J. Pharm. Pharmacol. 1987, 39: 118–123
Received May 29, 1986

Differences in the promotion mechanism of the colonic absorption of antipyrine, phenol red and cefmetazole

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The promotion of antipyrine, phenol red and cefmetazole absorption by sodium ethylenediaminetetraacetate (EDTA-Na) as a paracellular promoter, diethyl maleate (DEM) as a transcellular promoter, and sodium taurocholate (TC-Na), whose promotion mechanism is still unclear, has been investigated by the rat in-situ colonic loop technique. All these promoters increased AP absorption and water influx from the lumen to the blood. Ouabain treatment suppressed the increase in antipyrine absorption and water influx induced by TC-Na and EDTA-Na, but did not modify the enhancing effect of DEM. Thus, the promotion mechanism of TC-Na may be similar to that of EDTA-Na. Phenol red and cefmetazole absorption were increased by TC-Na and EDTA-Na but not by DEM. Accordingly, phenol red and cefmetazole absorption appears to be promoted via paracellular but not transcellular routes. The collection of blood for plasma samples reduced the influx of water which had been increased by TC-Na or EDTA-Na. Consequently, the enhancement in antipyrine plasma concentration by these promoters was reduced to the control level. The inhibitory mechanism for this is discussed on the basis of the blood-flow limitation of antipyrine and water absorption.

To enhance the epithelial membrane transport of poorly absorbed drugs, certain absorption promoters have been studied. Ethylenediaminetetraacetic acid (EDTA), which is considered to increase intercellular permeation by chelated depletion of calcium and magnesium in regions of the tight junction (Cassidy & Tidball 1967; Kunze et al 1972), is a paracellular promoter. Since interactions between salicylate and protein in intestinal membranes (Nishihata et al 1984a; Kajii et al 1985) and between diethyl maleate (DEM) and glutathione in membranes (Nishihata et al 1984b) increase epithelial permeability, these compounds qualify as a transcellular promoter.

We reported that EDTA sodium salt (EDTA-Na) and taurocholate sodium (TC-Na) increase the rectal absorption of antipyrine and water influx from the lumen to the blood, and that ouabain, a membrane Na^+-K^+ pump inhibitor, reduced antipyrine absorption clearance (CL_{AP}) and water influx which had been increased by EDTA-Na or TC-Na (Shiga et al 1985). The promotion of antipyrine absorption is thus considered to be related to increased water influx to the blood. Antipyrine absorption may be promoted by EDTA-Na and TC-Na via paracellular routes, since TC-Na shows promoting effects similar to those of EDTA-Na. It has also been reported that the promoting effects of bile salts on membrane permeability may be caused by Ca^{2+}-depletion in the paracellular pathway (Freel et al 1983; Murakami et al 1984).

In the present paper, the mechanism of enhancement of drug absorption by various promoters has been examined by the rat in-situ colonic loop technique (Doluisio et al 1969). The absorption conditions in this loop technique are more physiological than the recirculating perfusion used by Shiga et al (1985) and membrane damage due to forced perfusion can be avoided. Promoting effects are therefore considered capable of being detected sensitively by this loop technique. As model drugs, antipyrine, which is both water- and lipid-soluble and well absorbed, and phenol red and cefmetazole, which are water-soluble and poorly absorbed, were used. TC-Na, EDTA-Na and DEM were selected as representative promoters.

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 70 000), sodium taurocholate and ouabain were purchased from Sigma Chemical Co. (St Louis, MO, USA). EDTA-Na and diethyl maleate (purity 97%) were obtained from Wako Pure Chemical

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Industries Ltd (Osaka, Japan). Tritiated water (^3H_2O) and [^{14}C]antipyrine ([^{14}C]AP) were purchased from Amersham (Bucks, UK) and New England Nuclear (Boston, MA, USA), respectively. The sodium salt of cefmetazole was kindly provided by Sankyo Co. Ltd (Tokyo, Japan). All other drugs and reagents were the same as used by Shiga et al (1985).

