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Transport Characteristics of Propantheline Across Rat Intestinal Brush Border Membrane

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Abstract—The transport mechanism of propantheline, an anti-acetylcholine quaternary ammonium compound, has been studied using brush border membrane vesicles isolated from rat small intestine. The uptake of propantheline was facilitated by the transmembrane electrical potential difference (cell interior negative) induced by NaSCN, NaI or valinomycin. But this effect was a secondary action; in the initial phase of propantheline uptake (< 5 min), there was no facilitating effect. When the transmembrane potential difference was induced after propantheline uptake had reached a steady state, there was an overshoot of the drug. Therefore, it is suggested that the transport of propantheline across the brush border membrane consists of at least two processes. In the first, propantheline rapidly binds to the brush border membrane, in the second it enters into epithelium driven by the negative transmembrane electrical potential difference. Cationic tertiary amines such as chlorpromazine, imipramine and promethazine markedly inhibited propantheline uptake. These results suggest that there is a common absorption process for tertiary amines and quaternary ammonium compounds.

Although quaternary ammonium compounds (QACs) are completely ionized over the pH range in the gastrointestinal tract and generally have poor lipid solubility, it has been reported that they are absorbed rapidly in the initial phase after dosing (Levine 1966; Levine et al 1955; Turnheim & Lauterbach 1980). Therefore, it has been held that specialized transport mechanisms could contribute to this process (Levine 1966; Irwin et al 1969; Ruifrok & Mol 1983; Tsubaki & Komai 1986). However, the details of the absorption mechanism of QACs remain unclear.

Using rat intestinal brush border membrane vesicles, we have shown that propantheline, an anti-acetylcholine QAC, is highly bound to the membrane (Saitoh et al 1987). This binding was saturable, suggesting the presence of a specific binding site. The binding of propantheline was inhibited to varying extents by several QACs such as mepenzolate, methylbenactyzine and butylhyoscine. Although it is impossible to explain the initial rapid absorption directly by this binding property alone, it may be one of a succession of processes of transport through the intestinal epithelium.

It is well known (Rose & Schultz 1971; White & Armstrong 1971) that there is an electrical potential difference across the intestinal brush border membrane (ca 40–60 mV, cell interior negative). It is, therefore, probable that this electrical potential difference stimulates the permeation of QACs, which possess a positive charge, across the brush border membrane. Previously, Ruifrok (1981) reported that transport of *N*-methyldeptropine, a QAC, across the brush border membrane was a passive process and was stimulated by the transmembrane electrical potential difference. But *N*-methyldeptropine is much more lipophilic than many other QACs. Therefore, it is considered questionable whether the transmembrane electrical potential difference directly induces the transfer of less lipophilic QACs from the lumen into the epithelium, because the brush border membrane acts as a barrier.

We have investigated the effect of the transmembrane electrical potential difference on propantheline uptake by rat intestinal brush border membrane vesicles.

Moreover, if the ionized QAC binds to a specific site on the brush border membrane, cationic tertiary amines, which possess a similar chemical structure to propantheline, and are mostly ionized over the pH range in the gastrointestinal tract, may bind to the same site to inhibit propantheline uptake. So we have also studied the effect of tertiary amines such as chlorpromazine, imipramine, promethazine and diphenhydramine on propantheline uptake.

The results suggested that both rapid binding to the brush border membrane and transmembrane electrical potential difference across it might contribute to the permeation of QACs such as propantheline. And all tertiary amines tested in this study exhibited strong inhibition of propantheline uptake by brush border membrane vesicles.

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Materials and Methods

Materials

Propantheline bromide, chlorpromazine hydrochloride, imipramine hydrochloride, promethazine hydrochloride and valinomycin were purchased from Sigma Chemical Co. (St. Louis, MO., USA). Diphenhydramine hydrochloride was obtained from Nakarai Chemicals Ltd. (Kyoto, Japan). L-Lysine was obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Methylbenactyzine bromide (Yamanouchi Pharmaceutical Co., Tokyo, Japan) was kindly donated. All other reagents were of the highest grade available commercially and used without further purification.

