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Correlation Between Structures of Organic Cations and Their Binding Behaviours to Brush Border Membrane Isolated from Rat Small Intestine

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Abstract—We have investigated the correlation between the molecular structures of various organic cations and their binding to rat small intestinal brush border membrane. The binding of small quaternary ammonium compounds such as tetramethylammonium and choline to brush border membrane was not sufficient to inhibit methylchlorpromazine binding. However, lauryltrimethylammonium and cetyltrimethylammonium, both quaternary amines with a long carbon chain, inhibited binding significantly. The inhibition was competitive. When the unbranched hydrocarbon chain of the quaternary amines was extended in steps from C1 (methyl) to C16 (cetyl), the inhibitory effect increased sharply with length from C7 (heptyl) to C16. These results suggest that the size of the hydrophobic part of the molecule is an important factor in binding of quaternary ammonium compounds to the brush border membrane. The structure of the hydrophilic part was another factor. In imipramine-related compounds, the order of binding was *N*-didesmethylimipramine (primary amine) > desipramine (secondary) > imipramine (tertiary) > methylimipramine (quaternary). However, with the small molecular ethylamine-related compounds, binding properties did not reflect differences in the hydrophilic component. Therefore, the effect of the hydrophilic part may be secondary and may depend on the size of the hydrophobic part. We suggest that organic cations which are amphiphilic can bind to a common binding site on brush border membrane through hydrophobic and/or hydrophilic interactions.

Although quaternary ammonium compounds are cationic drugs with poor lipid solubility, they are immediately and rapidly absorbed from the gastrointestinal tract (Levine et al 1955; Levine 1966; Turnheim & Lauterbach 1980). This rapid absorption cannot be explained by a pH-partition hypothesis (Hogben et al 1959) and it has therefore been suggested that specialized transport mechanisms could contribute to this process (Levine 1966; Irwin et al 1969; Ruifrok 1981; Ruifrok & Mol 1983; Tsubaki & Komai 1986). We have suggested (Saitoh et al 1987, 1988a, b) that binding to the brush border membrane is a first step in the specialized transport mechanism. In the current study, we have examined the binding properties of various cationic drugs in relation to hydrophobicity and hydrophilicity.

Materials and Methods

Materials

Cetyltrimethylammonium bromide, choline chloride, lauryltrimethylammonium bromide, phenyltrimethylammonium bromide, (±)-propranolol hydrochloride, tetrabutylammonium bromide and tetramethylammonium bromide were purchased from Nakalai Tesque, Inc. (Kyoto, Japan). Tetraethylammonium chloride and chlorpromazine hydrochloride were obtained from Wako Pure Chemical Ind. (Osaka, Japan). Carbamazepine, chloroquine diphosphate, desipramine hydrochloride and imipramine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylamine hydrochloride, diethylamine hydrochloride and triethylamine hydrochloride were from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Ambenonium chloride (Nihon Shoji, Osaka, Japan), *N*-didesmethylimipramine hydrochloride (Ciba Geigy Japan, Takarazuka, Japan) and mepenzolate bromide (Fujisawa Pharmaceutical Co., Tokyo, Japan) were kindly donated. Methylchlorpromazine iodide, methylimipramine iodide, hexyltrimethylammonium iodide, heptyltrimethylammonium iodide and octyltrimethylammonium iodide were synthesized in our laboratory from the corresponding tertiary amines and methyl iodide using the method of Huang et al (1970). Nonyltrimethylammonium bromide, decyltrimethylammonium bromide and undecyltrimethylammonium bromide were synthesized from the corresponding n-alkylbromide and trimethylamine. The purities of synthesized quaternary amines were ascertained by elemental analysis and ^{1}H-NMR (100 MHz).

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Isolation of the brush border membrane from rat small intestine

Adult male Wistar rats, 250–300 g, were used. Entire small intestine was excised under anaesthesia with pentobarbitone sodium (3 mg/100 g, i.p.) from 3 to 5 rats. Brush border membranes were isolated using the calcium chloride precipitation method of Kessler et al (1978) as described previously (Iseki et al 1989).

