J. Pharm. Pharmacol. 1988, 40: 701-705
Received February 1, 1988

©1988 J. Pharm. Pharmacol.

Effect of Chlorpromazine on the Permeability of β-Lactam Antibiotics Across Rat Intestinal Brush Border Membrane Vesicles

KEN ISEKI, MITSURU SUGAWARA, HIROSHI SAITOH, KATSUMI MIYAZAKI AND TAKAICHI ARITA*

*Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, and * Faculty of Pharmaceutical Science, Mukogawa Women's University, 4-16 Edagawa-cho, Nishinomiya, Hyogo 663, Japan*

Abstract—The effect of chlorpromazine on the membrane permeability of β-lactam antibiotics (benzylpenicillin, ampicillin, cephradine and cephalexin) and actively transported substances (glycylglycine and D-glucose) has been studied using rat intestinal brush border membrane vesicles. Except for cephalexin, the initial uptakes at 25°C of these antibiotics were significantly enhanced in the presence of chlorpromazine. In contrast, the transport of glycylglycine and D-glucose was significantly inhibited. These results suggest that the two groups, drugs and actively transported substances, have a different permeation process. The effect of chlorpromazine concentration on membrane lipid fluidity, as assessed by the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-anilino-8-naphthalene sulphonate (ANS), was also examined. The fluorescence polarization of ANS decreased with increasing concentration of chlorpromazine, while that of DPH increased suggesting an increase of membrane surface fluidity might affect the permeation of β-lactam antibiotics and actively transported substances in a different manner.

Many investigations (Penzotti & Poole 1974; Iseki et al 1985; Kimura et al 1985; Okano et al 1986a, b; Tsuji et al 1987) have been made to clarify the intestinal absorption mechanisms of amino β-lactam antibiotics. Some reports (Penzotti & Poole 1974; Kimura et al 1985; Okano et al 1986a; Tsuji et al 1987) suggested that a carrier-mediated transport system for dipeptides or amino acids, which have a zwitterionic structure as do several amino β-lactam antibiotics, participates in the membrane transport of those drugs. However, in our previous reports (Miyazaki et al 1982; Iseki et al 1987a, b), we found the soluble protein fraction which binds amino β-lactam antibiotics in rat intestinal epithelial cells and indicated that this intracellular binding process played an important role in the absorption system of the antibiotics. Thus, it is considered that the absorption mechanisms of amino β-lactam antibiotics are complicated, and there remains controversy about the extent to which the carrier-mediated transport systems participate in the absorption process. In addition, despite many investigations, there are few reports relating to the other major function of epithelia, namely, their ability to serve as a barrier.

The effect of chlorpromazine, a major tranquillizer, on the many functions of biological membranes has been extensively studied revealing inhibition of L-methionine transport across the intestinal microvillous membranes (Sundaresan & Rivera-Calimlim 1977, 1978) and inhibition of the D-glucose transport system of human erythrocytes at relatively high concentrations of chlorpromazine (Baker & Rogers 1972; Lacko et al 1980). An increasing effect of chlorpromazine on membrane permeability for passively transported substances was reported by Tsao et al (1982) using isolated hepatocytes of rat and Maoi et al (1979) with a liposome membrane model.

Correspondence to: K. Miyazaki, Dept of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.

We have now compared the permeabilities of β-lactam antibiotics (benzylpenicillin, ampicillin, cephradine and cephalexin) and the actively transported substances, D-glucose and glycylglycine, using rat intestinal brush border membrane vesicles. We have also studied the effect of chlorpromazine on the perturbation of rat intestinal brush border membrane.

Materials and Methods

Materials

Ampicillin anhydrous (Takeda Chemical Industries, Osaka, Japan), cephalexin monohydrate (Shionogi & Co., Tokyo, Japan), cephradine (Sankyo Co., Tokyo, Japan) were kindly donated. Glycylglycine and chlorpromazine hydrochloride were purchased from the Sigma Chemical Co. (St. Louis, Mo, USA).

Potassium benzylpenicillin and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Wako Pure Chemical Industry Ltd (Osaka, Japan), and magnesium 8-anilino-1-naphthalene sulphonate (ANS) was obtained from Nakarai Chemicals Ltd (Kyoto, Japan). [U-^{14}C]Glycylglycine (sp. act. 100 mCi mmol^{-1}), D-[^{14}C]glucose (sp. act. 270 mCi mmol^{-1}), and benzyl[^{14}C]penicillin (sp. act. 59 mCi mmol^{-1}) were purchased from Amersham International, Ltd (Bucks, UK). All other chemicals were of the highest grade available and used without further purification.

