Transport Mechanism of Choline in Rat Renal Brush-Border Membrane

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The transport mechanism of choline was examined using rat renal brush-border membrane vesicles in comparison with tetraethylammonium transport. The stimulatory effect of an outward H^+ gradient on choline uptake was weak compared with that on tetraethylammonium uptake. [^{14}C]Tetraethylammonium uptake was *cis*-inhibited and *trans*-stimulated by choline, but the effects were less pronounced than those produced by unlabeled tetraethylammonium. [3H]Choline uptake was *trans*-stimulated by unlabeled choline, but not by tetraethylammonium. An interior-negative membrane potential induced marked stimulation of choline uptake against its concentration gradient (overshoot phenomenon), and the uptake was saturable with an apparent K_m of 0.77 mM. Various compounds such as hemicholinium-3 inhibited the choline uptake by renal brush-border membrane vesicles, but a sulfhydryl reagent did not. These findings suggest that choline can be actively transported by a carrier-mediated system driven by cell interior-negative membrane potential in renal brush-border membrane, and this system may play an important role in the tubular reabsorption of choline.

Keywords choline; carrier-mediated transport; renal reabsorption; brush-border membrane; membrane potential

Organic cations such as tetraethylammonium are actively secreted by renal proximal tubules and the mechanisms of the secretion have been studied by *in vivo* clearance technique, isolated proximal tubules and cortical slices.¹⁾ Recent developments in methods for isolating brush-border and basolateral membranes provided further information on the transport characteristics of organic cations in renal proximal tubules. Organic cation transport from blood into the proximal tubular cells across the basolateral membrane and that from cells into the lumen across the brush-border membrane are specific, carrier-mediated processes.^{2–5)} In basolateral membrane, cell interior-negative membrane potential^{4,5)} and/or exchange mechanism^{6–8)} serve as a driving force. H^+ gradient-dependent uphill transport of organic cations (electroneutral H^+ /organic cation antiport system) was demonstrated in renal brush-border membrane vesicles from rats,^{4,9–13)} rabbits,^{5,14–16)} dogs^{17,18)} and humans.¹⁹⁾ Since the luminal pH is more acidic than the intracellular pH in proximal tubules,²⁰⁾ it is reasonable to assume that the inward H^+ gradient (from lumen to cell) can serve as a driving force for the secretion of organic cations.

Choline is an endogenous organic cation which is important to normal cellular functions. The kidney plays a major role in the homeostatic control of the plasma levels of choline by means of tubular transport and metabolism of choline.^{21–25)} Bidirectional tubular transport of choline occurs in the kidney depending on its plasma concentration in several species.^{22–25)} When the plasma concentration is low, net reabsorption of choline occurs, whereas net secretion is observed when the plasma concentration of choline is elevated. Since the endogenous level of plasma choline is 10–20 μM ,²⁶⁾ choline is reabsorbed from the renal proximal tubules under physiological conditions. In addition, organic cation transport in the renal basolateral membrane was suggested to be mediated by exchange with intracellular organic cations such as choline.^{6–8)} Therefore, it is important to characterize the mechanism of choline reabsorption in the kidney. However, there has to date been

little information provided concerning the transport mechanism of choline in the renal brush-border membrane.

We therefore examined the characteristics of choline transport using rat renal brush-border membrane vesicles in comparison with the characteristics of tetraethylammonium, a typical exogenous organic cation, transport. We found that choline is transported by a carrier-mediated system which is distinct from the H^+ /organic cation antiport system in renal brush-border membrane and that this system is driven by cell interior-negative membrane potential.