Determination of organic cation binding to brush border membrane

The drugs were dissolved in 20 mM 2-(*N*-morpholino) ethanesulphonic acid (MES)/Tris (pH 6·5) containing 100 mM D-mannitol. Drug solution (800 μL) was mixed with 200 μL of membrane suspension, and the mixture incubated at

25°C for 30 min. The mixture was then centrifuged at 29 000 *g* for 20 min and 0·5 mL of supernatant fluid used for analysis. All drugs were assayed by HPLC. 1 mL of 5 mM sodium lauryl sulphate for quaternary amines or 1 mL 1 M NaOH for other organic cations, and 5 mL of chloroform were added to the supernatant, the mixture was then shaken for 15 min and centrifuged at 1500 *g* for 10 min. Three mL of the organic layer was taken and dried under vacuum. The residue was reconstituted with 0·5 mL of methanol containing internal standard (n-butyl *p*-aminobenzoate or *N,N*-diphenyl-*p*-phenylenediamine), and a sample of the solution was injected into the HPLC system.

A liquid chromatograph (Hitachi 638-50) equipped with a high pressure sampling valve (Hitachi 638-0801, 1–150 μL) was used (stationary phase; reversed-phase column (Hitachi gel #3053, 4 mm i.d. × 25 cm) at 55°C; mobile phase 0·05 M KH_2PO_4 adjusted to pH 2·5 with phosphoric acid-acetonitrile (38:62); flow rate 0·7 mL min^{-1}; variable wavelength UV monitor (Hitachi 638-41) at 250 nm and 0·04 aufs.) Binding was calculated from the difference between the initial amount of drug and that found in the supernatant fluid. Data were represented as total binding. Protein concentrations were determined by the method of Lowry et al (1951) with bovine serum albumin as standard.

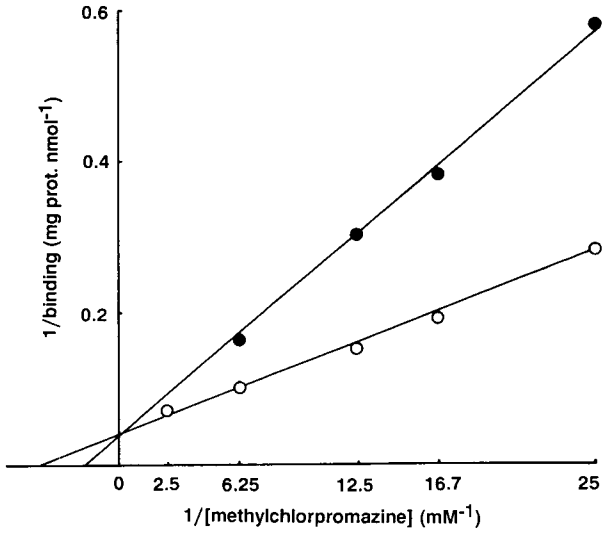

FIG. 2. Lineweaver-Burk plots of methylchlorpromazine in the presence of lauryltrimethylammonium. The concentration of methylchlorpromazine was 40–400 μM and that of lauryltrimethylammonium was 400 μM. Results are expressed as the means of 2 measurements using same membrane preparations. Symbols: ○ control; ● in the presence of lauryltrimethylammonium.

Results

Quaternary ammonium compound binding to rat small intestinal brush border membrane and its dependency on hydrocarbon-chain length

Fig. 1 shows the effect of quaternary ammonium compounds on methylchlorpromazine binding to rat small intestinal brush border membrane. Tetramethylammonium, tetraethylammonium, phenyltrimethylammonium and choline did not change methylchlorpromazine binding even at 800 μM. By contrast, lauryltrimethylammonium and cetyltrimethylammonium both decreased methylchlorpromazine binding in a dose-dependent manner, the cetyltrimethylammonium inhibiting methylchlorpromazine binding almost completely at 800 μM. Fig. 2 shows Lineweaver-Burk plots of methylchlorpromazine binding in the presence and absence of lauryltrimethylammonium. The lauryltrimethylammonium inhibited methylchlorpromazine competitively. The relationship between the length of unbranched hydrocarbons and effects on methylchlorpromazine binding is illustrated in Fig. 3. There was no inhibitory effect on methylchlorpromazine binding up to C6 (hexyltrimethylammonium), but from C7 (heptyltrimethylammonium) inhibitory effects clearly increased with each extension of the unbranched hydrocarbon chain up to C16 (cetyltrimethylammonium).

