PROMOTING MECHANISM BY BILE SALT RELATED TO WATER ABSORPTION IN DRUG RECTAL ABSORPTION

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(Received September 11, 1984)

The promoting mechanism by bile salts in rat rectal absorption of antipyrine was studied by the in situ recirculating perfusion. The significant correlation between rectal absorption clearance (CL_{AP}) of antipyrine (AP) and apparent water influx (influx') was found in the control without promoters, indicating the existence of solvent drag in the AP rectal absorption. Sieving coefficient of AP (b_{AP}), i.e., the slope of the regression line between CL_{AP} and influx', was 0.6 approximately equal to that in small intestine reported previously. The relation between the promoting effects and solvent drag was further studied, resulting that both CL_{AP} and influx' were significantly enhanced by sodium taurocholate (TC-Na), sodium glycocholate (GC-Na) and sodium cholate (C-Na). Accordingly the promoting effects of bile salts might be due to the increase in solvent drag. However TC-Na did not produce the significant change in b_{AP}. From these results, the enhancing mechanism in the epithelial cell membrane transport was discussed.

Keywords — promoting mechanism; rectal absorption; solvent drag; sieving coefficient; water influx; absorption clearance; antipyrine; rat; bile salt; sodium taurocholate

INTRODUCTION

Several studies on rectal absorption of water-soluble and low lipophilic drugs improved by various promoters have been reported, but the detailed promoting mechanism is still unknown. The cellular mechanism of epithelial permeability alteration can be separated to two routes, (i) paracellular route and (ii) transcellular route. For the former route, it is considered that widening of intercellular channel is induced by chelating depletions of calcium and magnesium in the region of tight junction by ethylenediaminetetraacetate (EDTA) and bile salts. And it is also reported as an example in the latter route that the interaction of sodium salicylate and its derivatives with the protein fraction in cell membrane may be essential for enhancing the permeability. On the other hand, we reported that there is the contribution of solvent drag to the intestinal absorption of salicylic acid and antipyrine, and the contribution ratio is approximately 12% or more in the total absorption. Therefore in this paper the possibility that the increase in solvent drag by the change of water channel improves the drug rectal absorption is investigated. (Received September 11, 1984)

MATERIALS AND METHODS

Materials — Deuterium oxide (D_{2}O, purity 99.75%) was obtained from E. Merck, Darm-
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stadt, Germany. Fluorescein isothiocyanate-dextran (FITC-dextran MW 39000), sodium taurocholate (TC-Na), sodium glycocholate (GC-Na), sodium cholate (C-Na), sodium deoxycholate (DC-Na) and sodium taurodeoxycholate (TDC-Na) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. All other drugs and reagents were the same as reported previously.10

Absorption Experiment — Wistar male rats weighing 220—250 g fasted overnight were anesthetized by intraperitoneal injection of ethyl carbamate (1.0 g/kg). The glass tubings (3 mm i.d., 5 mm o.d.) were put into the proximal end of rectum (3 cm length) and anus, and contents in lumen were washed out through the cannulas with saline solution (20—30 ml) warmed at 37 °C. The saline solution left in the lumen were expelled with air. Thereafter the recirculating perfusion of drug solution prewarmed at 37 °C was started through the cannulas at 2.5 ml/min. The decrease of the body temperature was prevented by the heat lamp. The other detailed surgery and absorption method were the same as reported in the intestinal absorption.10 The drug solution is 2 mM AP in bicarbonate buffer solution (5 mM KCl + 25 mM NaHCO₃, pH 8.0). This buffer is a modified one used by Binder et al.11 and can dissolve all bile salts used here. Finally 1.2 ml of D₂O and 1.5 mg of FITC-dextran as a nonabsorbable volume marker were added to 30 ml of the drug solution. And promoting effects were examined in the bile salts solutions adjusted to 1 or 2%. Also phosphate buffer solution (50 mM Na₂HPO₄ + 50 mM KH₂PO₄, pH 6.5) was used to compare the effects of ionic composition with the bicarbonate buffer. The tonicities were adjusted to the following NaCl equivalent values by adding NaCl: hypotonic 0.45; isotonic 0.9; hypertonic 1.8. Ten minutes (lag time) after the initiation of the perfusion, each 3 ml aliquot of the perfused solution was sampled out at 0 and 60 min.

Interaction between AP and TC-Na — A molecular sieve technique reported by Ashworth and Heard was used with a slight modification.12 To 2 ml of 2 mM AP was added 2 ml of the buffer solution with or without 1—4% TC-Na. The buffer solution is the isotonic bicarbonate buffer solution used in the absorption experiment. After the mixed solution was incubated for 1 h at 37 °C, the total volume was adjusted to 10 ml with Sephadex G-25 gel (fine grade, Pharmacia Fine Chemicals AB, Uppsalla, Sweden) well swollen in the buffer solution. Then the mixture was equilibrated by shaking for 2 h at 37 °C and allowed to stand for 5 min at 37 °C. After centrifuging the supernatant liquid for 5 min at 3000 rpm, the final supernatant was analyzed. External volume of the gel was determined in the same manner as described above with a solution of 0.25% Blue Dextran (MW 2000 000, Pharmacia Fine Chemicals AB, Uppsalla, Sweden) in place of AP. Finally the ratio of AP amount in external phase of the gel in the presence of TC-Na to the control was determined.

