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Carrier-mediated transport system for choline and its related quaternary ammonium compounds on rat intestinal brush-border membrane

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The characteristics of the intestinal transport system for choline were investigated using isolated brush border membrane vesicles from rat small intestine. In spite of the diminutive lipid solubility, the uptake of choline by membrane vesicles reflected its both permeation into intravesicular space rather than the binding to the membrane surface. Physiological conditions, present in the intact intestine, such as an inward-directed Na^+ or H^+ gradient and inside negative membrane potentials, didn't directly involve in choline transport across the brush-border membrane. Moreover, an outward-directed H^+ gradient had no significant effect on the time course of choline transport. However, in the absence of a driving-force,the initial uptake of choline exhibited a saturable manner. A kinetic analysis of the initial uptake rate gave an apparent K_m of 159 μM. Furthermore, unlabeled choline caused both cis-inhibition and trans-stimulation for labeled choline transport, suggesting the existence of a carrier-mediated transport system for choline, classified as so-called 'facilitated diffusion'. Since tetramethylammonium, acetylcholine, and N^1-methylnicotinamide caused both cis-inhibition and trans-stimulation, they appear to be accepted as the substrate of choline carrier. On the other hand, quaternary ammonium compounds (QACs) such as those which possessed hydrophobic parts in their molecules exhibited only cis-inhibition. They also inhibited Na^+-dependent D-glucose transport, indicating that they influenced various carrier-mediated transport systems non-specifically due to interaction with the membrane. These findings strongly suggest that the choline transport system on the brush-border membrane of rat intestine recognizes only small molecular QACs as its substrate.

Introduction

Choline (trimethyl-β-hydroxyethylammonium) is a quaternary ammonium compound (QAC) that is widely distributed amongst plants and animals. This substance is utilized to produce the essential components of biomembranes, such as phosphatidylcholine and sphingomyelin. Moreover, it is a precursor for the biosynthesis of the neurotransmitter acetylcholine. Since only a small amount of choline is produced in the body, most animals including human beings must depend upon their diet for the greater part of the choline supply [1]. It is, therefore, considered that choline is an essential nutrient [2].

It is known that choline is well absorbed from the intestine although it is completely ionized and extremely water-soluble [3–5]. It was, therefore, common to assume that the specialized transport mechanisms such as a carrier-mediated transport system facilitated its absorption process. Many attempts to clarify the mechanisms have been carried out using various experimental models [5,6–10]. However, the details of the carrier-mediated system remain unclear. Moreover, most of these methods using whole tissue preparations could not distinguish the transcellular choline transport from the extracellular one. It should be noted that the extracellular pathway via the tight junctions and

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Abbreviations: QAC, quaternary ammonium compound; TMA, tetramethylammonium; TEA, tetraethylammonium; NMN, N^1-methylnicotinamide; C6, hexyltrimethylammonium; C8, octyltrimethylammonium; C10, decyltrimethylammonium; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; DPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-[2-(5-phenyloxazolyl)]benzene.

the intercellular spaces behaved as aqueous pores with cation selectivity [11]. Therefore, the details of the transport across the lipoidal brush-border membrane, which is a substantial barrier to the absorption of ionic compound like choline, still remain to be fully characterized.

Transport study using brush-border membrane vesicles has proved useful as a model system to elucidate the absorption mechanism across the epithelial cell [12–14]. Up to now, using this membrane preparation from rat small intestine, we have reported the transport mechanism of QACs such as those which possess a variety of hydrophobic parts in their molecules [15,16]. The mechanism consisted of at least two consecutive processes. The first process was the rapid binding to the brush-border membrane, and the second was the entering into epithelium driven by the transmembrane electrical potential difference (cell interior negative).

We illustrated, as well, that the size of the hydrophobic part in QAC molecule was an important factor for the determination of the degree at which QAC bound to the brush-border membrane and that the absorption from rat intestine was correlated to the degree of binding [17,18]. However, the reason for the adequate absorption of choline, which has no hydrophobic parts in its molecule, is unable to be explained on the basis of the transport mechanism mentioned above.