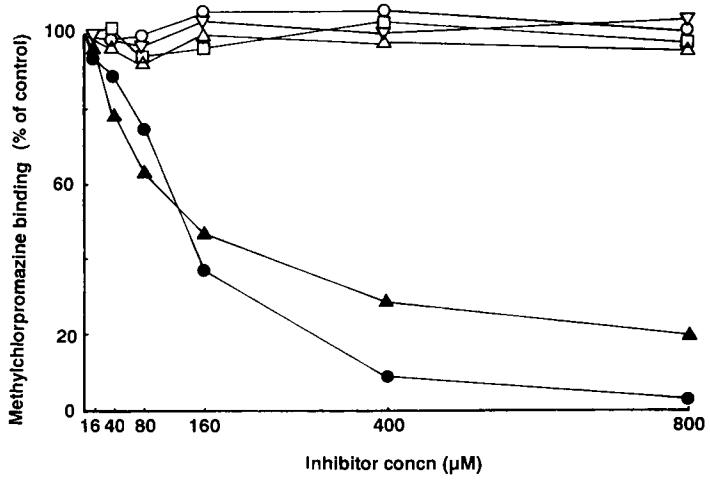

FIG. 1. Effect of six quaternary ammonium compounds on methylchlorpromazine binding to rat small intestinal brush border membrane. The concentration of methylchlorpromazine was 16 μM. Results are expressed as the means, each from 3 different membrane preparations. Symbols: △ tetramethylammonium; □ tetraethylammonium; ○ phenyltrimethylammonium; ▽ choline; ▲ lauryltrimethylammonium; ● cetyltrimethylammonium.

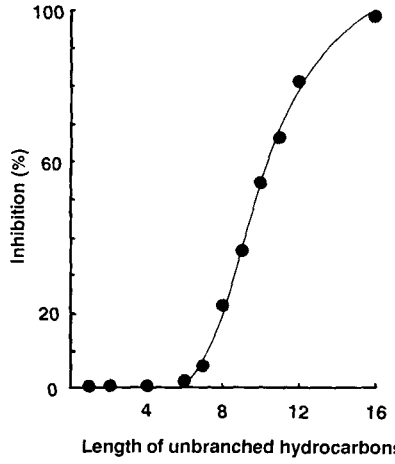

FIG. 3. Relationship between the length of unbranched hydrocarbons of eleven quaternary ammonium compounds on methylchlorpromazine binding to rat small intestinal brush border membrane. The concentration of methylchlorpromazine was 16 μM and that of inhibitors was 800 μM. Results are expressed as the means, each from 3 different membrane preparations.

Effect of hydrophilic structures on the binding of organic cations to rat small intestinal brush border membrane

Fig. 4 shows the binding properties of four imipramine-related compounds with different hydrophilic structures. The order of binding was *N*-didesmethylimipramine (primary amine) > desipramine (secondary amine) > imipramine (tertiary amine) > methylimipramine (quaternary). The lipid solubilities of these imipramine-related compounds were examined in an experimental buffer-chloroform system (Table 1). There was no relationship between the lipid solubilities of these imipramine-related compounds and their binding to the brush border membrane.

These imipramine-related compounds inhibited methylchlorpromazine binding roughly in proportion to their binding properties at 800 μM (Fig. 5). Also, imipramine binding was mutually inhibited by related compounds and methylchlorpromazine (Fig. 6). The results presented in Figs 5 and 6 suggest that these organic cations are bound to common binding sites.

