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Comparison of Water Influx and Sieving Coefficient in Rat Jejunal, Rectal and Nasal Absorptions of Antipyrine

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Permeability characteristics in rat jejunal, rectal and nasal absorption of antipyrine (AP) are discussed in terms of water influx and sieving coefficients. The sieving coefficient in nasal absorption was less than half of those in jejunal and rectal absorptions, which were not significantly different from each other. Apparent water influx in the jejunum was twice that in the rectum, and that in the nose was approximately three times larger than that in the jejunum. The pore size of the water channels in each site was calculated from the sieving coefficient and molecular radius of AP by using the modified Levitt equation. It was concluded that: (1) the nasal membrane is composed of smaller-sized pores but is richer in water channel distribution than the jejunal membrane; (2) the rectal membrane has similar-sized pores to the jejunal membrane but the water channel distribution is poorer.

Keywords——jejunal absorption; rectal absorption; nasal absorption; antipyrine; solvent drag; sieving coefficient; water influx; water channel; pore size; rat

Recently Hirai *et al.* reported that water-soluble phenol red and cephazolin, which are poorly absorbed from the gastrointestinal tract, can be well absorbed through the nasal membrane.[1] Nishihata *et al.* showed that rectal absorption of poorly absorbable lidocaine and cefmetazole as ionic forms was promoted by several adjuvants.[2] Considering that such water-soluble and weakly lipophilic drugs should permeate through the aqueous phase rather than the lipoidal phase in membranes, it would be useful to compare the permeability characteristics of water channels of the jejunum, rectum and nose. Although proof of the existence of water channels in membranes has not been obtained as yet, the concept of a pathway where water-soluble compounds can permeate has often been used. There have been several reports where the pore sizes in membranes are discussed in terms of the solute radius and the reflection coefficient in solvent drag.[3] We estimated the solvent drag effect in intestinal absorption of water-soluble drugs such as antipyrine, salicylic acid and cephalexin from the relationship between drug absorption clearance and water influx, *i.e.*, a unidirectional water flux from the lumen to the blood.[4] Sieving coefficients ($=1-$reflection coefficient) between zero and one were obtained, suggesting that such water-soluble drugs are partly absorbed through water channels by solvent drag.

In this paper, we studied the characteristics of water channels at various absorption sites. Namely, we compared the sieving coefficients of antipyrine (AP) and the water influxes in rat jejunum, rectum and nose. The pore size of water channels in each site was calculated from the sieving coefficient and the molecular radius of AP by using the modified Levitt equation, which Granger and Taylor adopted to calculate the pore size of the intestinal capillary wall.[5]

Experimental

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

Fluorescein isothiocyanate-dextran (FITC-dextran, MW 39000) was purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). All other drugs and reagents were the same as in the previous paper.[6]

Absorption Experiments——Wistar male rats (250±30 g) fasted overnight were used under anesthesia with pentobarbital (30 mg/kg) or ethyl carbamate (1 mg/kg) administered intraperitoneally. AP jejunal absorption was studied by the *in situ* single-pass perfusion technique in individual rats. The technique was described in detail by Hirasawa *et al.*[7] For nasal absorption, the *in situ* recirculating perfusion method at 2.5 ml/min was used according to Hirai *et al.* with slight modifications.[1,8] Rectal absorption was also studied by the *in situ* recirculating perfusion method at 2.5 ml/min. The proximal end of the rectum (about 3 cm length) and the anus were cannulated with glass tubes (3 mm i.d., 5 mm o.d.) and the contents of the lumen were washed out through the cannulas with saline solution (20—30 ml) warmed to 37 °C. Then, saline solution left in the lumen was expelled with air and 15 ml of the perfusion solution prewarmed to 37 °C was perfused. In all the experiments, the perfusion solution was 50 mM phosphate buffer (pH 6.5) containing 2 mM AP, 1.7 g/100 ml inulin or 5 mg/100 ml FITC-dextran as a nonabsorbable volume marker and 4 mg/100 ml D_2O. Its osmotic pressure was adjusted with sodium chloride to the following sodium chloride equivalent values: hypertonic 1.8; isotonic 0.9; hypotonic 0.45. In rectal and nasal absorptions, 2 ml aliquots of the perfused solution were sampled at 0 and 50 min, timed from ten minutes (lag time) after the start of the perfusion.

Assay——The sample solution was centrifuged for 5 min at 3000 rpm and the supernatant was used for assay. The concentrations of AP, D_2O and inulin were determined as described previously.[6] FITC-dextran concentration was determined fluorometrically (495 nm for excitation and 515 nm for emission).