Absorption experiments

Male Wistar rats (200 ± 20 g) fasted overnight were anaesthetized by intraperitoneal injections of ethyl carbamate (1·1 mg kg^{-1}). The proximal and distal ends of the colon (about 10 cm length) were cannulated according to Doluisio et al (1969), and the contents in the lumen were washed through the cannulae with 0·9% NaCl (saline) (about 50 mL) at 37 °C. The saline remaining in the lumen was expelled with air and a 10 mL plastic injection syringe was attached to each cannula. The luminal solution was a 50 mM phosphate buffer (Na_2HPO_4 + KH_2PO_4, pH 6·5) containing 2 mM antipyrine, 0·1 mM phenol red or 20 mM cefmetazole. The solution also contained 0·0025% FITC-dextran as a non-absorbable volume marker, 2% (v/v) D_2O or 0·1 μCi mL^{-1} ^3H_2O as a measure of water influx and 2% TC-Na, 2% EDTA-Na or 1% DEM as a promoter. Five mL of the solution was injected into the lumen and then stored. The method of withdrawing samples from the lumen was in accordance with that of Doluisio et al (1969). Two mL of the luminal solution was taken at the beginning of the experiment, just after injection of the solution into the lumen, and at the end of the experiment, at 30 min for antipyrine, 30 or 60 min for phenol red and at 60 min for cefmetazole. To obtain the plasma concentration of antipyrine, 0·01 μCi mL^{-1} [^{14}C]AP was added to the luminal solution. For blood antipyrine and cefmetazole concentrations, 0·3 mL of blood was collected from the jugular vein cannula every 15 min and centrifuged for 2 min to obtain the plasma sample. A cefmetazole absorption experiment was also performed with an isotonic citrate buffer (pH 2·5) prepared according to Kakemi et al (1965) to examine pH dependency. For the ouabain treatment, a 15 mM ouabain solution was first placed in the loop for 15 min. The luminal solution was then changed to one containing a drug, a promoter and ouabain. Each experiment, i.e. the control experiment without a promoter and the experiment to examine promoting effects or ouabain inhibitory effects, was carried out in random order for each combination of a drug and promoter.

The osmotic pressure of all luminal solutions 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.

Assay

After centrifugation of the luminal 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 (1982). The phenol red concentration was determined at 590 nm (λ_1) and 560 nm (λ_2) by a dual beam-spectrophotometer after the supernatant (0·25 mL) had been made alkaline with 1 M NaOH (3 mL). The cefmetazole concentration was measured by HPLC as reported by Sekine et al (1982). The assay of the FITC-dextran concentration was in accordance with that reported by Shiga et al (1985). To determine the [^{14}C]AP plasma and ^3H_2O luminal concentrations, a 10 mL scintillator solution (0·3 g of POPOP, 12 g of DPO, 2 L of toluene and 1 L of Triton X-100) was added to 0·1 mL of the plasma sample or 0·5 mL of the luminal solution. All samples were counted in a liquid scintillation counter (Aloka 903, Tokyo, Japan).

Data analysis

Absorption clearance, net flux of water and water influx were determined as described by Karino et al (1982).

RESULTS

Promotion of antipyrine absorption

The clearance of antipyrine after absorption (CL_{AP}) from the control (in the absence of a promoter) is about 4 μL min^{-1} cm^{-1} (Table 1). The enhancement of antipyrine absorption and water influx by the promoters, and their inhibition by ouabain are also shown. TC-Na and EDTA-Na increased both CL_{AP} and the water influx by as much as 1·5 times the control values,and DEM caused the values to almost double. The secretory net flux of water (negative value) tended to be larger in the presence of TC-Na or EDTA-Na than in the control, but the net flux became absorptive (positive value) in the presence of DEM. Ouabain treatment almost completely inhibited the promoting effects of TC-Na and EDTA-Na on both CL_{AP} and water influx, but it caused no significant decrease in DEM effects.

Promotion of phenol red absorption

Table 1 also shows the enhancement of phenol red absorption by promoters and its inhibition by oua-

Table 1. Promoting effects of sodium taurocholate (TC-Na), sodium ethylenediaminetetraacetate (EDTA-Na) and diethyl maleate (DEM) on absorption clearances of antipyrine (CL_{AP}), phenol red (CL_{PR}) and water flux (influx and net flux), and inhibitory effects of ouabain on these promoting effects.