In contrast to these results, the inhibitory effect of all ethylamine-related compounds (ethylamine, diethylamine, triethylamine and tetraethylammonium) was negligible (< 3%, binding, nmol (mg protein)$^{-1}$ 9·6 ± 0·4). These

Table 1. Partition of imipramine-related compounds between chloroform and experimental buffer.

Compound	% dissolved in $CHCl_3$
N-Didesmethylimipramine	89·6
Desipramine	93·5
Imipramine	100
Methylimipramine	6·2

Drugs (40 μM) were dissolved in 20 mM MES/Tris (pH 6·5) containing 100 mM D-mannitol. Partition was measured by shaking aqueous drug solution with equal volume of water-saturated chloroform at 25°C for 1 h. Values represent the mean of two experiments.

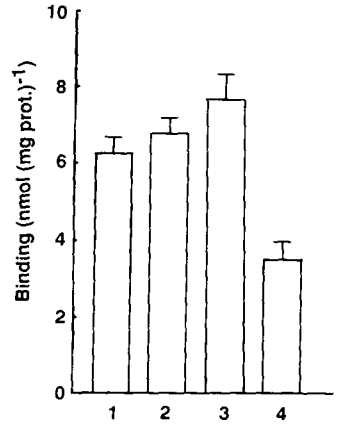

FIG. 4. Binding of four imipramine-related compounds to rat small intestinal brush border membrane. The concentration of each drug was 16 μM. Results are expressed as the means, each from 3 different membrane preparations. 1 imipramine; 2 desipramine; 3 *N*-didesmethylimipramine; 4 methylimipramine.

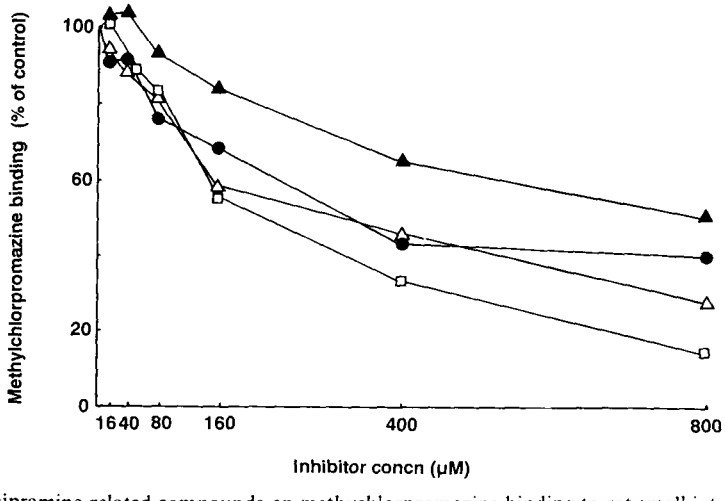

FIG. 5. Effect of imipramine-related compounds on methychlorpromazine binding to rat small intestinal brush border membrane. The concentration of methylchlorpromazine was 16 μM. Results are expressed as the means, each from 3 different membrane preparations. ● imipramine; △ desipramine; □ *N*-didesmethylimipramine; ▲ methylimipramine.

Table 2. Effect of various organic cations on methylchlorpromazine binding to rat small intestinal brush border membrane.

Inhibitor	% inhibition
Chlorpromazine	99·9±0·14
Propranolol	67·8±4·17
Chloroquine	69·7±4·16
Mepenzolate	36·6±2·55
Ambenonium	28·5±3·43
Carbamazepine	18·0±5·43

The concentration of methylchlorpromazine was 16 μM and that of inhibitor was 800 μM. Values represent the mean±s.d. from measurements on 3 different membrane preparations.

compounds possess much smaller hydrophobic components than the imipramine-related drugs. Thus, the effect of hydrophilic structures is apparently negligible in the case of small molecular organic cations.