Preparation of the brush border membrane vesicle

Brush border membrane vesicles were isolated from the small intestine of male Wistar rats (180–230g) according to the calcium chloride precipitation method of Kessler et al (1978). The membrane vesicles were suspended in a final concentration of about 2 mg protein mL^{-1} with 20 mM 2-(*N*-morpholino)ethanesulphonic acid (Mes)/Tris (pH6·0), 100 mM NaCl, and 100 mM D-mannitol. The purity of the

membranes was routinely evaluated with alkaline phosphatase (E.C.3.1.3.1.), an enzyme specific for the intestinal brush border membrane. The specific activity of this enzyme increased 13-fold in the final membrane suspension. Protein was determined by the method of Lowry et al (1951) with bovine serum albumin as standard.

Transport studies

The uptake of substrates was measured by a rapid filtration technique. In the regular assay, the reaction was initiated by the addition of 100 μL of a buffer (20 mM Mes/Tris (pH6·0), 100 mM NaCl, and 100 mM D-mannitol) containing the substrate (4·0 mM) to 100 μL of membrane suspension at 25°C. For D-glucose uptake, the membrane vesicles were suspended in 20 mM Mes/Tris (pH 6·0) buffer containing 100 mM D-mannitol, and the incubation medium contained, in 200 μL final volume, 20 mM Mes/Tris (pH 6·0) buffer, 100 mM D-mannitol, 100 mM NaCl, and 2·5 mM D-glucose. At a stated time the reaction was stopped by diluting the reaction mixture with 5 mL of ice-cold buffer (150 mM NaCl, 20 mM Tris/HCl, pH 7·5). The tube contents were immediately filtered through a Millipore filter (HAWP, 0·45 μm, 2·5 cm diameter), which was washed once with 8 mL of the same ice-cold buffer.

In a separate experiment, non-specific adsorption onto Millipore filter was determined using the incubation medium instead of brush border membrane suspension. This value was subtracted from the uptake data.

Analytical methods

The radiolabelled substances (benzylpenicillin, glycylglycine and D-glucose) on the filter were determined by a liquid scintillation counting. Ampicillin, cephradine and cephalexin were determined by high-performance liquid chromatography using a fluorometric detection according to Miyazaki et al (1983).

Membrane fluidity

Brush border membrane fluidity was studied by the fluorescence polarization method of Merrill et al (1987). Fluorescence intensities were measured with a Hitachi fluorescence spectrophotometer equipped with polarizing filters and a temperature-controlled cell (25°C). The fluorescence polarization of fluorophore, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 8-anilino-1-naphthalene sulphonate (ANS), provided the relative measure of the fluidity of the lipid regions of the membrane. To 2·5 mL of brush border membrane suspension (0·2–0·25 mg protein mL^{-1}), 5 μL of 0·5 mM DPH in tetrahydrofuran was added. After thorough mixing with a Vortex shaker and incubation at room temperature (20°C) for 60 min, at which time little or no odour of tetrahydrofuran could be detected. To the vesicles with ANS, a small volume of the ANS solution (3·0 mM) was added directly to the vesicle suspension (final 30 μM) in the cuvette and the fluorescence determined.

Excitation and emission wavelengths were 360 and 430 nm for DPH, or 385 and 480 nm for ANS, respectively. Corrections for background fluorescence and light scattering were made with blanks containing brush-border membranes alone and DPH suspension alone. Steady-state polarization (**P**) was calculated from the fluorescence intensities measured parallel ($\mathbf{I}_1$) and perpendicular ($\mathbf{I}_2$) to the plane of the light of excitation according to the following equation:

$$\mathbf{P}=\frac{\mathbf{I}_1-(\mathbf{G})\,\mathbf{I}_2}{\mathbf{I}_1+(\mathbf{G})\,\mathbf{I}_2}$$

where (**G**) = HV/HH, a correction factor for the polarization of the instrument (Miller & Bronner 1981).