In this paper, we have investigated the transport mechanism of choline across the brush-border membrane, and suggested that choline might pass through the membrane via a carrier-mediated transport system, which recognize only small molecular QACs as its substrate.

Materials and Method

Materials

[*methyl*-^{14}C]Choline chloride (2.0 GBq/mmol) and D-[U-^{14}C]glucose (0.5 GBq/mmol) were obtained from Du Pont/NEN Research Products (Wilmington, DE, USA) and Amersham International (Buckinghamshire, UK), respectively. Unlabeled choline chloride, tetramethylammonium bromide (TMA), and phenyltrimethylammonium bromide were purchased from Nakalai Tesque (Kyoto, Japan). Unlabeled D-glucose, tetraethylammonium chloride (TEA), and acetylcholine bromide were from Wako Pure (Osaka, Japan). N^1-Methylnicotinamide chloride (NMN) was from Sigma (St. Louis, MO, USA). Hexyltrimethylammonium iodide (C6), octyltrimethylammonium iodide (C8) were synthesized from the corresponding tertiary amines and methyl iodide in our laboratory using the method of Huang et al. [19]. Decyltrimethylammonium bromide (C10) was synthesized from n-decyl bromide and trimethylamine. The purities of synthesized QACs were checked by elemental analysis and ^{1}H-NMR (100 MHz). All other chemicals were of the highest grade available and used without further purification.

Preparation of brush-border membrane vesicles

Adult male Wistar rats (250–300 g) were used. Entire small intestine was excised under anesthesia (pentobarbital sodium (30 mg/kg body weight, i.p.)). Brush-border membrane vesicles were isolated according to the calcium chloride precipitation method of Kessler et al. [20] as described previously [21]. Membrane vesicles were finally suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5) in a concentration of 3–4 mg membrane protein per ml. All steps were performed on ice or at 2°C. The purity of the membrane was routinely evaluated by measuring the enrichment of alkaline phosphatase (EC 3.1.3.1), an enzyme specific to the intestinal brush-border membrane. The specific activity of this enzyme increased more than 12-fold in the final membrane suspension compared with concentrations found in the homogenate of intestinal scrapings. In some cases, the membrane vesicles were frozen at −80°C until use and thawed in iced water within 2 days for transport experiments.

Transport experiments

The transport of [^{14}C]choline into the isolated membrane vesicles was measured by a rapid filtration technique as described previously [21]. Briefly, in the regular assay, the transport was initiated by mixing 50 μl of membrane vesicle suspension with 100 μl of experimental buffer containing [^{14}C]choline. The specific conditions for each experiment are given in the legends of figures and tables. All transport experiments were carried out at 25°C. After appropriate time intervals, the mixture was diluted with 3 ml of ice-cold stop solution (150 mM NaCl, 1 mM Tris-HCl (pH 6.5)) and filtered under vacuum through a Millipore membrane filter (HAWP, 0.45 μm pore size, 25 mm filter diameter), which was previously washed with 2 ml of ice-cold stop solution containing 5 mM phenyltrimethylammonium in order to reduce non-specific adsorption of [^{14}C]choline. The filter was washed once with 3 ml of the same ice-cold stop solution and dissolved in 10 ml of scintillation fluid (1% DPO, 0.05% POPOP in toluene/polyoxyethylene (10) octyl-phenyl ether (2:1, v/v)). The radioactivity was counted by liquid scintillation spectrophotometer. In parallel, membrane-free incubation media were handled in an identical manner, and the radioactivity on the filter was subtracted from the uptake values determined in the presence of the membranes. Protein concentration of membrane suspension was determined by the method of Lowry et al. [22], using bovine serum albumin as the standard.

Statistical method

Transport experiments were routinely carried out in triplicate or quadruplicate, and the variation among the replicate values was < 10% of the mean value in most cases. All experiments were performed with two to three membrane preparations. Statistical analysis was performed using the Student's *t*-test and a $P < 0.05$ was considered significant.