	CL_{AP} μL min^{-1} cm^{-1}	Water influx μL min^{-1} cm^{-1}	Net flux μL min^{-1} cm^{-1}	CL_{PR} μL min^{-1} cm^{-1}
Promoting effects of TC-Na, EDTA-Na and DEM				
Control	4·33 ± 0·26	6·73 ± 0·23	−0·18 ± 0·21	ND
TC-Na	6·82 ± 0·49[a]	10·8 ± 0·89[a]	−0·58 ± 0·11	1·39 ± 0·08[a]
EDTA-Na	6·86 ± 0·53[a]	10·3 ± 0·92[a]	−0·64 ± 0·75	1·39 ± 0·24[a,b]
DEM	9·68 ± 0·37[a]	14·0 ± 0·66[a]	2·35 ± 0·36[a]	ND
Inhibitory effects of ouabain				
Control	4·35 ± 0·19	7·00 ± 0·24	−1·08 ± 0·25	ND
TC-Na	4·08 ± 0·49[d]	6·22 ± 0·65[d]	−0·83 ± 0·06	1·10 ± 0·05[c,e]
EDTA-Na	4·78 ± 0·29[d]	6·63 ± 0·12[d]	−0·27 ± 0·18	ND[b]
DEM	9·97 ± 0·36[c]	14·0 ± 0·56[c]	2·41 ± 0·23[c]	ND

Values are the mean ± s.e., obtained during 30 min of the experimental period, from three to eight rats. The inhibitory effects of ouabain were compared with the control values under ouabain treatment and the promoting effects of the respective promoters.
[a] Significantly greater than control value ($P < 0·01$).
[b] Values obtained during 60 min of the experimental period.
[c] Significantly greater than the control value under ouabain treatment ($P < 0.01$).
[d] Significantly less than the values enhanced by the respective promoting effects ($0·01 < P < 0·05$).
[e] Significantly less than the values enhanced by the respective promoting effects ($P < 0·01$).

bain. The effects of all promoters and ouabain on water influx were similar to those in the case of antipyrine absorption. Phenol red was hardly absorbed and in the control could not be detected. TC-Na significantly enhanced phenol red absorption clearance (CL_{PR}). The promoting effects of EDTA-Na on CL_{PR} could not be found during the first 30 min of the experiment. Over 60 min, EDTA-Na caused CL_{PR} to increase to approximately the same amount as that induced by TC-Na. However, no DEM effects were seen. Ouabain significantly reduced CL_{PR} which had been increased by TC-Na (Table 1). The promoting effects of EDTA-Na on CL_{PR} over 60 min were inhibited completely by ouabain (Table 1).

Promotion of cefmetazole absorption

Since cefmetazole absorption at pH 6·5 is low, even in the presence of a promoter, it cannot be detected from luminal concentration change. Therefore, the promoting effects were determined by examination of the increase in its plasma concentration (Fig. 1). This was measured every 15 min for 60 min and the results clearly indicated enhancement of its absorption by TC-Na (Fig. 1a) and EDTA-Na (Fig. 1b) but not by DEM (Fig. 1c). The EDTA-Na effects were observed to be markedly delayed, but the extent of promotion after 45 min was greater than that of TC-Na. This delay was similar to that with phenol red. In contrast to antipyrine and phenol red, there

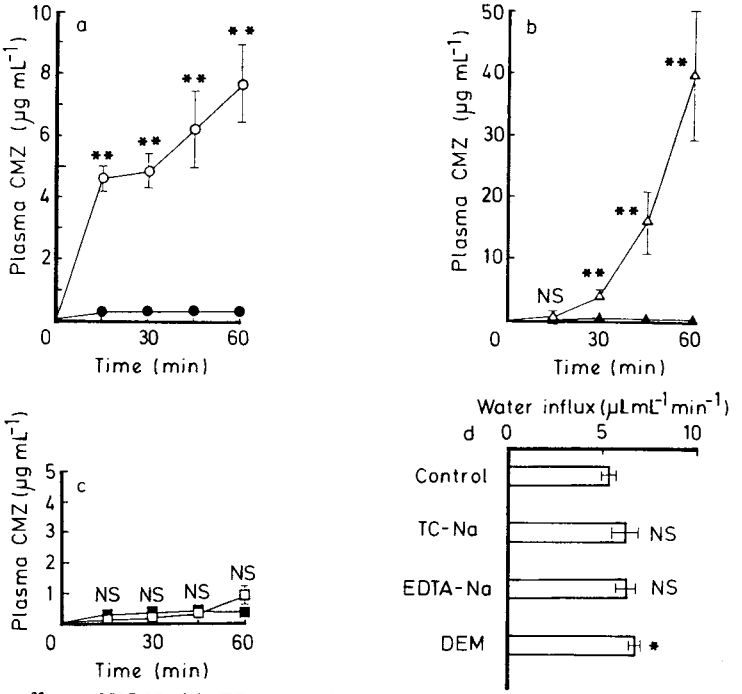

FIG. 1. The promoting effects of TC-Na (a), EDTA-Na (b) and DEM (c) on cefmetazole (CMZ) colonic absorption and water influx (d) at pH 6·5. The closed and open symbols represent the plasma CMZ concentrations in the control and in the presence of promoters, respectively. The values represent the means ± s.e. of four to six rats. $^{**}P < 0·01$ vs control; $^{*}0·01 < P <$ vs control; NS, not significant ($P > 0·05$ vs control).