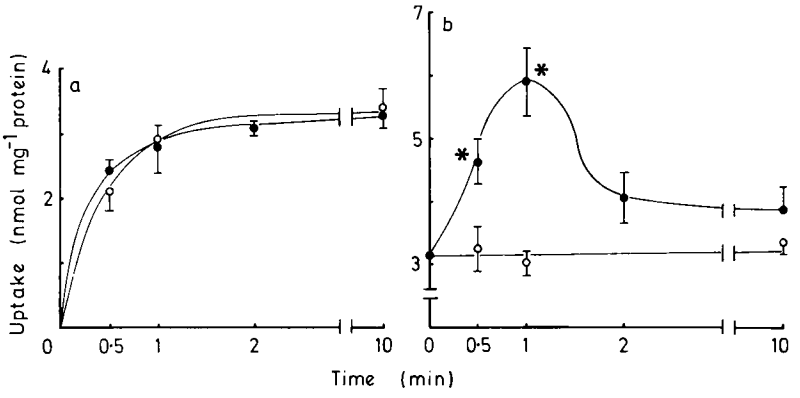

FIG. 1 Effect of NaSCN and Na gluconate gradient on propantheline uptake by brush border membrane vesicles. Membrane vesicles were suspended in buffer A. (a) Vesicle suspension (100 μL) was incubated with buffer A (100 μL) containing 5 mM propantheline bromide, 200 mM NaSCN (●) or Na gluconate (○) (pH 7·5) at 25°C. (b) Vesicle suspension (100 μL) was preincubated with buffer A (100 μL) containing 5 mM propantheline bromide at 25°C for 10 min, and then the mixture (200 μL) was incubated with buffer A (100 μL) containing 2·5 mM propantheline bromide and 300 mM NaSCN (●) or Na gluconate (○) at 25°C. Time 0 represents NaSCN or Na gluconate addition. Each point represents the means ±s.d. of five to seven measurements with different preparations of vesicles. *$P < 0.05$, significantly different from Na gluconate addition.

Preparation of brush border membrane vesicles

Brush border membrane vesicles were isolated from the entire small intestine of male Wistar rats (250–300 g) according to the calcium chloride precipitation technique of Kessler et al (1978). The membrane vesicles were suspended in a final concentration of 3–4 mg protein mL^{-1} with 100 mM D-mannitol, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes)/Tris, pH 7·5 (buffer A) or 100 mM D-mannitol, 20 mM 2-(*N*-morpholino) ethanesulphonic acid (Mes)/Tris, pH 6·5 (buffer B). The purity of the membrane was routinely evaluated by the enrichment of alkaline phosphatase (E.C.3.1.3.1.), an enzyme specific to the intestinal brush border membrane. The specific activity of this enzyme increased 12-fold in the final membrane suspension compared with concentrations found in the homogenate of intestinal scrapings. The membrane vesicles were equilibrated for 1 h at 0°C before use.

Uptake experiments

The uptake of a substrate into the isolated membrane vesicles was measured by a rapid filtration technique using 0·45 μm Millipore membrane filters (HAWP 02500). The specific conditions for each experiment are given in the Figure legends. All experiments were carried out at 25°C. At a stated time the reaction was stopped by dilution of the incubated sample by addition of 10 mL of ice-cold buffer (150 mM NaCl, 1 mM Tris/HCl, pH 7·5) to the reaction tube followed by rapid filtration through a membrane filter. The filter was washed once with 15 mL of the same ice-cold buffer. Non-specific adsorption of propantheline onto the Millipore filter was determined using the incubation medium instead of vesicle suspension. This value was subtracted from the uptake data.

Analytical methods

Propantheline was analysed by high performance liquid chromatography as described previously (Saitoh et al 1987). Protein was determined by the method of Lowry et al (1951) with bovine serum albumin as standard.