Materials and Methods

Preparation of Brush-Border Membrane Vesicles Brush-border membrane vesicles were isolated from the renal cortex of male Wistar albino rats (200–230 g) by the Mg/EGTA precipitation method of Biber *et al.*²⁷⁾ and our previous report²⁸⁾ with several modifications. All steps were performed on ice or at 4 °C. The cortex was homogenized with an Ace Homogenizer AM-4 (Nihonseiki Co., Ltd., Tokyo, Japan) at 18000 rpm for 2 min in the buffer consisting of 300 mM mannitol, 12 mM Tris-HCl (pH 7.1) and 5 mM EGTA, and then the same buffer was added to make a 10% (w/v) homogenate. After dilution with distilled water (1 : 1), $MgCl_2$ was added to a final concentration of 10 mM, and the homogenate was allowed to stand for 15 min. The homogenate was centrifuged at $1900 \times g$ for 15 min in a Hitachi high speed refrigerated centrifuge SCR 20B (rotor: RPR20-2). The supernate was centrifuged at $24000 \times g$ for 30 min. The pellet was resuspended in the buffer consisting of 150 mM mannitol, 6 mM Tris-HCl (pH 7.1) and 2.5 mM EGTA, and homogenized in a glass/Teflon Potter homogenizer with 10 strokes at 1000 rpm. $MgCl_2$ was added to a final concentration of 10 mM, and the suspension was allowed to stand for 15 min. The suspension was then centrifuged at $1900 \times g$ for 15 min, and then the supernate was centrifuged at $24000 \times g$ for 30 min. The final pellet (purified brush-border membrane) was resuspended in a buffer consisting of either 100 mM mannitol and 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 6.0) or 100 mM mannitol and 10 mM Hepes (pH 7.5). Aminopeptidase (EC 3.4.11.2) and γ -glutamyltransferase (EC 2.3.2.2), marker enzymes for brush-border membrane, were enriched approximately 14-fold, whereas $Na^+-K^+-ATPase$ (EC 3.6.1.3), a marker enzyme for basolateral membrane, was not detectable. The membrane vesicles were used for transport studies on the day they were prepared.

In some experiments, the brush-border membrane was pretreated with *p*-chloromercuribenzenesulfonate (PCMBS) as previously described.¹¹⁾

Transport Studies The uptake of labeled substrate by brush-border membrane vesicles was measured by a rapid filtration technique.²⁹⁾ The reaction was rapidly initiated by the addition of a buffer (80–180 μl)

Fig. 1. Effect of Outward H⁺ Gradient on Tetraethylammonium (A) and Choline (B) Uptake by Brush-Border Membrane Vesicles

Membrane vesicles were suspended in 100 mM mannitol, 10 mM MES (pH 6.0; ●) or 10 mM Hepes (pH 7.5; ○), and 100 mM KCl. The aliquots (20 μ l) were incubated with the substrate mixture (80 μ l) containing 100 mM mannitol, 10 mM Hepes (pH 7.5), 100 mM KCl, and 0.3125 mM [¹⁴C]tetraethylammonium (A) or 62.5 μ M [³H]choline (B). Each point represents the mean $\pm$ S.E. of three separate experiments performed in two to three determinations.

containing labeled substrate to 20 μ l of membrane suspension (5–10 mg of protein per ml) at 25 $^{\circ}$ C. At the stated times, the incubation was stopped by diluting the reaction mixture with 1 ml of ice-cold stop solution. The composition of the stop solution was: 150 mM KCl, 20 mM Hepes-Tris (pH 7.5) and 0.1 mM HgCl₂. The contents of the tube were poured immediately onto Millipore filters (HAWP, 0.45 μ m, 2.5 cm diameter) and washed once with 5 ml of ice-cold stop solution. Under each experimental condition, nonspecific adsorption was estimated by the addition of labeled substrate mixture to 1 ml of ice-cold stop solution containing 20 μ l of membrane vesicles. This value was subtracted from each uptake data for background correction.

Analytical Methods After precipitation with ice-cold 10% (w/v) trichloroacetic acid, protein was determined by the method of Lowry *et al.*³⁰⁾ with bovine serum albumin as a standard. The marker enzymes were assayed as previously described.²⁹⁾

Materials [1-¹⁴C]Tetraethylammonium bromide (3.7 mCi/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). [Methyl-³H]choline chloride (75.0–79.3 mCi/mmol) was purchased from Amersham International, Ltd. (Buckinghamshire, U.K.). Valinomycin was obtained from Sigma Chemical Co. (St. Louis, MO). Hepes, MES, Tris and PCMBs were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals used were of the highest purity available.