Results

Transport ability of isolated membrane vesicles for D-glucose

To ascertain the viability of membrane vesicles prepared in this study, the uphill transport of D-glucose was measured. The transport of D-glucose (50 μM) by brush-border membrane vesicles demonstrated its usual characteristics of a distinct 'overshoot' phenomenon in the presence of an inward-directed Na^+ gradient (outside, 50 mM; inside, 0 mM). At the peak of the overshoot, the intravesicular concentration of D-glucose was about 3-fold greater than the equilibrium value. And the magnitude of overshoot was nearly identical in both freshly-prepared and frozen-thawed membrane vesicles. These results indicated that both membrane vesicles retained intact transport properties to the same degrees.

Characteristics of choline transport by brush-border membrane vesicles

The initial uptake of [^{14}C]choline as a function of time was examined at the two concentrations of 0.2 mM and 0.5 mM. As shown in Fig. 1, at both concentrations the uptake was nearly linear over at least 40 s. The extrapolation of the lines to time zero were extremely small and the value was only 40.3 pmol/mg protein even at the concentration of 0.5 mM.

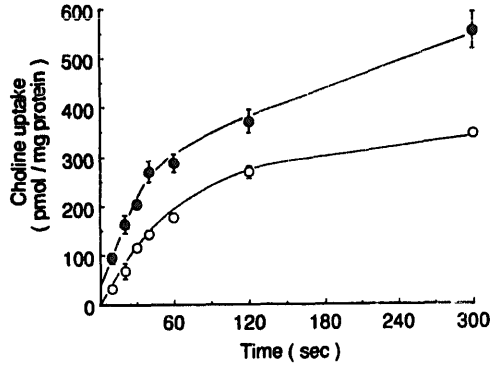

Fig. 1. Time-course of choline uptake by brush-border membrane vesicles. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5). Choline uptake was initiated by adding 50 μl of membrane suspension to 100 μl of an incubation medium composed of 0.3 mM (○) or 0.75 mM (●) [^{14}C]choline, 100 mM D-mannitol and 20 mM Mes-Tris (pH 6.5). Each point represents the mean ± S.E. of four determinations.

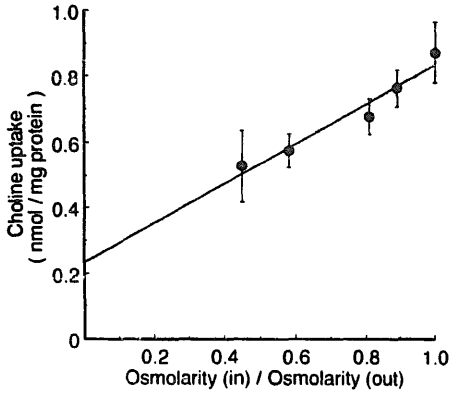

Fig. 2. Effect of medium osmolarity on choline uptake by brush-border membrane vesicles. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5). Choline uptake was measured after 30 min incubation in medium containing 0.5 mM [^{14}C]choline, 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5) and various concentrations (75–360 mM) of D-cellobiose. The osmolarity was checked using a Fiske Osmometer. Each point represents the mean ± S.E. of six determinations.

To clarify that choline uptake by membrane vesicles represented the transport into the intravesicular space rather than the binding to the membrane, the effect of extravesicular medium osmolarity on the uptake of choline (0.5 mM) at the steady state (30 min) was investigated by adding D-cellobiose to the extravesicular medium as a membrane-impermeable solute. The amount of choline taken up by vesicles was found to decrease in a linear fashion inversely proportional to the increase of extravesicular medium osmolarity (Fig. 2). Extrapolating choline uptake to infinite osmolarity, i.e., to zero intravesicular space, was 231 pmol/mg protein. From this value, the percentage of membrane binding at the steady state was estimated to be 26% of the total uptake from the incubation medium normally used, and the remaining (74% of total uptake) was transported into the intravesicular space. The data shown in Figs. 1 and 2 suggest that in spite of the diminutive lipid-solubility the permeation of choline across the brush-border membrane is comparatively smoother than expected.

In order to clarify whether the various physiological conditions present in intact intestine involve in choline transport across the brush-border membrane, several experiments were performed. Table I shows the effect of an inward-directed Na^+ gradient (outside, 100 mM; inside, 0 mM) on the uptake of choline (0.1 mM). The initial uptake value (30 s) under Na^+ gradient was almost identical to those under K^+ and sucrose gradient. It was, therefore, considered that choline transport across the brush-border membrane was independent of Na^+.