Results

Effect of chlorpromazine on the initial uptake of β-lactam antibiotics and actively transported substances by brush border membrane vesicles

To compare the permeation characteristics of β-lactam antibiotics and the actively transported substances, glycylglycine and D-glucose, we investigated the effect of chlorpromazine on the initial uptake (1 min) of these two groups across the brush border membrane vesicles. The result is shown in Fig. 1. The initial uptakes of benzylpenicillin, ampicillin and cephradine clearly increased in the presence of chlorpromazine (1·0 mM). Cephalexin uptake was not significantly influenced by chlorpromazine.

On the other hand, the initial uptake of glycylglycine and D-glucose, which are transported across the brush border membrane by a carrier-mediated system, was decreased significantly by the same concentration of chlorpromazine. This inhibitory effect on the actively transported substances agreed well with the findings of Sundaresan & Rivera-

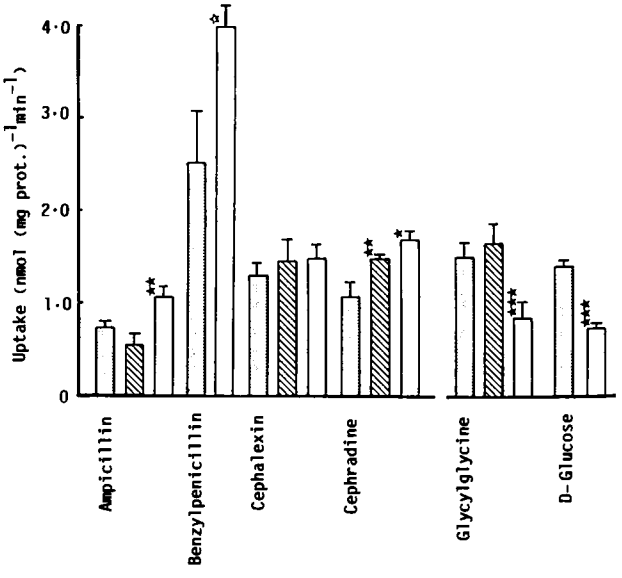

FIG. 1. Effect of chlorpromazine on the initial uptake (1 min) of glycylglycine, D-glucose and some β-lactam antibiotics by intestinal brush border membrane vesicles. Membrane vesicles (100 μL), suspended in 100 mM NaCl, 100 mM D-mannitol and 20 mM Mes/Tris (pH 6·0), were incubated with the substrate mixture (100 μL) comprising 100 mM NaCl, 100 mM D-mannitol, 20 mM Mes/Tris (pH 6·0) and 5·0 mM each substrates in the presence of chlorpromazine. In the case of D-glucose uptake, membrane vesicles were suspended in 100 mM D-mannitol and 20 mM Mes/Tris (pH 6·0) buffer, and the reaction was initiated by addition of 5·0 mM D-glucose solution in a buffer (20 mM Mes/Tris (pH 6·0), 200 mM NaCl, and 100 mM D-mannitol). Each value represents the mean ± s.e. of 3–5 experiments performed in triplicate. Statistically significant at $P<0·005$ (★★), $P<0·01$ (★★★) and $P<0·025$ (☆) when compared with the control. Key: (▨) control, (▩) 0·2 mM and (□) 1·0 mM chlorpromazine treated.

Calimlim (1977, 1978) in that chlorpromazine inhibited the transport of L-methionine and D-galactose across the rat small intestine. The osmotic gradient across the vesicles did not affect the carrier-mediated transport of glucose nor did the pH of 6·0 provide different results from pH 7·0 in preliminary experiments.

Time course of β-lactam antibiotic uptake by brush border membrane vesicles

Fig. 2 shows the time course of uptake of ampicillin and cephradine at 2·5 mM. Initially, the uptake of both antibiotics clearly increased in the presence of chlorpromazine. Then their equilibrium uptakes (30 min) both in the presence and absence of chlorpromazine was almost the same. This suggests that chlorpromazine does not alter the volume of intravesicular space of brush border membrane vesicles.

For cephalexin, the time course of uptake was not altered in the presence of chlorpromazine (not shown).

Membrane fluidity alteration in the presence of chlorpromazine

The perturbation of the brush border membrane induced by chlorpromazine was estimated by fluorescence polarization of ANS and DPH. DPH provides information on fluidity in the hydrophobic interior of the plasma membrane (Shinitzky & Inbar 1974), while ANS is presumed to monitor the bilayer closer to the aqueous interface (Podo & Blasie 1977). As shown in Fig. 3, fluorescence polarization of the DPH-labelled vesicles increased significantly with increasing chlorpromazine concentration, while the ANS fluorescence polarization decreased significantly with the same concentration of chlorpromazine. These results suggest that chlorpromazine might modify membrane fluidity by increasing it at the membrane surface and then decreasing it in the interior region.

