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Transport characteristics of [^{3}H]-chlorpromazine across rat small intestinal brush border membrane

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Abstract—The transport mechanism of chlorpromazine, a tertiary amine, has been investigated using brush border membrane vesicles isolated from rat small intestine. Chlorpromazine was taken up rapidly by the vesicles the uptake being mainly due to binding to the membrane. The transport of chlorpromazine into the intravesicular space was facilitated by the transmembrane electrical potential difference (inside negative) induced by valinomycin or sodium thiocyanate. This facilitating effect was observed only when the transmembrane electrical potential difference was induced after chlorpromazine uptake had reached a steady state. In the initial phase of chlorpromazine uptake, there was no effect. Therefore, it is suggested that both rapid binding to brush border membrane and transmembrane electrical potential difference (inside negative) across the membrane plays a significant role in the transport processes of chlorpromazine through the intestinal epithelium.

Chlorpromazine, a tertiary amine compound (TAC) used orally as a potent major tranquilizer, is a cationic amphiphilic drug possessing both a hydrophobic side chain and a hydrophilic residue in its structure. It has been shown that chlorpromazine binds to liposomes and biological membranes such as erythrocytes (Sheetz & Singer 1974; Zachowski & Durand 1988). Although it is mostly ionized over the pH range in the gastrointestinal tract (Green 1967), it is well known that its absorption is rapid (Curry et al 1971). This phenomenon is common for other TACs such as promethazine, imipramine and diphenhydramine. But there are few reports on the transport mechanism of these TACs across brush border membrane.

In our previous reports dealing with the transport mechanism of quaternary ammonium compounds (QACs) across the brush border membrane isolated from rat small intestine, we have shown that there might be at least two processes in the transport of propantheline, an anti-acetylcholine QAC, across the membrane (Saitoh et al 1987, 1988a). Initially, propantheline binds rapidly to brush border membrane and then enters into the epithelium stimulated by the transmembrane electrical potential difference (inside negative). Moreover, we have indicated that the binding of propantheline to the brush border membrane is competitively inhibited by several TACs such as chlorpromazine. Therefore, it is suggested that there is a common transport mechanism across brush border membrane between TACs and QACs.

As one approach to clarify the absorption mechanism of TACs, we have examined the transport characteristics of chlorpromazine across the brush border membrane.

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Materials and methods

Chlorpromazine hydrochloride and valinomycin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [^{3}H]-Chlorpromazine hydrochloride (27·1 Ci mmol^{-1}) was obtained from New England Nuclear Co. (Boston, MA, USA). All other chemicals were of the highest grade available commercially and were used without further purifications.

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 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 about 12-fold in the final membrane suspension compared with concentrations found in the homogenate of initial scrapings. The membrane vesicles were equilibrated for 1 h at 0°C before use.

The uptake of chlorpromazine into the isolated membrane vesicles were measured by a rapid filtration technique using Toyo GC-50 glass fibre filters (0·5 μm, Toyo Roshi Co., Tokyo, Japan). The filters were prewetted with 2 mL of the ice-cold buffer (1 mM Tris/HCl, pH 6·5, containing 150 mM NaCl) containing 1 mM non-labelled chlorpromazine before use. The specific conditions for each experiment are given in the Figure legends. All experiments were carried out at 25°C. At stated times, the reaction was stopped by dilution of an incubation sample with 3 mL of the same ice-cold buffer followed by rapid filtration through a glass fibre filter. The filter was washed once with 5 mL of the same ice-cold buffer. Nonspecific adsorption of chlorpromazine onto the filter was determined using the incubation medium instead of vesicle suspension. This value was subtracted from uptake data.

Uptake was determined by counting radioactivity in brush border membrane vesicles trapped on the filter. Protein concentration was determined by the method of Lowry et al (1951) with bovine serum albumin as standard. Results were expressed as nmol chlorpromazine taken up per mg of membrane protein.

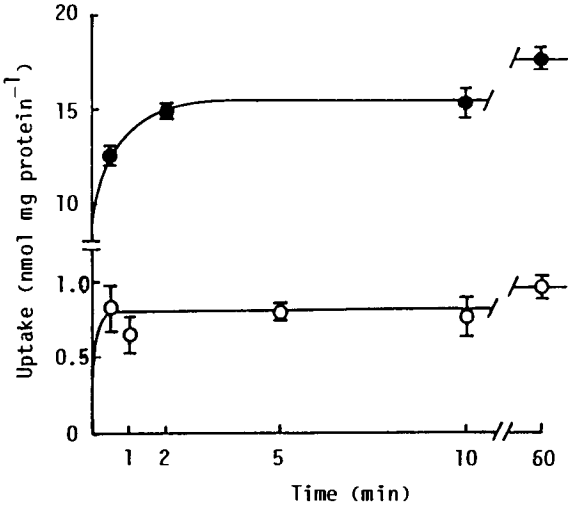

FIG. 1. Time course of chlorpromazine uptake by rat small intestinal brush border membrane vesicles. Vesicles were suspended in 20 mM 2-(N-morpholino)-ethanesulfonic acid (Mes)/Tris, pH 6·5, containing 100 mM D-mannitol (buffer A). 50 μL of vesicles suspension was incubated at 25°C with 100 μL of buffer A containing 50 μM (○) or 1 mM (●) chlorpromazine. Each point represents the mean ± s.e.m. of three to five measurements with different preparations of vesicles.