Effect of transmembrane electrical potential difference (inside negative) on choline uptake was examined using lipophilic anion which could permeate the

TABLE I

Effect of inward-directed Na^+ gradient on choline uptake by the brush-border membrane vesicles

Choline uptake was measured in medium containing 0.1 mM [^{14}C]choline, 100 mM D-mannitol, 20 mM Mes-Tris (pH. 6.5) and 100 mM NaCl or KCl, or 200 mM sucrose at final concentrations. Results represent the means ± S.E. of three to six determinations.

Condition	Choline uptake (pmol/mg protein)	
	30 s	30 min
Na^+ gradient	118.7 ± 13.0	204.0 ± 23.1
K^+ gradient	103.1 ± 14.0	211.8 ± 28.9
Sucrose gradient	115.6 ± 7.1	246.0 ± 18.1

brush-border membrane much faster than paired cation [23]. As shown in Table II, the uptake of D-glucose (50 μM) over 15 s in the presence of NaSCN gradient was about 4-fold greater than equilibrium value (30 min). Under Na gluconate gradient, the stimulation of initial D-glucose uptake was extremely small. This result reconfirmed that Na^+-dependent D-glucose transport across the brush-border membrane was also dependent to inside negative membrane potential [24]. In the case of choline (0.1 mM), however, there was no difference in the uptake between these two experimental conditions, demonstrating that choline transport was irrelevant to membrane potential. This result was compatible with that obtained from membrane preparation from rabbit intestine [20].

Table III shows the effect of an outward-directed H^+ gradient on the uptake of choline (0.2 mM) by brush-border membrane vesicles. Choline uptake didn't alter significantly in the presence or absence of a H^+ gradient. Although a type of endogenous organic cation is known to pass through the brush-border membrane via an organic cation-H^+ antiport system [25], there

TABLE II

Effect of inside negative transmembrane electrical potential difference on the uptake of D-glucose and choline by the brush-border membrane vesicles

Uptake was measured in medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5), either 100 mM NaSCN or Na gluconate, and substrate. Results represent the means ± S.E. of three to four determinations. The uptake of D-glucose and choline was measured using different membrane preparations, respectively. * $P < 0.01$, significantly different from Na gluconate.

Substrate	Time	Uptake (pmol/mg protein)	
		NaSCN	Na gluconate
D-Glucose	15 s	88.4 ± 3.0 *	31.1 ± 2.3
(50 μM)	30 min	23.9 ± 4.4	27.4 ± 5.9
Choline	30 s	105.4 ± 5.5	98.7 ± 14.7
(100 μM)	30 min	185.7 ± 40.1	156.2 ± 25.7

TABLE III

Choline uptake by the brush-border membrane vesicles in the presence and absence of an outward-directed H^+ gradient

Brush-border membrane vesicles were suspended in medium containing either 100 mM D-mannitol, 20 mM Mes-Tris (pH 5.5) or 100 mM D-mannitol 20 mM Hepes-Tris (pH 7.5). Choline uptake was initiated by adding 20 μl of membrane suspension to 100 μl of an incubation medium composed of 100 mM D-mannitol, 20 mM Hepes-Tris (pH 7.5) and 0.24 mM [^{14}C]choline. Results represent the means ± S.E. of four determination. n.s., not significant.

Time (min)	Choline uptake (pmol/mg protein)		
	pH in = 5.5	pH in = 7.5	
0.5	225.6 ± 26.4	206.3 ± 7.6	n.s.
1	304.0 ± 29.8	297.9 ± 14.7	n.s.
30	389.9 ± 61.7	410.6 ± 26.8	n.s.

was no indication of active transport of choline via the antiport system. The result in Table III also suggest that inside negative H^+-diffusion potential had no effect on choline uptake.

Moreover, an inward-directed H^+ gradient (pH_{in} = 7.5, pH_{out} = 5.5), which is known to induce the uphill transport of dipetides [26,27], had no stimulatory effect on choline transport (data not shown).