Exsorption Absorption — In situ rectal single-pass perfusion of the isotonic or hypertonic buffer solution with or without TC-Na was performed at 2.5 ml/min in the same manner as the recirculating method. After the perfusion of the buffer solution with or without TC-Na for 30 min, 20 μmol of AP was injected intravenously and the exsorption amount of AP in the perfused solution for 60 min was determined.

Data Analysis — Water net flux was obtained by the change of FITC-dextran concentration in the perfused solution. Suitability of FITC-dextran as a nonabsorbable volume marker in the presence of TC-Na was preliminarily ascertained from the recovery ratio after perfusion. Apparent water influx (influx') and outflux (outflux') including D₂O diffusive permeability were calculated from the change of D₂O amount in the perfused solution and water net flux as reported previously.8 The calculation of drug absorption clearance was also described previously.8,10

Assay — All sample solutions were centrifuged for 5 min at 3000 rpm and the superna-
nants were used for assay. The concentrations of AP and D$_2$O were determined as reported previously. FITC-dextran concentration was determined fluorometrically at 495 nm for excitation and 515 nm for emission after a 250-fold dilution of the supernatant with the same buffer as used in the perfusion experiment.

RESULTS

The promoting effects of 1% bile salts on the AP rectal absorption are listed in Table I. The results were obtained by the perfusion of isotonic bicarbonate buffer (pH 8.0). TC-Na, GC-Na and C-Na enhanced significantly influx', outflux' and absorption clearance of AP (CL$_{AP}$). For water net flux, only TC-Na apparently induced the absorptive change but the increase was not significant from the control. All other bile salts changed the net flux to secretory direction but the changed values were not significantly different from the control value except C-Na. Since there was occasionally a bleeding in the large enhancing effect of C-Na, the contribution of solvent drag to the promotion was further studied in the effect of TC-Na where such damage to membrane was not observed.

Fig. 1a and b show the relationship between CL$_{AP}$ and influx' when the hypo-, iso-, and hypertonic AP solutions were perfused in the control and in the presence of TC-Na, respectively. The significant correlation indicated the existence of solvent drag in the AP rectal absorption. Kitazawa et al. described that water absorption is changeable according to the ionic composition of the perfused solution. Therefore we also perfused the phosphate buffer solution (pH 6.5) used in the intestinal absorption, resulting in the significant correlation in the same manner as shown in Fig. 1 (not shown in Figure). And the values of sieving coefficient ($b_{AP}$, the slopes) and the intercepts of the regression lines in the control and in the presence of TC-Na are listed for both buffer solutions in Table II. The values of $b_{AP}$ in the control were approximately equal each other in both buffer solutions and they were about 0.6 significantly smaller than one in all cases regardless of the presence of TC-Na. On the other hand, the variances in the intercept were so large that the TC-Na effects were not found.

The ratio of AP amount in the external phase of Sephadex G-25 (fine grade) gel in the presence of TC-Na to the control was 1.00–1.04 at 1–4% TC-Na. The result rules out the interaction between TC-Na micelle and AP. The exsorption amount of AP into the rectal lumen for 60 min after the intravenous administration of AP was less than 1% of the intravenous dose.

| Table I. Effects of Bile Salts on Apparent Water Influx (Influx') Apparent Water Outflux (Outflux'), Water Net Flux and Absorption Clearance (CL$_{AP}$) in Antipyrine (AP) Rectal Absorption |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number of expts | Influx' (µl/min) | Outflux' (µl/min) | Net flux (µl/min) | CL$_{AP}$ (µl/min) |
| Control         | 35.8 ± 4.2      | 34.3 ± 11.4     | 1.5 ± 1.5        | 22.9 ± 3.0       |
| TC-Na           | 96.1 ± 9.9      | 89.3 ± 10.1     | 6.8 ± 0.9        | 58.3 ± 8.2       |
| GC-Na           | 83.8 ± 9.5      | 84.5 ± 7.8      | -1.1 ± 2.1       | 50.2 ± 2.1       |
| C-Na            | 98.7 ± 12.0     | 108.7 ± 12.7    | -10.1 ± 1.6      | 54.6 ± 6.8       |
| DC-Na           | 42.8 ± 6.8      | 48.7 ± 11.9     | -5.9 ± 5.2       | 16.2 ± 7.6       |
| TDC-Na          | 59.3 ± 15.3     | 60.4 ± 15.1     | -1.2 ± 3.5       | 37.6 ± 4.5       |

*TC-Na: Sodium Taurocholate, GC-Na: Sodium Glycocholate, C-Na: Sodium Cholate, DC-Na: Sodium Deoxycholate, TDC-Na: Sodium Taurodeoxycholate Values are mean ± S.E. in the perfusion of isotonic bicarbonate buffer solution (pH 8.0). The concentration of all bile salts is 1%.

a) Significantly different from control ($p < 0.01$). b) Significantly different from control (0.01 < $p < 0.05$).
regardless of the presence of TC-Na and the amount was not influenced by the tonicity.