Discussion

It has been reported that chlorpromazine inhibited the activity of the carrier-mediated transport system of the small intestine and the erythrocyte (LeFevre & Sternberg 1982) by binding to the carrier proteins and/or their boundary lipids in the plasma membrane (Goldstein 1984; Zimmer 1984). In the present study, we have compared the transport behaviour of endogenous compounds and β-lactam antibiotics in the presence of chlorpromazine by using brush border membrane vesicles isolated from rat small intestine. Both glycylglycine and D-glucose are well known as actively transported substances while the antibiotics are drugs whose transport mechanisms are still unclear, except for benzylpenicillin, a passively transported compound. As a result, there was a distinct difference between the effect of chlorpromazine on the actively transported substances (glycylglycine and D-glucose) and those on β-lactam antibiotics (benzylpenicillin, ampicillin, cephradine and cephalexin). Chlorpromazine extensively inhibited the initial uptake of glycylglycine and D-glucose. In contrast, the initial uptake of benzylpenicillin, ampicillin and cephradine was increased significantly by chlorpromazine, while cephalexin was not influenced.

It has been pointed out that the transport of cephalexin and cephradine across the rat intestinal brush border membrane depends on a H^+-gradient (outside to inside) (Okano et al 1986a) and that both drugs could be transported, in part, by a carrier-mediated system for dipeptides in the presence of an inward H^+-gradient (Okano et al 1986b). In contrast, our present results show that the transport system of β-lactam antibiotics (ampicillin, cephalexin, cephradine and benzylpenicillin) differ from those of glycylglycine and D-glucose in a medium that lacks a H^+-gradient. Additionally, some reports (Cheeseman & Devlin 1985; Rajendran et al 1985, 1987) indicated that the carrier-mediated transport system for dipeptides retains its activity in the medium lacking an H^+-gradient. Moreover, Sundaresan & Rivera-Calimlim (1977, 1978) indicated that chlorpromazine at 1·0 mM inhibited L-methionine and D-galactose transport across the rat small intestine in the absence of an H^+-gradient. They also pointed out that the diffusional (passive) transport process of L-methionine was not influenced by chlorpromazine. Thus, although the driving forces for the dipeptide transport system were insufficient under the conditions of our study, chlorpromazine would be expected to inhibit the carrier-mediated transport system for glycylglycine and D-glucose. From these results, it is suggested that the transport systems for amino β-lactam

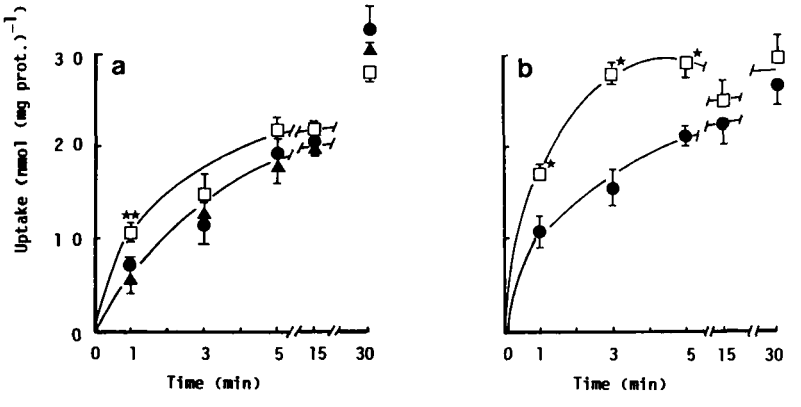

FIG. 2. Effect of chlorpromazine on the time-courses of ampicillin (a) and cephradine (b) uptake by intestinal brush border membrane vesicles. The medium conditions were as given in the legend for Fig. 1. Each point represents the mean ± s.e. of 3–4 experiments performed in triplicate, some values lie within the symbol area. Statistically significant at $P < 0.001$ (★) and $P < 0.005$ (★★) when compared with the control. Key: (●) control, (▲) 0·2 mM and (□) 1·0 mM chlorpromazine treated.