Concentration dependence of choline uptake by brush-border membrane vesicles

Fig. 3 shows the rate of choline uptake at various extravesicular concentrations of choline over a range of 0.025 to 1 mM. There was a non-linear relationship, saturability, between the concentration and the rate of uptake. In order to analyze the saturation of the rate of choline uptake, total uptake was divided into sat-

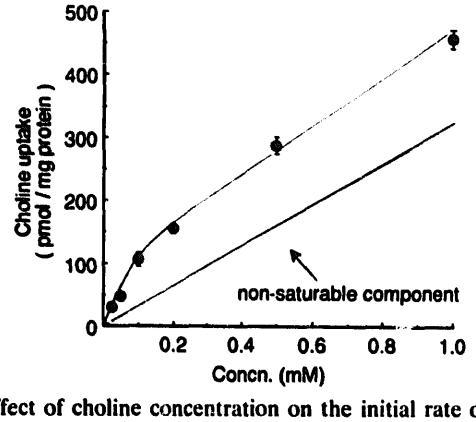

Fig. 3. Effect of choline concentration on the initial rate of choline uptake by brush-border membrane vesicles. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5). Choline uptake over a 30 s period was measured in medium containing [^{14}C]choline at the indicated concentrations, 100 mM D-mannitol and 20 mM Mes-Tris (pH 6.5). Results represents the means ± S.E. of six determinations.

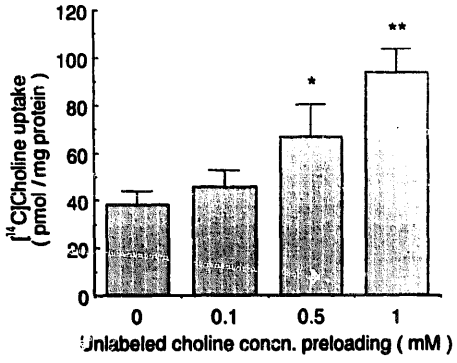

Fig. 4. Effect of preloading of unlabeled choline on [^{14}C]choline uptake by brush-border membrane vesicles. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5). Firstly, membrane vesicles were preincubated in a medium containing unlabeled choline at indicated concentrations, 100 mM D-mannitol and 20 mM Mes-Tris (pH 6.5) at 25°C for 30 min. And then [^{14}C]choline uptake over a 30 s period was initiated by adding 40 μl of the mixture to 200 μl of an incubation medium containing 30 μM labeled choline, 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5) and appropriate amount of unlabeled choline to adjust its extravesicular concentration to 166 μM. Results represent the means ± S.E. of six determinations. * $P < 0.01$, ** $P < 0.05$, significantly different from 0 mM.

urable and non-saturable components by solving the following equation by computer analysis:

$$V = \frac{V_{max}[S]}{K_m + [S]} + K_d[S]$$

where V is the initial uptake rate, [S] is the initial concentration, V_{max} is the maximum uptake by saturable component, K_m is the Michaelis constant, and K_d is the coefficient of non-saturable component (i.e., simple diffusion). The calculated values of apparent K_m, V_{max}, and K_d were 159 μM, 166 pmol/mg protein per 30 s, and 322 pmol/mg protein per 30 s per mM, respectively.

Effect of unlabeled choline on [^{14}C]choline uptake by brush-border membrane vesicles

To determine whether the saturability shown in Fig. 3 indicates the presence of carrier-mediated transport of choline across the brush-border membrane, the effect of unlabeled choline on [^{14}C]choline uptake was examined. The uptake of 0.1 mM [^{14}C]choline over 30 s significantly decreased in the presence of a large quantity of unlabeled choline in the extravesicular medium. The degree of cis-inhibition by unlabeled choline was about 40% at 2.5 mM and 65% at 5 mM, respectively.

Fig. 4 shows the effect of preloading of unlabeled choline on the uptake of [^{14}C]choline (25 μM) over 30 s. In these experiments unlabeled choline coexisting in the extravesicular medium at the start of [^{14}C]choline uptake was kept constant in every samples. [^{14}C]Choline uptake was gradually enhanced with the increase of preloading concentrations of unlabeled choline. The data indicate that the presence of unlabeled choline inside the vesicles was capable of trans-stimulating the transport of labeled choline from outside to inside.