Results and Discussion

Effect of transmembrane electrical potential difference (inside negative) on propantheline uptake by brush border membrane vesicles

SCN^- and I^- are lipophilic anions and permeate the intestinal brush border membrane much faster than Na^+ (Gunther et al 1984). So when NaSCN or NaI is added to the extravesicular medium, the transmembrane electrical potential difference (cell interior negative) is rapidly induced (Kessler & Semenza 1979). On the other hand, when Na gluconate is added to the extravesicular medium, no such potential difference is induced, since gluconate ion is less

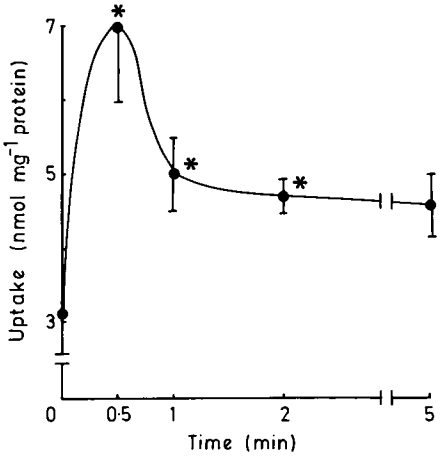

FIG. 2 Effect of valinomycin-induced potassium diffusion potential difference on propantheline uptake by brush border membrane vesicles. Membrane vesicles were suspended in buffer A containing 100 mM K gluconate. Vesicle suspension (100 μL) was preincubated with buffer A (100 μL) containing 100 mM K gluconate and 5 mM propantheline bromide at 25°C for 10 min, and then the mixture (200 μL) was incubated with buffer A (100 μL) containing valinomycin (5 μg). Each point represents the means ±s.d. of at least three measurements from a typical experiment. *$P < 0.05$, significantly different from time 0.

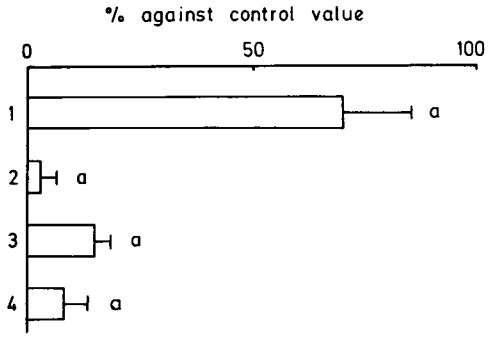

FIG. 3 Effect of several tertiary amines on propantheline uptake by brush border membrane vesicles. Membrane vesicles were suspended in buffer B. Vesicle suspension (100 μL) was preincubated with buffer B (100 μL) containing 10 mM tertiary amine at 25°C for 3 min, and then the mixture (200 μL) was incubated with buffer B (100 μL) containing 5 mM propantheline bromide at 25°C for 10 min. Results are expressed as percent against the control value obtained from experiments without tertiary amines. Absolute control value for propantheline uptake was 3·1 ± 0·52 nmol (mg protein)$^{-1}$. Each column represents the mean ± s.d. of 6–11 measurements with different preparations of vesicles. (a) $P < 0{\cdot}001$, significantly different from control. Key: 1,diphenhydramine; 2, chlorpromazine; 3, imipramine; 4, promethazine.

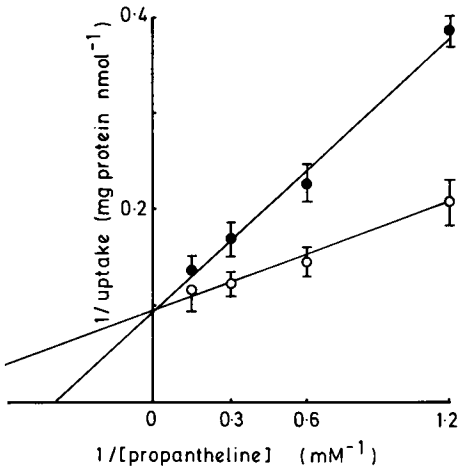

FIG. 4 Lineweaver-Burk plots of propantheline uptake by brush border membrane vesicles in the presence of methyl-benactyzine. Membrane vesicles were suspended in buffer A. Vesicle suspension (100 μL) was preincubated with buffer A (100 μL) containing 10 mM methylbenactyzine bromide (●) at 25°C for 3 min, and then the mixture (200 μL) was incubated with buffer A (100 μL) containing various concentration of propantheline bromide at 25°C for 10 min. In the control experiment (○), vesicle suspension (100 μL) was preincubated with buffer A (100 μL) not containing methylbenactyzine. Each point represents the mean ± s.d. of three measurements from a typical experiment. The lines were drawn by the least square method.

permeable than Na^+. We have investigated the effect of the transmembrane electrical potential difference on propantheline uptake by using these salts.