Data Analysis——Water net flux was obtained from the change in inulin or FITC-dextran concentration. Apparent water influx including D_2O diffusive permeability (abbreviated as water influx) and antipyrine absorption clearance (CL_{AP}) were calculated as described previously.[4] Namely, water influx was calculated from the change in luminal D_2O concentration and water net flux. The sieving coefficient of AP (b_{AP}) was obtained as the slope of the regression line calculated by linear regression analysis between CL_{AP} and water influx.[4] Pore size of water channels was calculated by using the following modified Levitt equation:

$$b_{AP}=1-(1/3)(16R^2-20R^3-7R^4)$$

where R is the ratio of AP radius to pore radius.

Results

For jejunal absorption, the result in the *in situ* single-pass perfusion reported by Hirasawa *et al.* was used since b_{AP} obtained by the single-pass method was not essentially different from that in the recirculating method and was more accurate.[7] On the other hand,

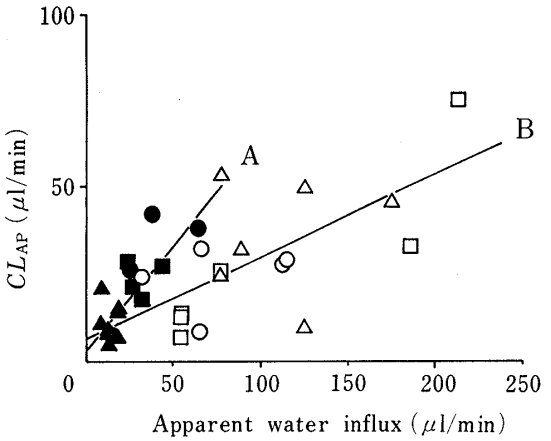

Fig. 1. Relationship between Rectal (A, Closed Symbol) and Nasal (B, Open Symbol) Absorption Clearances of Antipyrine (CL_{AP}) and Apparent Water Influx

Each line was obtained by linear regression analysis. Regression line (A): $y=0.612x+4.01$ ($r=0.756$, $p<0.01$), (B): $y=0.238x+6.13$ ($r=0.662$, $p<0.01$). ■□, hypertonic; ▲△, isotonic; ●○, hypotonic perfusion solution.

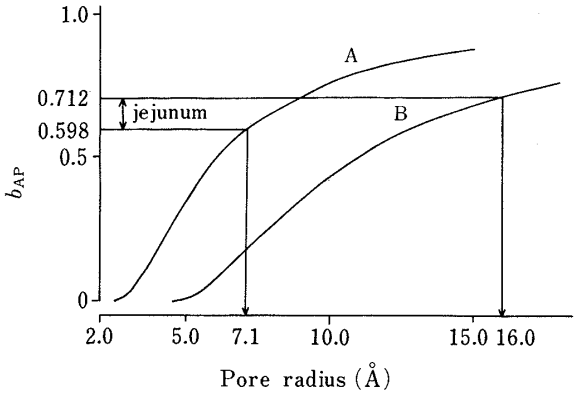

Fig. 2. Relationship between Sieving Coefficient of Antipyrine (b_{AP}) and Pore Radius Obtained by Using the Modified Levitt Equation

Curves A and B indicate the cases of minimum radius (2.5 Å) and maximum radius (4.5 Å) of the antipyrine molecule, respectively. The method for obtaining the pore radius from the sieving coefficient, including the S.E. range, in the jejunum is also shown as an example.

TABLE I. Sieving Coefficient (b_{AP}), Apparent Water Influx and Water Net Flux in Jejunal, Rectal and Nasal Absorptions of Antipyrine

Absorption site	b_{AP}[a]	Water influx[b] (μl/min)	Water net flux[b] (μl/min)
Jejunum	0.655±0.057[c] (19) [h]	32.5±2.2[d] (10) [e]	−5.6±0.8[d] (10) [h]
Rectum	0.612±0.137[c] (17) [g] [e]	15.9±2.4 (9) [e] [e]	−7.9±2.4 (9) [h] [f]
Nose	0.238±0.070[c] (17)	111.2±15.8 (6)	−18.1±7.1 (6)

Values are each the mean±S.E. Values in parentheses are numbers of experiments. *a*) Slope of the regression line of drug absorption clearance *versus* apparent water influx. *b*) Values for the perfusion of isotonic solution. *c*) Significantly smaller than one ($p<0.01$) in the jejunum and nose, and ($0.01<p<0.05$) in the rectum. *d*) Values per 3 cm (approximately corresponding to the rectal length). *e*) Significantly different ($p<0.01$). *f*) Significantly different ($0.01<p<0.05$). *g*) Significantly different ($0.05<p<0.10$). *h*) Not significantly different ($p>0.10$).