Considering above results (saturability, cis-inhibition, and trans-stimulation), it is strongly suggested that a carrier-mediated transport is involved in choline uptake by the brush-border membrane vesicles from rat small intestine.

Cis-inhibition and trans-stimulation effect of other quaternary ammonium compounds on choline uptake by brush-border membrane vesicles

Since there is considerable variety within the structures of QACs, it would be worthwhile interesting as to whether the presumable choline transport system on the brush-border membrane accepts other QACs as its substrate. Initial uptake of choline (0.1 mM) was significantly inhibited to varying extents by other QACs with the single exception of TEA (Fig. 5). Decyltrimethylammonium was the strongest inhibitor among QACs tested. Tetramethylammonium, C6, and C8 inhibited choline uptake to similar degrees. The effect of acetylcholine and NMN was weaker than that of other QACs but still significant. The inhibitory effect of C8 and C10 on choline uptake was observed even at 40 min, whereas TMA, NMN, and C6 had little or no effect (data not shown). Since C8 and C10 interact with the brush-border membrane, the long-acting inhibition of these QACs might take place as a result of this action.

In order to investigate as to whether the inhibition of choline uptake shown above (Fig. 5) was the result of a competition of tested compounds with choline for transport carrier or not, the ability of QACs to trans-stimulate the uptake of choline was studied. Membrane vesicles were preloaded at the concentration of

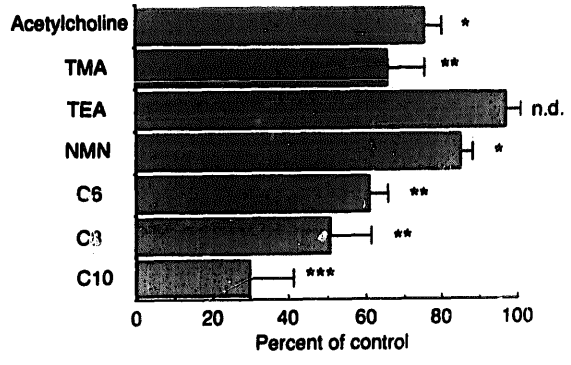

Fig. 5. Effect of other QACs on choline uptake by brush-border membrane vesicles. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5). Choline uptake over a 30 s period was measured in medium containing 0.1 mM [^{14}C]choline, 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5) and 2.5 mM QAC at final concentrations. Results represent the means ± S.E. of six determinations. n.d., not different from control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly smaller than control.

0.1 mM for 30 min. As shown in Fig. 6, the presence of 0.1 mM unlabeled choline inside the vesicles stimulated the uptake of radiolabeled choline (25 μM) by 50%. TMA and acetylcholine obviously enhanced choline uptake. Although the effect observed with NMN was less significant as compared to unlabeled choline, it exhibited a tendency to stimulate choline uptake. On the contrary, TEA, C6, and C8 had no stimulatory effect and C10 caused considerable inhibition even under the experimental conditions used here.

Effect of quaternary ammonium compounds on Na^+-dependent D-glucose uptake by brush-border membrane vesicles

As previously reported [15,17,28,29], QACs possessing a hydrophobic part in their structures, such as C8, C10, and propantheline, interact with the brush-border membrane. It is, therefore, probable that the inhibition of these QACs on choline transport (Fig. 5) is non-specifically caused as a result of membrane binding of these QACs. In order to substantiate the possibility, their effect on the well-characterized carrier-mediated transport system was examined. Table IV shows the effect of four QACs on D-glucose uptake in the presence of an inward-directed Na^+ or K^+ gradient. Although TMA and C6 failed to inhibit D-glucose transport under Na^+ gradient, C8 and C10 significantly inhibited it. On the other hand, all QACs exhibited no noteworthy effects on D-glucose the uptake in the presence of K^+ gradient. Since there was no difference in D-glucose uptake at a steady state (40 min) in the presence or absence of C10 (data not shown), it seems to indicate that the decrease of choline transport at 40 min by C10 mentioned above was not due to a morphological change such as a decrease of vesicular volume.