Inhibitory effect of various organic cations on methylchlorpromazine binding to rat small intestinal brush border membrane

Table 2 shows the inhibitory effect of various organic cations on methylchlorpromazine binding. Chlorpromazine, a parent compound of methylchlorpromazine, inhibited the binding of its quaternary derivative completely, the strength of inhibition being comparable to that of cetyltrimethylammonium (Fig. 1). Dicyclic cations, propranolol and chloroquine, markedly inhibited methylchlorpromazine binding. The mono-quaternary ammonium compound, mepenzolate slightly inhibited binding. Ambenonium, having two quaternary nitrogen atoms, one at each end of the molecule, exhibited a weak inhibitory effect. Although carbamazepine has a tricyclic structure similar to imipramine-related compounds, its inhibitory effect was extremely low in comparison.

Fig. 7 shows the relationship between the binding properties of various organic cations tested in this study and their inhibitory effect on methylchlorpromazine binding. There is a good correlation (r=0·947). We therefore suggest that all

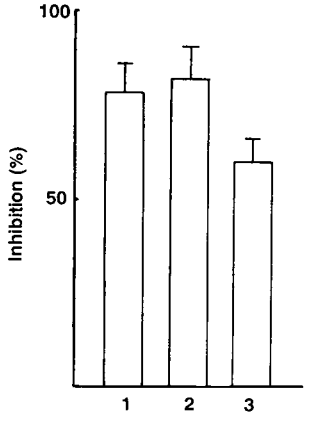

FIG. 6. Effect of three organic cations on imipramine binding to rat small intestinal brush border membrane. The concentration of imipramine was 16 μM and that of inhibitors was 800 μM. Results are expressed as the means±s.d., each from 3 different membrane preparations. Key: 1, methylchlorpromazine; 2, *N*-didesmethylimipramine; 3, methylimipramine.

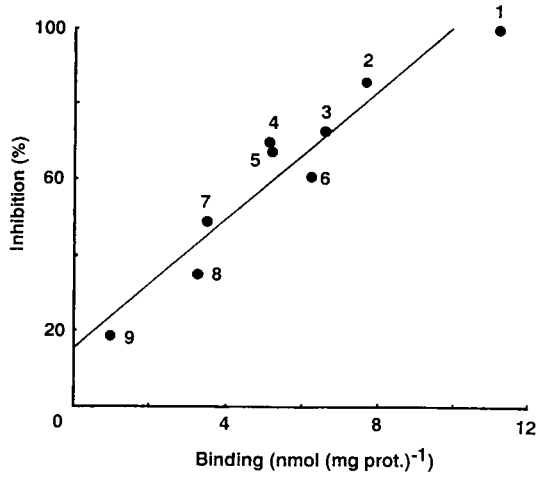

FIG. 7. Relationship between binding behaviours of various organic cations and their inhibitory effect on methylchlorpromazine binding. Binding of organic cations was determined at 16 μM. Inhibitory effect of organic cations to methylchlorpromazine binding was determined at 16 μM for methylchlorpromazine and 800 μM for inhibitors. Line was determined by linear regression analysis (r=0·947). Key: 1, chlorpromazine; 2, *N*-didesmethylimipramine; 3, desipramine; 4, chloroquine; 5, propranolol; 6, imipramine; 7, methylimipramine; 8, mepenzolate; 9, carbamazepine.

organic cations tested in this study bind to a common site on rat small intestinal brush border membrane.