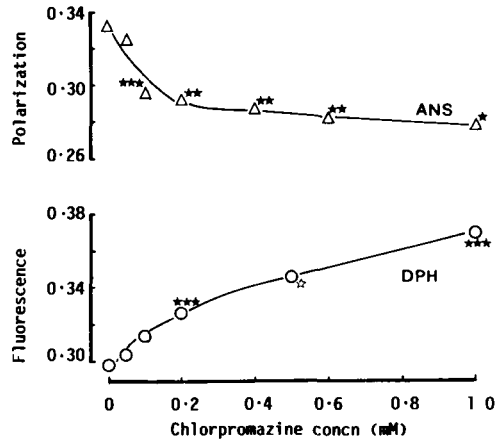

FIG. 3. Effects of chlorpromazine on the fluorescence polarization of ANS (△) and DPH (○)-labelled brush border membrane vesicles from rat small intestine. Each point represents the mean values of three or four determination in a single experiment. The protein concentrations in the vesicles were 0·2–0·25 mg mL^{-1}. Excitation (Ex) and emission (Em) wavelength: ANS; Ex = 385 nm, Em = 480 nm, DPH; ex = 365nm, Ex = 430 nm. ★; $P < 0.001$, ★★; $P < 0.005$, ★★★; $P < 0.01$, ☆; $P < 0.025$ compared with the control value.

antibiotics are different from those of glycylglycine and D-glucose. We also found that chlorpromazine produces an alteration of membrane fluidity. As has been pointed out by Sundaresan & Rivera-Calimlim (1977, 1978) and Elsenhans et al (1985), a decrease of the fluidity of the interior region might participate in the inhibition of glycylglycine and D-glucose transport.

On the other hand, little is known about the relation between chlorpromazine acceleration on membrane surface fluidity and drug permeation. Recently, however, Kajii et al (1986) reported that salicylate, which increases the fluidity of the membrane surface, accelerated the transport of 6-carboxyfluorescein across the brush border membrane vesicles. Also, Kimura et al (1980) reported that intestinal lipid liposomes were permeable to β-lactam antibiotics and that the permeability or fluidity of the lipid bilayer was increased by ampicillin and cephalothin. Thus, it is considered that an increase of membrane surface fluidity plays a role in the intestinal transport of amino β-lactam antibiotics.

In conclusion, our results demonstrate that the permeation characteristics of the amino β-lactam antibiotics, ampicillin, cephradine and cephalexin, differ from those of the actively transported substances, glycylglycine or D-glucose.

References

Baker, G. F., Rogers, H. J. (1972) Effects of psychotropic drugs on the erythrocyte permeability to glucose and ethylidene glucose. Biochem. Pharmacol. 21: 1871–1878

Cheeseman, C. I., Devlin, D. (1985) The effect of amino acids and dipeptides on sodium-ion transport in rat enterocytes. Biochim. Biophys. Acta 812: 767–773

Elsenhans, B., Blume, R., Lembcke, B., Caspary, W. F. (1985) In vitro inhibition of rat small intestinal absorption by lipophilic organic cations. Ibid. 813: 25–32

Goldstein, D. B. (1984) The effects of drugs on membrane fluidity. Ann. Rev. Pharmacol. Toxicol. 24: 43–64

Iseki, K., Iemura, A., Miyazaki, K., Arita, T. (1985) Uptake of amino β-lactam antibiotics into rat intestinal brush border membrane vesicles. J. Pharm. Pharmacol. 37: 374–375

Iseki, K., Mori, K., Miyazaki, K., Arita, T. (1987a) Binding of amino β-lactam antibiotics to soluble protein from rat intestinal mucosa I. Biochem. Pharmacol. 36: 1837–1842

Iseki, K., Mori, K., Miyazaki, K., Arita, T. (1987b) Binding of amino β-lactam antibiotics to soluble protein from rat intestinal mucosa II. Ibid. 36: 1843–1846

Kajii, H., Horie, T., Hayashi, M., Awazu, S. (1986) Effects of salicylic acid on the permeability of the plasma membrane of the small intestine of the rat: a fluorescence spectroscopic approach to elucidate the mechanism of promoted drug absorption. J. Pharm. Sci. 75: 475–478

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M., Semenza, G. (1978) A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes; their use in investigating some properties of D-glucose and choline transport systems. Biochim. Biophys. Acta 506: 136–154

Kimura, T., Yoshikawa, M., Yasuhara, M., Sezaki, H. (1980) The use of liposomes as a model for drug absorption: β-lactam antibiotics. J. Pharm. Pharmacol. 32: 394–398