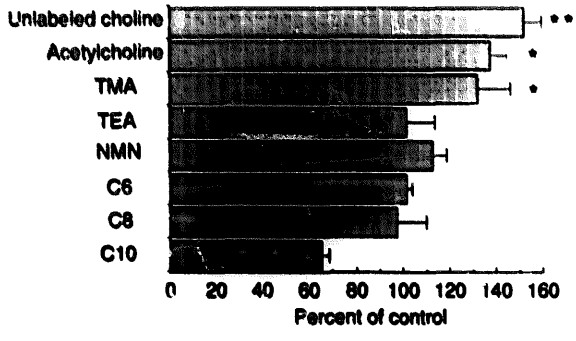

Fig. 6. Effect of preloading of other QACs on choline uptake by brush-border membrane vesicles. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5). Firstly, membrane vesicles were preincubated in a medium containing 0.1 mM QAC, 100 mM D-mannitol and 20 mM Mes-Tris (pH 6.5) at 25°C for 30 min. And then choline uptake over a 30 s period was initiated by adding 40 μl of the mixture to 200 μl of an incubation medium comprised of 30 μM [^{14}C]choline, 100 mM D-mannitol and 20 mM Mes-Tris (pH 6.5). Results represent the means ± S.E. of six determinations. * $P < 0.05$, significantly greater than control.

TABLE IV

Effect of four QACs on D-glucose uptake by the brush-border membrane vesicles

D-Glucose uptake over a 30 sec period was measured in medium containing 50 μM D-[^{14}C]glucose, 100 mM D-mannitol, 20 mM Mes-Tris (pH 6.5) and 2.5 mM QAC at final concentrations. Results represent the means ± S.E. of four determinations. * $P < 0.05$, ** $P < 0.01$, significantly different from control.

QAC	D-Glucose uptake (pmol/mg protein)	
	Na^+ gradient	K^+ gradient
None (control)	126.5 ± 5.6	7.9 ± 1.0
TMA	125.6 ± 1.4	9.8 ± 3.8
C6	123.0 ± 3.6	8.1 ± 0.3
C8	107.2 ± 2.8 *	8.0 ± 0.3
C10	72.7 ± 8.8 **	7.9 ± 1.1

Discussion

The present results demonstrate the existence of a carrier-mediated transport system for choline in the brush-border membrane from rat small intestine. The evidence can be summarized as follows: (1) initial uptake of choline was accomplished in a saturable manner with an apparent K_m of 159 μM (Fig. 3), (2) unlabeled choline caused cis-inhibition and trans-stimulation of [^{14}C]choline uptake (Fig. 4), and (3) some structurally-related QACs had the same effect as unlabeled choline on the transport of labeled one (Figs. 5 and 6). Although it is possible that saturability of initial uptake and cis-inhibition are observed in the permeation through cation-selective pores or channels on the membrane, trans-stimulation is considered to be characteristic of the carrier-mediated transport [30,31]. In vitro results obtained with everted intestinal sacs of rat [5,6] and hamster [6], intestinal segments of chicken [7], guinea pig [8] and rat [10], isolated enterocytes of guinea pig [9], or brush-border membrane vesicles from rabbit intestine [20] have indicated the saturability of choline transport. The affinity of transport system such as K_m value shown in this study was within the range reported in previous papers.

The conflicting aspect in the literature concerned the sodium dependence of the choline transport. Sodium-dependent choline transport was indicated using intestinal segment of chick [7] and isolated enterocytes of guinea pig [8]. Our results illustrated that choline transport into rat intestinal brush-border membrane vesicles was unaffected in the presence of an inward-directed Na^+ gradient (Table I). A similar result was reported in the same experimental model with rabbit intestine. Although species difference in choline transport can not be excluded, choline transport

through the brush-border membrane of small intestine may be characterized as a sodium-independent process. It is generally known that such physiological conditions present in intact intestine as inward-directed Na^+ or H^+ gradient, inside negative membrane potential and outward-directed H^+ gradient, participate in the membrane transport of various substance as the driving-force. In the present study, however, these physiological conditions had no significant effect on choline transport, indicating a possibility that the carrier-mediated transport system for choline didn't necessarily demand a driving-force; assuming this was true, the transport system would be characterized as a sort of facilitated diffusion. Since Ruifrok and Mol [11] reported the contribution of the extracellular route to the absorption of small molecular QACs such as TMA, TEA and choline, choline would be efficiently absorbed via the carrier-mediated transport system in addition to the extracellular route.