Discussion

We (Saitoh et al 1987, 1988a) have already presented two consecutive processes as one possible absorption mechanism in the case of propantheline, an anti-acetylcholine quaternary amine. In the first absorption process propantheline binds rapidly to brush border membrane. It then enters the epithelium, stimulated by the transmembrane electrical potential difference (cell interior negative). We have also shown that chlorpromazine, a well absorbed cationic tertiary amine, could be transported through the brush border membrane by the same mechanism (Saitoh et al 1989a). We have found that methylchlorpromazine, a quaternary derivative of chlorpromazine, is also transported by the absorption mechanism (Saitoh et al 1989c). Therefore, the binding properties of these organic cations should be examined in more detail. Many organic cations administered orally, including chlorpromazine and propantheline, have both hydrophobic and hydrophilic components. In this study, we have demonstrated that both hydrophobic and hydrophilic components are significant factors in the determination of binding properties in rat small intestinal brush border membrane. Tetramethylammonium, tetraethylammonium and choline are absorbed through the rat small intestine by a common carrier-mediated transport system (Tsubaki & Komai 1986). In the present study, these compounds did not bind to brush border membrane sufficiently to inhibit methylchlorpromazine binding. It is suggested that this lack of interaction at binding sites is due to their small hydrophobic components. Apparently, the molecular size has to be larger than n-heptyl (C7) for interaction with the binding

sites (Fig. 3). It is therefore reasonable to suggest that the binding site involved in this study is different from the choline and tetramethylammonium carrier system.

The binding of organic cations was saturable and inhibited by structurally related organic cations as shown previously by Saitoh et al (1988b). The question therefore arises whether these binding sites belong to a separate carrier on brush border membrane for absorption of organic cations possessing a relatively large chemical structure. There has been much interest in biology and physiology in the interaction between cationic amphiphilic drugs and membranes. Several reports (Lullmann & Wehling 1979; Bondy & Remien 1981; Surewicz & Leyko 1981; Kubo et al 1986; Joshi et al 1988) suggest that both electrostatic and hydrophobic interactions are involved in binding of cationic amphiphiles to phospholipid vesicles, but the mechanism of binding is not yet well understood. Although the details of the binding sites on the brush border membrane remain unclear, we have recently reported that propantheline binding to the brush border membrane was significantly decreased when the membrane was pretreated with neuraminidase and that the binding capacity of liposomes for propantheline and methylchlorpromazine was significantly increased when GM_3 ganglioside (isolated from rat small intestinal brush border membrane) was incorporated (Saitoh et al 1989b). This suggests that sialic acid plays a role in the binding of these organic cations to the membrane.

It is clear that differences in hydrophilic structures can influence binding properties (Fig. 4). However, this might be a secondary effect dependent on the size of hydrophobic components, because in small molecular ethylamine-related compounds differences in hydrophilicity had no effect on binding. The binding of imipramine-related compounds decreased with incorporation of methyl groups around the nitrogen atom; i.e. the order of binding being $-NH_2 > -NHCH_3 > -N(CH_3)_2 > -N^+(CH_3)_3$. This could be due to the decrease of electrostatic interaction with binding sites by steric hindrance.

It is interesting that ambenonium, having two quaternary nitrogen atoms, one at each end of the molecule, exhibited an inhibitory effect on methylchlorpromazine binding. Chung et al (1985) have proposed that polycations such as spermine lie flat on the surface of the vesicles of acidic phospholipids. Although the binding mechanism of ambenonium is unclear, it might also behave like spermine, on the outer surface of the brush border membrane, and consequently inhibit methylchlorpromazine binding indirectly.

Apart from carbamazepine, which is non-ionizable, all organic cations tested would ionize in our experimental conditions (pH 6·5). Perhaps the organic cations possessing amphiphilic structures bind to common sites on rat small intestinal brush border membrane. As previously mentioned, this binding would be an indispensable process in absorption from intestine. Moe et al (1988) investigated a weak base binding phenomenon in pig intestinal brush border membrane and indicated that binding involved predominantly the non-ionized form of benzylamine. It would be interesting to determine whether ionization is necessary by using other cationic drugs.

In conclusion, it has been shown that both hydrophobic and hydrophilic structures of various organic cations are significant determinants of binding to rat small intestinal brush border membrane. A molecular size larger than n-heptyl seems to be necessary for hydrophobic structures to interact with the binding sites on the membrane. The effect of the hydrophilic structure might be a secondary action dependent on the size of the hydrophobic one. Organic cations possessing amphiphilic structures bind to the common binding sites on the brush border membrane through both hydrophobic and/or hydrophilic interactions.

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