In one experiment, we added either NaSCN or Na gluconate to a vesicle suspension containing propantheline. As shown in Fig. 1a, there was no difference in the uptake of propantheline in the presence of either salt.

Since we showed that propantheline was rapidly bound to rat intestinal brush border membrane (Saitoh et al 1987), it seemed that the effect of the electrical potential difference on propantheline uptake was negated by this binding in the initial phase of propantheline uptake. So, in another experiment, we first saturated the binding of propantheline to the brush border membrane by incubating vesicles for 10 min with propantheline in the absence of NaSCN or Na gluconate. And then NaSCN or Na gluconate was added to the extravesicular medium. As shown in Fig. 1b, with Na gluconate addition, the uptake of propantheline was not altered. But an overshoot was observed on addition of NaSCN. The values at 0·5 and 1 min after NaSCN addition were significantly increased compared with Na gluconate addition. It was obvious that this overshoot was substantially different from those found in the Na^+-cotransport system of D-glucose (Murer & Hopfer 1974; Kessler et al 1978) or some amino acids (Sigrist-Nelson et al 1975; Stevens et al 1982; Berteloot 1984), since the Na^+ gradient (outside to inside) existed when Na gluconate was added. A similar overshoot was observed when NaI was added to the extravesicular medium after preloading propantheline for 10 min (data not shown).

The effect of the transmembrane electrical potential difference on propantheline uptake was also examined by adding valinomycin, potassium ionophore, to potassium-preloaded vesicles. As shown in Fig. 2, overshoot was observed when valinomycin was added to extravesicular medium after preloading of propantheline for 10 min. The values of 0·5, 1 and 2 min after valinomycin addition were significantly increased compared with the value at time 0. But when valinomycin and propantheline were added simultaneously, overshoot was not seen in the time course of propantheline uptake (data not shown). These results were consistent with those obtained from the experiment using NaSCN (Fig. 1).

From the above results, it was suggested that the effect of the transmembrane electrical potential difference on propantheline uptake was not a direct but a secondary action. As one absorption mechanism of a QAC like propantheline, the following was considered: firstly the rapid binding to the brush border membrane would take place and thereafter the QAC would enter into the epithelium stimulated by the transmembrane electrical potential difference (interior negative). Our present results are partly consistent with those of Ruifrok (1981) in that the transmembrane potential difference stimulates the transport of the QAC across brush border membrane, but he made no mention of rapid binding to the brush border membrane.

Effect of several tertiary amines on propantheline uptake by brush border membrane vesicles

Tertiary amines such as chlorpromazine, promethazine, imipramine and diphenhydramine are mostly ionized over the pH range in the gastrointestinal tract, since their pK_a values are above 9 (Green 1967; deRoos et al 1970). But these drugs are efficiently absorbed after oral administration (Gram & Christiansen 1975; Taylor & Houston 1983; Curry et al 1971) and their chemical structures are similar to that of propantheline. We previously reported (Saitoh et al 1987) that several quaternary ammonium compounds had an inhibitory effect to varying extents on the equilibrium uptake of propantheline. To clarify whether the binding property of

