL_{AP}$ and water influx caused by CA-Na were reduced to the control levels by ouabain, as was also found in the case of EDTA-Na. However, the promoting effect of DEM was decreased only slightly (not significantly) by ouabain treatment. These results indicate that the promotion mechanisms of CA-Na and DEM are different and that CA-Na works as a paracellular promoter in a manner similar to EDTA-Na. Antipyrine absorption through the paracellular route may possibly be promoted by water absorption dependent on active sodium transport.

**Keywords**——promoting effect; rectal absorption; water absorption; paracellular route; transcellular route; sodium caprate; diethyl maleate; sodium ethylenediaminetetraacetate

The cellular mechanism of the enhancement of epithelial permeability of poorly absorbable drugs by promoters may involve two routes, a paracellular route and transcellular route. In the former route, loss of calcium and magnesium intercellularly chelated by ethylenediaminetetraacetate (EDTA) is considered to occur in the region of tight junctions.[1,2] In the latter route, Nishihata *et al.* reported an interaction between sodium salicylate and the protein fraction in the membrane, and a reaction of diethyl maleate (DEM) with glutathione in the membrane, resulting in increased epithelial permeability.[3,4]

In the previous paper, water absorption was found to promote antipyrine rectal absorption.[5] EDTA disodium salt (EDTA-Na) and sodium taurocholate (TC-Na) increased the unidirectional water influx as well as antipyrine absorption clearance ($CL_{AP}$) in rat rectum. Ouabain, a metabolic inhibitor blocking the membrane sodium–potassium pump, reduced both the water influx and $CL_{AP}$ increased by EDTA-Na to the control level. The increase in the water influx and $CL_{AP}$ by TC-Na was only partly (but significantly) reduced by ouabain. We thus concluded that ouabain inhibits the promotion of antipyrine absorption through the paracellular route by water influx, and that TC-Na may promote antipyrine absorption through both transcellular and paracellular routes.

In order to clarify the relationship between the mechanisms of promotion of antipyrine and water absorption reported in the previous paper,[5] we investigated the influence of ouabain on the promotion of antipyrine absorption by sodium caprate (CA-Na), which, like EDTA-Na, has affinity for calcium,[6,7] or by DEM, which reacts with gluthathione in the membrane[8] as a promoter, and compared the results with those for EDTA-Na.[5]

## Experimental

**Materials**——Deuterium oxide ($D_2O$, purity 99.75%) was obtained from E. Merck (Darmstadt, Germany).

Fluorescein isothiocyanate dextran (FITC-dextran, $M_r$ 39000) and ouabain were purchased from Sigma Chemical Co. (St Louis, Mo, U.S.A.). DEM (purity 97%) and CA-Na (purity 97%) were obtained from Wako Pure Chemical Industries (Osaka, Japan) and Tokyo Chemical Industry Co. (Tokyo, Japan), respectively. All other drugs and reagents were the same as used in the previous paper.[5]

**Absorption Experiments**——The *in situ* recirculating perfusion method was used in the rectum of Wistar male rats (200—280 g) which had been fasted overnight. Procedures for animal surgery and absorption experiments were the same as in the previous paper.[5] For the ouabain treatment, a 15 mM ouabain solution was first perfused for 30 min and then was added to the drug solution which was subsequently perfused. The concentration of either CA-Na or DEM as a promoter was 1% (w/v). The DEM solution was prepared with the isotonic phosphate buffer after being dissolved in a small amount of ethyl alcohol. The final concentration of ethyl alcohol in the DEM solution was 1% (v/v).

**Assay**——The concentrations of $D_2O$, antipyrine and FITC-dextran in the perfusate were determined as described previously.[5,9]

**Data Analysis**——$CL_{AP}$, net flux of water and water influx were obtained as described by Karino *et al.*[10]

## Results and Discussion

### The Promoting Effects of CA-Na and DEM

The promoting effects of CA-Na or DEM on antipyrine and water absorption were quite similar to those of EDTA-Na or TC-Na reported in the previous paper.[5] The water influx was significantly increased by both promotors but no change in the net flux of water was observed. The changes in $CL_{AP}$ and the water influx caused by CA-Na or DEM are shown in Fig. 1, together with that by EDTA-Na taken from the previous paper.[5] CA-Na enhanced $CL_{AP}$ and the water influx by about 1.7 and 2.7 times over the control, respectively. DEM increased these values to 2.6 and 4.6 times those of the control, respectively. A solution of 1% (v/v) ethyl alcohol (vehicle) also had a slight promoting effect but the effect of DEM was significantly greater.