**DISCUSSION**

The promotion of the AP absorption by trihydroxy bile salts such as TC-Na, GC-Na and C-Na was larger than that by dihydroxy bile salts such as DC-Na and TDC-Na. This result contradicts the promoting effect of bile salts related to chelating abilities on the rectal absorption of sodium ampicillin reported by Murakami et al. The differences might be due to the physicochemical properties of drugs, the difference in tonicity of the drug solution such as hypertonicity in their experiment and isotonicity in our experiment (Table I), and/or the different con-

![Graphs showing relationships between rectal absorption clearance of antipyrine and apparent water influx](image)

**FIG. 1. Relationships between Rectal Absorption Clearance of Antipyrine ($CL_{AP}$) and Apparent Water Influx ($I_{app}$) in Control (a) and in the Presence of 1% Sodium Taurocholate (b).**

The results were obtained by the in situ recirculating perfusion of bicarbonate buffer solution (pH 8.0). The line was calculated by the linear regression analysis.

○ ●, hypotonic; △ ▲ , isotonic; □ ■ , hypertonic perfused solution.

**TABLE II. Effects of Sodium Taurocholate (TC-Na) on Relationship between Rectal Absorption Clearance of Antipyrine ($CL_{AP}$) and Apparent Water Influx ($I_{app}$)**

<table>
<thead>
<tr>
<th>Perfused solution</th>
<th>Number of expts</th>
<th>Sieving coefficient ($b_{AP}$)</th>
<th>Intercept ($I_{app}$)</th>
<th>Correlation coefficient ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>Control</td>
<td>18</td>
<td>0.644 ± 0.144&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−0.39 ± 6.73</td>
</tr>
<tr>
<td>Buffer</td>
<td>1% TC-Na</td>
<td>19</td>
<td>0.676 ± 0.103&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−7.60 ± 7.69</td>
</tr>
<tr>
<td></td>
<td>2% TC-Na</td>
<td>12</td>
<td>0.512 ± 0.132&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.53 ± 10.43</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Control</td>
<td>17</td>
<td>0.612 ± 0.137&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.01 ± 3.25</td>
</tr>
<tr>
<td>Buffer</td>
<td>1% TC-Na</td>
<td>15</td>
<td>0.452 ± 0.115&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.10 ± 9.40</td>
</tr>
</tbody>
</table>

Values of $b_{drug}$ and intercept indicate mean ± its estimated S.E.

- <sup>a</sup> Slope of the regression line in Fig. 1.
- <sup>b</sup> All values are not significantly different from zero ($p>0.05$).
- <sup>c</sup> Significantly smaller than one ($p<0.01$).
- <sup>d</sup> Significantly smaller than one ($0.01<p<0.05$).
centration of bile salts such as their 12.5 mM (0.52—0.67%) and our 1%, but the detail is as yet unknown. Here the effect of TC-Na was further studied since TC-Na had the largest promoting effects on the AP absorption and such damage to membrane as bleeding was not observed.

TC-Na increased influx’ as well as CL_{AP} by more than two times that of the control as shown in Table I. Since Fig. 1a indicated the solvent drag in the AP rectal absorption, the simultaneous enhancement of influx’ and CL_{AP} suggests the contribution of solvent drag to the rectal absorption became larger in the presence of the bile salts. Considering that the pore size of water channel can be calculated from the molecular radius of permeant and its sieving coefficient,^{14} the result that b_{AP} was not changed by TC-Na implies that TC-Na did not affect the pore size where AP molecule is permeable (Fig. 1b and Table II). Further the interaction between TC-Na micelle and AP which might affect the AP absorption was ruled out by a molecular sieve method. The exsorption ratio of AP to the dose for 60 min in the whole small intestine was reported 26% in the hypertonic perfused solution.^{8} Since the length of the whole small intestine is more than 20 times larger than that of the rectum (about 3 cm) and outflux’ per cm of the small intestine^{8} and rectum is approximately equal in the control (Table I), it is acceptable that the exsorption ratio in rectum was less than 1%. Accordingly it is considered that the effects of the back flux of the absorbed AP from blood to lumen on the rectal absorption was negligibly small since the exsorption was less than 1% even in the presence of TC-Na where outflux’ was increased.

The enhancement in solvent drag, i.e., the increase in influx’, is considered due to the increase in the numbers of water channel since the change in pore size of water channel was ruled out. However, its detailed cellular mechanism is now unknown. TC-Na surely has a chelating ability to Ca^{2+} but its affinity is reported to be low.^{3,6} Further, since TC-Na is a surface active agent with a large solubilizing ability to Sudan III,^{3} the direct interaction with the membrane can be anticipated. Accordingly the mechanism of the promotion is not always confined to the paracellular route but the contribution of the transcellular route is also needed to be considered.

Acknowledgement The authors wish to thank Miss Sachiko Hagiwara for her technical assistance.

REFERENCES