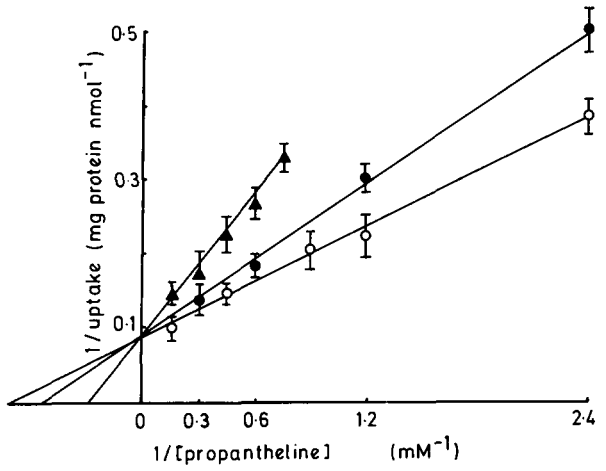

FIG. 5 Lineweaver-Burk plots of propantheline uptake by brush border membrane vesicles in the presence of chlorpromazine and diphenhydramine. Membrane vesicles were suspended in buffer B. Vesicle suspension (100 μL) was preincubated with buffer B (100 μL) containing 1 mM chlorpromazine hydrochloride (▲) or 10 mM diphenhydramine hydrochloride (●) at 25°C for 3 min, and then the mixture (200 μL) was incubated with buffer B (100 μL) containing various concentration of propantheline bromide at 25°C for 10 min. In the control experiment (○), vesicle suspension (100 μL) was preincubated with buffer B (100 μL) not containing tertiary amines. Each point represents the mean ± s.d. of three measurements with different preparations of vesicles. The lines were drawn by the least square method.

propantheline was common to these tertiary amines, the effect of tertiary amines on propantheline uptake was examined. A high concentration of tertiary amines (10 mM) did not dissolve in buffer A (pH 7·5) completely, so we used buffer B (pH 6·5) and confirmed that the equilibrium value of propantheline uptake obtained therefrom did not change compared with that obtained from buffer A. As shown in Fig. 3, the equilibrium uptake of propantheline was significantly decreased when the vesicles were preincubated in the medium containing these tertiary amines. The inhibitory effect of chlorpromazine, imipramine and promethazine was much greater than that of diphenhydramine. We previously showed that equilibrium uptake of propantheline did not alter under the various medium osmolarities (Saitoh et al 1987). Therefore, it was obvious that the decrease of propantheline uptake on addition of the tertiary amines was not due to the increase of medium osmolarity.

To further elucidate the inhibitory effect, kinetic experiments were carried out. Fig. 4 shows the Lineweaver-Burk plots of propantheline uptake in the presence of methylbenactyzine, an anti-acetylcholine QAC that exhibited a competitive type of inhibition as did the results for chlorpromazine and diphenhydramine (Fig. 5). From these results, it is reasonable to postulate that propantheline and these amines bind to the same site of the brush border membrane in their absorption process, since they have similar structures.

Cassano et al (1983) reported that basic amino acid, L-lysine was highly bound to the inner surface of rat intestinal brush border membrane vesicles. But, as shown in Fig. 6, L-lysine exhibited no inhibitory effect on propantheline uptake. Therefore, it seems that binding process of propantheline differs from that of L-lysine.

In conclusion, it was suggested that there might be at least two processes in the transport of QACs, like propantheline across the brush border membrane. Initially the QAC binds rapidly to brush border membrane and then enters into the epithelium stimulated by transmembrane electrical potential difference (cell interior negative). The transport of the QAC across the brush border membrane might be partly shared with that of cationic tertiary amines such as chlorpromazine, imipramine, promethazine and diphenhydramine. In the present study, the interaction of a QAC and tertiary amines has been shown at the binding step to brush border membrane. Whether a QAC and tertiary amines interact during the subsequent entry phase has got to be established.

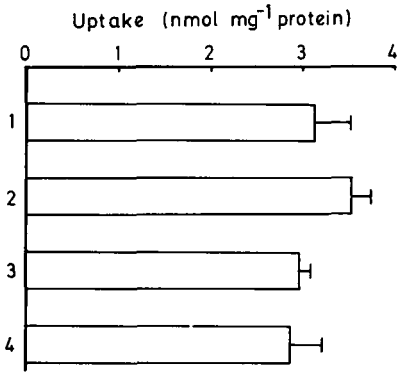

FIG. 6 Effect of L-lysine on propantheline uptake by brush border membrane vesicles. Membrane vesicles were suspended in buffer A. The vesicle suspension (100 μL) was preincubated with (100 μL) containing 10 mM (Key 2), 20 mM (Key 3) and 40 mM (Key 4) L-lysine at 25°C for 3 min, and then the mixture (200 μL) was incubated with buffer A (100 μL) containing 5 mM propantheline bromide at 25°C for 10 min. In the control experiment (Key 1), vesicle suspension (100 μL) was preincubated with buffer A (100 μL) not containing L-lysine. Each column represents the mean ± s.d. of at least three measurements with different preparations of vesicles.

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