### Effects of Ouabain on the Promotion by CA-Na and DEM

The inhibition by ouabain of the increase in $CL_{AP}$ and water influx caused by CA-Na or DEM is also summarized in Fig. 1. As described in the previous paper, ouabain had no effect on the control.[5] The increases in $CL_{AP}$ and water influx caused by CA-Na were reduced to the control level by ouabain, indicating its effect to be the same as that of EDTA-Na.[5] However,

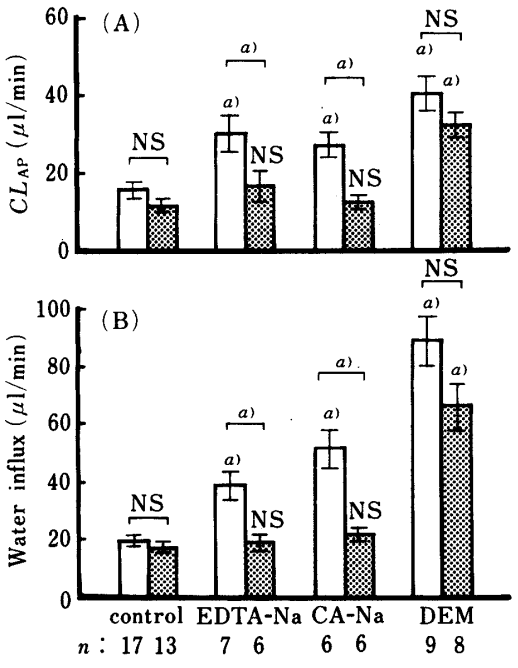


Fig. 1. Promoting Effects (Open Columns) of Disodium Ethylenediaminetetraacetate (EDTA-Na), Sodium Caprate (CA-Na) and Diethyl Maleate (DEM) on the Absorption Clearance of Antipyrine ($CL_{AP}$) (A) and the Water Influx (B), and the Inhibitory Effects of Ouabain (Dotted Columns) on These Effects

The numbers of experiments is shown as *n*. Values for EDTA-Na are taken from our previous paper.[5] Values of the open and dotted columns in each promoter group are compared with the values of the control (open column) and the control with ouabain (dotted column), respectively. Values in the open and dotted columns in each group are also compared. The significance of differences was tested by means of Student's *t* test. Each value represents the mean ± S.E.

*a*) $p<0.01$; NS, not significant ($p>0.05$).

$CL_{AP}$ and the water influx increased by DEM were only slightly decreased (not significantly) by ouabain treatment.

**Relationship between the Promotion Mechanism and Water Absorption**

CA-Na, which has an affinity for calcium ion,[6,7] increased $CL_{AP}$ and the water influx, but this effect was reduced by ouabain (Fig. 1). Thus, promoters such as EDTA-Na and CA-Na chelated to metal ions increase paracellular permeation where water absorption, dependent on sodium transport, participates in the promotion mechanism. The main promoting effect of DEM has been reported to be an improvement in transcellular permeation by bringing about a change in the integrity of the membrane.[4] The increases in $CL_{AP}$ and the water influx caused by DEM were not observed to be reduced by ouabain. Thus, the increase in water absorption is considered to be one of the factors influencing the promotion mechanism. The water flux in the paracellular route (EDTA-Na and CA-Na) differs from that in the transcellular route (DEM), the former being dependent on active sodium transport and the latter not necessarily being dependent on it.

Since ouabain is poorly absorbable, differences in its absorbability induced by various promoters may affect water absorption dependent on active sodium transport. However, digitoxin, which is readily absorbable and is a metabolic inhibitor for the sodium–potassium pump, had the same inhibitory effect as ouabain on the promotion of antipyrine absorption by EDTA-Na and DEM (data not shown). Ouabain may thus be considered to effectively block the sodium–potassium pump in all promotion systems.

In regard to the relationship of promotion mechanism to water absorption, three possibilities may be considered: (i) a concentration effect owing to water absorption at the membrane interface, resulting in enhancement of the drug concentration gradient, (ii) promotion by solvent drug or (iii) promotion by increase in the absorption site blood flow. Further research will be necessary to determine which is correct.

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