Moreover, the additional significant finding in the present study is the substrate specificity of the carrier involved in choline transport. From the results shown in Figs. 5 and 6, tested QACs were divided into at least three groups on the basis of their effect on choline uptake. The first group, exhibiting both cis-inhibition and trans-stimulation, includes TMA, acetylcholine, and most likely NMN. QACs such as those which caused cis-inhibition but no trans-stimulation are in the second group, including C6, C8, and C10. The third group contains compounds like TEA, which exhibited no effect on choline uptake. A special feature of QACs in the first group is that their structures are comparatively small and there are no hydrophobic parts in their molecules, as well as choline. These QACs are considered to be the substrates of the carrier-mediated transport system of choline in the brush-border membrane, because of their trans-stimulation effect which is a convincing demonstration of a carrier-mediated transport system. On the other hand, it seems that QACs in the second group are not directly recognized as substrates of the choline transport system. It is of importance that C8 and C10 in this group influenced a separate carrier-mediated transport system. As shown in Table IV, the magnitude of overshoot in Na^+-dependent D-glucose transport significantly decreased in the presence of these QACs. We have already shown [17,18] that the binding of n-alkyltrimethylammonium to the brush-border membrane gradually increased with each extention of unbranched hydrocarbon chain from C8 to tetradecyltrimethylammonium (C14), and yet, C6 and heptyltrimethylammonium (C7) failed to bind to the membrane significantly.

It is well known that a variety of amphiphilic organic cations strongly interact with biomembrane [28,32,33] and interfere in various membrane functions [34–36]. If the inhibition of Na^+-dependent D-glucose transport by C8 and C10 was due to the interaction between these QACs and the brush-border membrane, their inhibitory effect on choline transport as shown in Fig. 5 would take place non-specifically. These QACs didn't change D-glucose transport under K^+ gradient instead of Na^+ (Table IV), suggesting that they had little or no effect on the simple diffusion process. It is, therefore, possible that these QACs may possibly suppress various carrier-mediated transports by means of random binding to carrier proteins rather than by the change in membrane fluidity. We have previously found that sialic acids, which occupy a terminal position in carbohydrate chains of glycoprotein or glycolipid, play an important role in the high binding of QACs to the brush-border membrane [29]. This finding may serve as a possible explanation for the direct interaction between QACs and carrier proteins.

The transport of TEA, a prototypical substrate for the well-known organic cation/proton antiport in kidney, into renal brush-border membrane vesicles was significantly inhibited by choline [37]. It is, therefore, noteworthy that TEA had no effect on choline transport across intestinal brush-border membrane. This may indicate that TEA is unable to interact with the binding sites of the carrier on rat intestinal brush-border membrane, due to steric hindrance caused by the four ethyl groups around the nitrogen atom. The TEA result was compatible with that reported by Tsubaki and Komai [5]. The greater part of the characteristics of the choline transport system in this study seem to be quite consistent with the translocation model of the choline carrier of erythrocyte as reported by Deves and Krupka [38].

In conclusion, the rat intestinal brush-border membrane was shown to possess a carrier-mediated process for choline transport and this process quite possibly plays an important role in choline absorption from the intestine. Since this transport system operated fully independent of a driving-force, it might be characterized, for the present, as a type of facilitated diffusion. The choline carrier accepts only small molecular QACs, such as TMA, acetylcholine, and NMN, as its substrate. Moreover, QACs such as those possessing hydrophobic parts in their structures are able to bind to the choline carrier, but are unable to permeate the membrane via the transport system. It is, therefore, considered that the transport mechanism between small molecular and larger QACs is substantially distinct.

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