rectal and nasal absorptions were studied by the recirculating perfusion method, since the difference between input and output concentration used in the analysis by the single-pass method was not significant in such small absorption areas. Figure 1 shows the regression lines between CL_{AP} and water influx calculated by linear regression analysis for rectal and nasal absorptions. A significant correlation was obtained in both absorption sites. The values of b_{AP} as well as water influx and water net flux in isotonic perfusion are listed in Table I. Water influx and net flux in the jejunum are values per 3 cm (approximately corresponding to the rectal length). The b_{AP} values in jejunal and rectal absorptions were not significantly different ($p>0.10$) but that in nasal absorption was less than half of those at the other absorption sites. On the other hand, water influx in isotonic perfusion was the largest in the nose, approximately three times larger than in the jejunum, and that in the rectum was half that in the jejunum. Water net flux, which is the difference between influx and outflux showed negative values, indicating secretion from the blood to the lumen in all sites. The absolute value of water net flux was largest in the nose, as was the case for water influx, but there was no significant difference between the jejunum and rectum. There were larger deviations as compared with water influxes at all sites. Figure 2 shows the relationship between the sieving coefficient and pore radius obtained by using the modified Levitt equation. Curves A and B indicate the cases of minimum radius (2.5 Å) and maximum radius (4.5 Å) assuming that the AP molecule is spherical, respectively. Consequently the pore sizes were 7.1—16.0 Å in jejunal, 5.9—17.0 Å in rectal and 3.9—8.4 Å in nasal membrane based on the mean and S.E. range of each sieving coefficient.

Discussion

The pore size in the jejunal membrane, 7.1—16.1 Å, is larger than the values of 3.9 Å determined from the epithelial permeability of polyalcohol *in situ*[9] and 4 Å determined *in vitro*, based on the radius of mannitol.[10] However, the value agrees with that of 12—16 Å calculated from the reflection coefficients and molecular radii of urea and water.[11] Comparing the pore size at three sites, the pores of the jejunal and rectal membrane have similar size and those of the nasal membrane show a slightly smaller size. On the other hand, water influx in the nose, jejunum and rectum decreases in that order. The volume of the nasal cavity, 0.2—0.3 ml as reported by Hirai *et al.*, is similar to that of the rectum (approximately 3 cm length). Accordingly, as compared with the jejunum, it is suggested that there is a greater water flux in the nose, *i.e.*, the nasal membrane is enriched with water channels of smaller pore size, and the rectum has a poorer water channel distribution.

The pore sizes as discussed above are approximate values since the Levitt equation is derived from a physical model on the assumption that the permeant molecule is spherical and the water channel is cylindrical in shape. However, the above results clearly reveal characteristic differences in water channels of the three absorption sites.

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References

1) S. Hirai, T. Yashiki, T. Matsuzawa and H. Mima, *Int. J. Pharmaceut.*, **7**, 317 (1981).
2) T. Nishihata, J. H. Rytting and T. Higuchi, *J. Pharm. Sci.*, **71**, 865 (1982).
3) J. S. Fordtran, F. C. Rector, Jr., M. F. Ewton, N. Soter and J. Kinney, *J. Clin. Invest.*, **44**, 1935 (1965).
4) A. Karino, M. Hayashi, S. Awazu and M. Hanano, *J. Pharmacobio-Dyn.*, **5**, 670 (1982).
5) D. N. Granger and A. E. Taylor, *Am. J. Physiol.*, **238**, H457 (1980).
6) A. Karino, M. Hayashi, T. Horie, S. Awazu, H. Minami and M. Hanano, *J. Pharmacobio-Dyn.*, **5**, 410 (1982).
7) T. Hirasawa, T. Muraoka, A. Karino, M. Hayashi and S. Awazu, *J. Pharmacobio-Dyn.*, **7**, 246 (1984).
8) A. Kotani, M. Hayashi and S. Awazu, *Chem. Pharm. Bull.*, **31**, 1097 (1983).
9) D. Winne and J. Remischovsky, *Naunyn-Schmiedebergs Arch. Pharmakol.*, **270**, 22 (1971).
10) D. H. Smyth and E. M. Wright, *J. Physiol.* (London), **182**, 591 (1966).
11) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **279**, 133 (1973).