Kimura, T., Yamamoto, T., Ishizuka, R., Sezaki, H. (1985) Transport of cefadroxil, an aminocephalosporin antibiotic, across the small intestinal brush border membrane. Biochem. Pharmacol. 34: 81–84

Lacko, L., Wittke, B., Lacko, I. (1980) Interaction of chlorpromazine with the transport system of glucose in human erythrocytes. Arzneimittel-Forsch./Drug Res. 30: 1852–1855

LeFevre, P. G., Sternberg, D. M. (1982) Discordant findings concerning the stimulation by chlorpromazine of erythrocyte suger transfer. Biochem. Pharmacol. 31: 463–466

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) *Protein measurement with the Folin phenol reagent.* J. Biol. Chem. 193: 265–275

Maoi, M., Suzuki, T., Yagi, K. (1979) Effects of chlorpomazine and other phenothiazine derivatives on the permeability of liposomes. Biochem. Pharmacol. 28: 295–299

Merrill, A. R., Aubry, H., Proulx, P., Szabo, A. G. (1987) Relation between Ca^{2+} uptake and fluidity of brush-border membranes isolated from rabbit small intestine and incubated with fatty acids and methyl oleate. Biochim. Biophys. Acta 896: 89–95

Miller, A., III, Bronner, F. (1981) Calcium uptake in isolated brush-border vesicles from rat small intestine. Biochem. J. 196: 391–401

Miyazaki, K., Iseki, K., Arita, T. (1982) Intestinal absorption of several β-lactam antibiotics. IV. Binding to the various components in the intestinal mucosa of rat and role in absorption process. J. Pharmacobiodyn. 5: 593–602

Miyazaki, K., Ohtani, K., Sunada, K., Arita, T. (1983) Determination of ampicillin, amoxicillin, cephalexin, and cephradine in plasma by high-performance liquid chromatography using fluorometric detection. J. Chromatogr. 276: 478–482

Okano, T., Inui, K., Takano, M., Hori, R. (1986a) H^+ gradient-dependent transport of aminocephalosporins in rat intestinal brush-border membrane vesicles. Biochem. Pharmacol. 35: 1781–1786

Okano, T., Inui, K., Maegawa, H., Takano, M., Hori, R. (1986b) H^+ coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes. J. Biol. Chem. 261: 14130–14134

Penzotti, S. C., Poole, J. W. (1974) Transport mechanisms of β-lactam antibiotics across everted rat gut. J. Pharm. Sci. 63: 1803–1806

Podo, F., Blasie, J. K. (1977) Nuclear magnetic resonance studies of lecithin bimolecular leaflets with incorporated fluorescent probes. Proc. Natl. Acad. Sci. 74: 1032–1036

Rajendran, V. M., Ansari, S. A., Harig, J. M., Adams, M. B., Khan, A. H., Ramaswamy, K. (1985) Transport of glycyl-L-proline by human intestinal brush border membrane vesicles. Gastroenterology 89: 1298–1304

Rajendran, V. M., Harig, J. M., Ramaswamy, K. (1987) Characteristics of glycyl-L-proline transport in intestinal brush-border membrane vesicles. Am. J. Physiol. 252: G281–G286

Shinitzky, M., Inbar, M. (1974) Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. J. Mol. Biol. 85: 603–615

Sundaresan, P. R., Rivera-Calimlim, L. (1977) Effect of chlorpromazine on L-methionine transport in the rat intestine. Biochem. Pharmacol. 26: 1411–1415

Sundaresan, P. R., Rivera-Calimlim, L. (1978) Mechanism of chlorpromazine action on rat intestinal transport processes. Ibid. 27: 2781–2786

Tsao, S. C., Iga, T., Sugiyama, Y., Hanano, M. (1982) Effect of chlorpromazine on isolated rat hepatocytes. Ibid. 31: 491–497

Tsuji, A., Hirooka, H., Terasaki, T., Tamai, I., Nakashima, E. (1987) Saturable uptake of cefixime, a new oral cephalosporin without an α-amino group, by the rat intestine. J. Pharm. Pharmacol. 39: 272–277

Zimmer, G. (1984) Fluidity of cell membranes in the presence of some drugs and inhibitors. In: Kates, M., Manson, L. A. (eds) Membrane Fluidity (Biomembranes; V. 12) Plenum Press., New York & London, pp 169–208