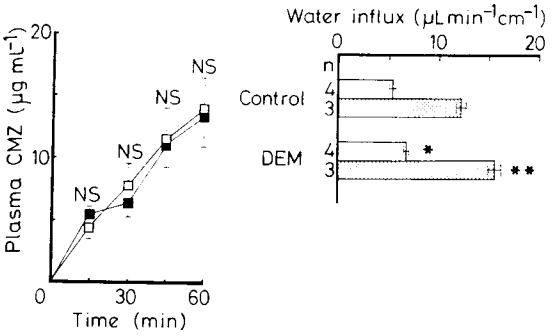

FIG. 2. The promoting effects of DEM on cefmetazole (CMZ) colonic absorption and water influx at pH 2·5. The closed and open squares show the plasma CMZ concentrations in the control and in the presence of DEM, respectively. Water influxes at pH 6·5 (open column), which is already depicted in Fig. 1, and at pH 2·5 (dotted column) are shown together and the effects of pH and DEM are compared. The values at pH 2·5 represent the mean ± s.e. of three rats. DEM effects on water influx are compared between each pH group. ** $P < 0.01$; * $0.01 < P < 0.05$; NS not significant ($P > 0.05$) vs the control.

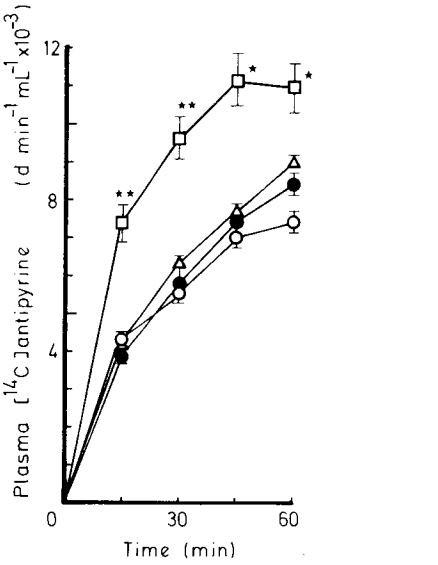

FIG. 3. The promoting effects of TC-Na, EDTA-Na and DEM on antipyrine (AP) colonic absorption. ○ TC-Na; △ EDTA-Na; □ DEM; ● control. The values represent the mean ± s.e. of three rats. ** $P < 0.01$ vs control; * $0.01 < P < 0.05$ vs control; No * not significant ($P > 0.05$ vs control).

was no increase in water influx by TC-Na or EDTA-Na, but a small enhancement occurred in the presence of DEM (Fig. 1d). Ouabain's inhibitory effects were not examined for cefmetazole absorption since there was no obvious promoting effects on water influx. DEM's promoting effects on cefmetazole absorption and water influx were also examined at pH 2·5 (Fig. 2). Both cefmetazole absorption and water influx at this pH were much higher than those at pH 6·5 in the control. DEM significantly increased water influx at pH 2·5 to the same extent as that at pH 6·5. However, no promoting effects of DEM on the plasma cefmetazole concentration were noted.

Promotion of antipyrine absorption examined with its plasma concentration data

Antipyrine absorption obtained by measurement of luminal clearance (Table 1) was examined with the [^{14}C]AP plasma concentration data in Fig. 3. The promoting effects of TC-Na and EDTA-Na on antipyrine absorption disappeared, although DEM gave a significantly higher plasma concentration than the control.

DISCUSSION

The promoting effects of TC-Na and EDTA-Na on CL_{AP} and water influx (Table 1) were similar to those found for in-situ recirculating perfusion in our previous paper (Shiga et al 1985). However, the net flux of water tended to increase in a secretory direction in the presence of TC-Na and EDTA-Na, and in an absorptive direction in the presence of DEM (Table 1). Such changes are noted here for the first time, indicating the in-situ loop method to be better than the recirculating perfusion method for examining small water transport such as net flux.