Results and discussion

Fig. 1 shows the time course of chlorpromazine uptake at two different concentrations. Chlorpromazine was taken up very rapidly by brush border membrane vesicles at both concentrations.

To distinguish between binding to brush border membrane and transport into the intravesicular space, the effect of extravesicular medium osmolarity on chlorpromazine uptake was investigated using D-cellobiose. Normally, the equilibrium uptake is decreased in inverse proportion to increase of extravesicular medium osmolarity. For chlorpromazine, the equilibrium uptake did not alter under the various medium osmolarities.

Therefore, it was suggested that chlorpromazine uptake by brush border membrane vesicles was mainly due to the binding to the membrane. This high binding property may explain the rapid attainment of a steady state. The results herein presented are consistent with our previous observation in that TACs such as chlorpromazine are highly bound to the brush border membrane. Moreover, we found that chlorpromazine possessed two types of binding site on the membrane (Saitoh et al 1988b). As it is impossible to explain its rapid absorption from the gastrointestinal tract by this high binding property alone, we

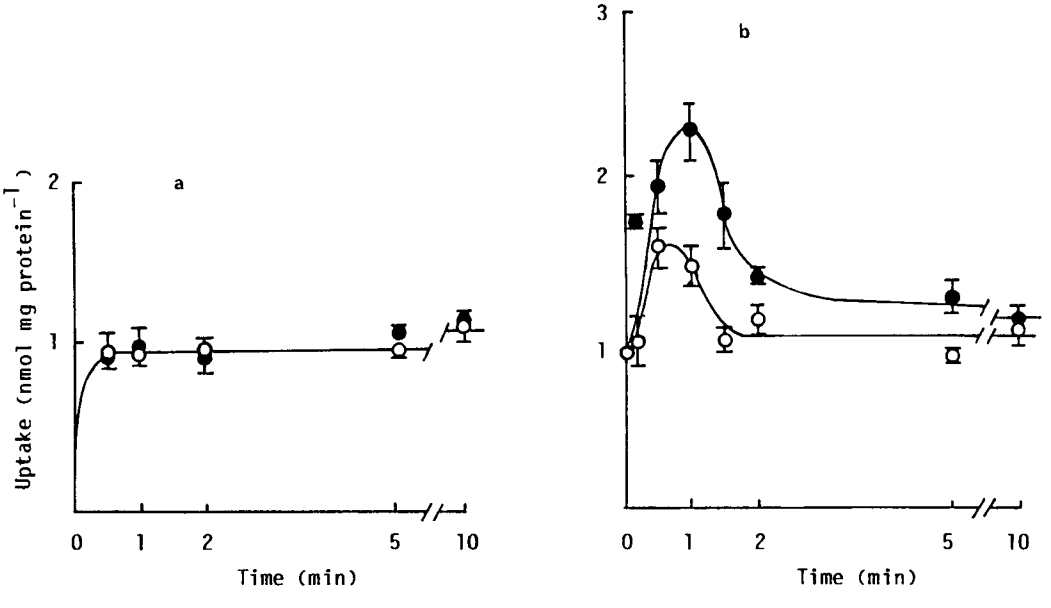

FIG. 2. Effect of valinomycin-induced potassium diffusion potential difference on chlorpromazine uptake by brush border membrane vesicles. (a) Membrane vesicles were suspended in buffer A containing 150 mM K-gluconate. 50 μL of vesicle suspension was incubated at 25°C with 100 μL of buffer A containing 75 μM clorpromazine with (●) or without (○) valinomycin (50 μg mL^{-1}). (b) Membrane vesicles were suspended in buffer A containing 200 mM K-gluconate. 50 μL of vesicle suspension was incubated at 25°C with buffer A (50 μL) containing 200 mM K-gluconate and 100 μM chlorpromazine. After 10 min, 100 μL of the mixture was incubated at 25°C with 100 μL of buffer A containing 50 μM chlorpromazine with (●) or without (○) valinomycin. Each point represents the mean ± s.e.m. of three to five measurements with different preparations of vesicles.