Although ouabain reduced to the control level CL_{AP} and water influx that had increased through the action of TC-Na and EDTA-Na, it did not have an inhibiting action on the enhancing effects of DEM (Table 1). The results for the effects of TC-Na and EDTA-Na correspond to those of Shiga et al (1985), except that CL_{AP} and water influx, enhanced by TC-Na, decreased significantly but without falling to the control level found by Shiga et al (1985). This small discrepancy may arise from differences in method. That the promotion mechanism of TC-Na was similar to that of EDTA indicates that TC-Na may be considered to be a paracellular promoter. The change in net water flux from secretory to absorptive, observed only in the presence of DEM, and the differences in the inhibitory effects of ouabain, may therefore be considered to reflect differences in the promotion mechanisms of the TC-Na and EDTA-Na groups and DEM.

In contrast to antipyrine, the absorption of phenol red and cefmetazole was promoted by TC-Na and EDTA-Na but not by DEM (Table 1, Fig. 1), indicating them to be capable of permeating mainly via the paracellular route. Antipyrine is absorbed relatively easily without promoters but its absorption

is also affected by both promoter groups. Thus it is considered that antipyrine absorption rates are increased both through paracellular and transcellular routes. There are obvious differences in how quickly the effects of TC-Na and EDTA-Na on phenol red and cefmetazole absorption become evident, the effect of TC-Na being seen much sooner (Table 1, Fig. 1). Therefore there are differences in the effects of TC-Na and EDTA-Na. The finding that cefmetazole absorption is not enhanced by DEM is at variance with the results of Nishihata et al (1984b), possibly owing to differences in the mode of administration, and composition and pH of the drug solution administered. We used the in-situ loop with phosphate buffer (pH 6·5) as a luminal solution while they used a microenema with an aqueous solution of distilled water. However, pH difference was not found to have an effect on the action of DEM (Figs 1 and 2).

The increase by TC-Na and EDTA-Na of water influx that accompanied the increase in antipyrine disappearance from the colonic lumen (Table 1), was not observed in the absorption of cefmetazole into the plasma (Fig. 3). This raises the question of why in the cefmetazole experiment, water influx ceased to increase in the presence of TC-Na or EDTA-Na. The remarkable differences in the results of the two experiments could be attributable to blood collection in the cefmetazole experiment but not in the antipyrine experiment. To examine this further, the promoting effects on antipyrine absorption were examined in the light of plasma concentration data. There was no enhancement of its plasma concentration in the presence of TC-Na and EDTA-Na (Fig. 3), so the disappearance of the enhancing effects on water influx and antipyrine absorption could be due to blood collection. Water absorption has been reported to be nearly blood flow-limited (Mailman 1981) and antipyrine absorption, partly blood flow-limited (Ochsenfahrt & Winne 1974). The increase in absorption site blood flow (ASBF) is considered to be related to water absorption (Mailman 1984) and, consequently, water influx enhanced by promoters should increase ASBF while blood collection should decrease it. The enhancement of water influx, which is blood flow-limited, is thus reduced. Such an interaction between water influx and ASBF may inhibit the promotion of antipyrine absorption. That this promotion is related to water influx (Table 1) may be explained to some extent on the basis of the increase in ASBF induced by enhanced water influx. We previously reported that the increase in the rectal absorption of antipyrine by water influx arose from solvent drag (Hirasawa et al 1985). The relation between increased ASBF and solvent drag as a promotion mechanism of antipyrine absorption remains unclear. Overlapping aspects in both promotion mechanisms are conceivable since water absorption is nearly blood flow-limited (Mailman 1981). The DEM effect is so large that it could be detected from antipyrine absorption as assessed from its plasma concentration data (Fig. 3). Cefmetazole absorption is less blood flow-limited since it can be enhanced by TC-Na or EDTA-Na even during blood collection (Fig. 1).

In summary, the promotion mechanisms of three drugs were examined and the following results obtained: phenol red and cefmetazole are absorbed mainly via the paracellular route and antipyrine through both paracellular and transcellular routes. It took longer for the paracellular promoting effects of EDTA-Na on phenol red and cefmetazole absorption to appear than those of TC-Na. The increase in ASBF by water influx may be a promotion mechanism of drug absorption with blood flow limitation. Blood collection, which may reduce ASBF, inhibits an increase in water influx and antipyrine absorption, both of which are blood flow-limited, even in the presence of promoters.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, Science and Culture of Japan (No. 59460197 and No. 60571094). The authors wish to thank Mr Kazutoshi Chiba for his technical assistance.

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