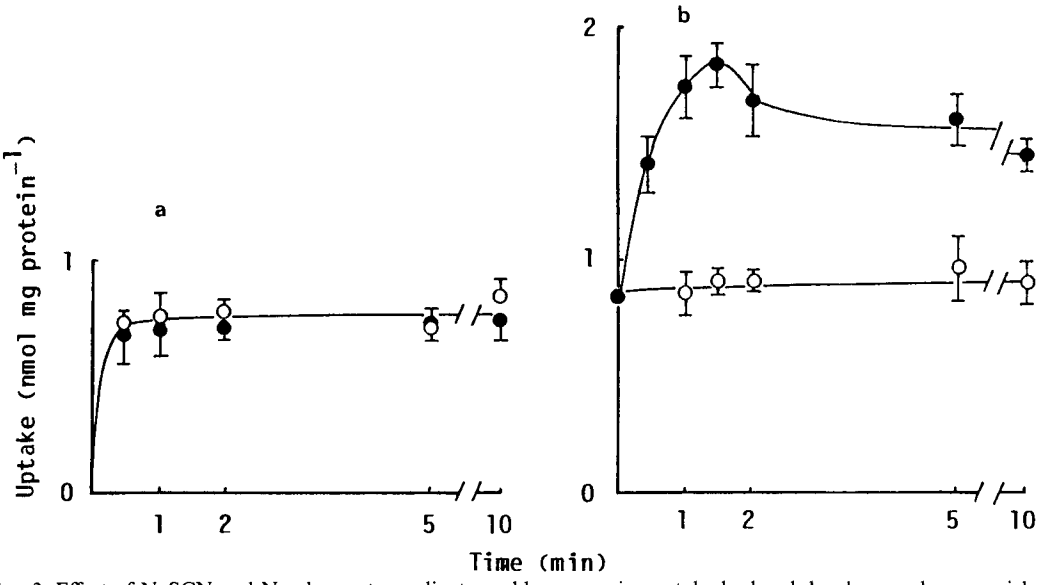

FIG. 3. Effect of NaSCN and Na gluconate gradient on chlorpromazine uptake by brush border membrane vesicles. Vesicles were suspended in buffer A. (a) 50 μL of vesicle suspension was incubated with 100 μL of buffer A containing 75 μM of chlorpromazine, 150 mM NaSCN (●) or Na gluconate (○). (b) 50 μL of vesicle suspension was incubated at 25°C with buffer A (50 μL) containing 100 μM chlorpromazine. After 10 min, the mixture was incubated at 25°C with 100 μL of buffer A containing 50 μM chlorpromazine and 200 mM NaSCN (●) or Na gluconate (○). Time 0 represents NaSCN or Na gluconate addition. Each point represents the mean ± s.e.m. of three to five measurements with different preparations of vesicles.

examined whether the transmembrane electrical potential difference (inside negative) facilitated the movement of the drug into the intravesicular space, as reported for propantheline (Saitoh et al 1988a).

When valinomycin, a potassium ionophore, is added to brush border membrane vesicles preloaded with K-gluconate, outward flow of potassium is specifically facilitated, and potassium diffusion potential difference (inside negative) is induced transiently (Murer & Hopfer 1974; Ganapathy & Leibach 1983). When valinomycin and chlorpromazine were simultaneously added to the vesicle suspension, there was no facilitating effect (Fig. 2a).

As shown in Fig. 2b, on the other hand, when valinomycin was added to the vesicle suspension after chlorpromazine uptake had reached a steady state by incubating the vesicles for 10 min with chlorpromazine, clear overshoot was observed, and the uptake was facilitated transiently.

The effect of transmembrane electrical potential difference on chlorpromazine uptake was also examined by adding NaSCN or Na-gluconate. It has been indicated that the ion permeability through intestinal brush border membrane is $SCN^- > K^+ > Na^+ >$ gluconate$^-$ (Gunther et al 1984). So when NaSCN is added to the extravesicular medium, the transmembrane electrical potential difference (inside negative) is rapidly induced (Kessler & Semenza 1979).

As shown in Fig. 3a, when NaSCN and chlorpromazine were added simultaneously, the facilitating effect was not observed. On the contrary, when NaSCN was added to the extravesicular medium after preloading of chlorpromazine for 10 min, overshoot was observed (Fig. 3b). These results were consistent with those obtained from the experiment using valinomycin (Fig. 2). In the case of Na-gluconate addition, any overshoot was not observed in both cases.

From these results, the folowing is considered as one absorption mechanism of chlorpromazine; firstly the drug is rapidly bound to the brush border membrane and then it enters into the epithelium stimulated by the transmembrane electrical potential difference (inside negative). Our observations suggest the existence of a common absorption mechanism between TACs and QACs, since propantheline uptake by brush border membrane vesicles was significantly inhibited by chlorpromazine (Saitoh et al 1988a).

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