Results

Effect of an Outward H⁺ Gradient on Tetraethylammonium and Choline Uptake To clarify the contribution of H⁺/organic cation antiport system to choline transport in renal brush-border membrane, the effect of an outward H⁺ gradient on choline uptake was examined. Figure 1 shows the time course of tetraethylammonium (A) and choline (B) uptake by brush-border membrane vesicles in the presence ([pH]_{in}=6.0, [pH]_{out}=7.5) or absence ([pH]_{in}=[pH]_{out}=7.5) of an outward H⁺ gradient. H⁺ coupled uphill transport of tetraethylammonium *via* the H⁺/organic cation antiport system was observed in brush-border membrane. On the contrary, the outward H⁺ gradient stimulated choline uptake only slightly (but significantly at 30 s; *p*<0.05). The stimulation of choline uptake by an outward H⁺ gradient was inhibited in the presence of 5 mM unlabeled tetraethylammonium, but was not altered when the vesicles were voltage-clamped by valinomycin (data not shown).

Effect of Unlabeled Tetraethylammonium and Choline on [¹⁴C]Tetraethylammonium Uptake Figure 2A shows the *cis*-inhibition effect of unlabeled tetraethylammonium and

Fig. 2. Effect of Unlabeled Tetraethylammonium and Choline on [¹⁴C]Tetraethylammonium Uptake by Brush-Border Membrane Vesicles

(A) *cis*-Inhibition effect. Membrane vesicles were suspended in 100 mM mannitol, 10 mM MES (pH 6.0), and 100 mM KCl. The aliquots (20 μ l) were incubated for 10 s with the substrate mixture (80 μ l) containing 100 mM mannitol, 10 mM Hepes (pH 7.5), 100 mM KCl, 0.3125 mM [¹⁴C]tetraethylammonium (TEA), and unlabeled tetraethylammonium or choline. (B) *trans*-Stimulation effect. Membrane vesicles were suspended in 100 mM mannitol, 10 mM Hepes (pH 7.5), and 100 mM KCl, with or without 5 mM organic cations. The aliquots (20 μ l) were incubated with the substrate mixture (180 μ l) containing 100 mM mannitol, 10 mM Hepes (pH 7.5), 100 mM KCl, and 0.278 mM [¹⁴C]tetraethylammonium. Control (○); tetraethylammonium preloaded (●); and choline preloaded (△). Each point represents the mean $\pm$ S.E. of six determinations from two separate experiments.

choline on [¹⁴C]tetraethylammonium uptake in the presence of an outward H⁺ gradient. Choline (10 mM, but not 1 mM) inhibited the tetraethylammonium uptake by brush-border membrane vesicles. However, the inhibitory effect was weaker than that of 1 mM unlabeled tetraethylammonium.

To study further the interaction of H⁺/organic cation antiport system with choline, the *trans*-stimulation effect of unlabeled choline on [¹⁴C]tetraethylammonium uptake was examined. As shown in Fig. 2B, the uptake of tetraethylammonium was stimulated by preloading the membrane vesicles with unlabeled choline. However, a much

Fig. 3. *trans*-Stimulation Effect on Choline Uptake by Brush-Border Membrane Vesicles

Membrane vesicles were suspended in 100 mM mannitol, 10 mM Hepes (pH 7.5), and 100 mM KCl, with or without 5 mM organic cations. The aliquots (20 μ l) were incubated with the substrate mixture (180 μ l) containing 100 mM mannitol, 10 mM Hepes (pH 7.5), 100 mM KCl, and 55.6 μ M [3 H]choline. Control (○); choline preloaded (●); and tetraethylammonium preloaded (△). Each point represents the mean $\pm$ S.E. of six determinations from two separate experiments.

greater effect was observed when the vesicles were preloaded with unlabeled tetraethylammonium.

***trans*-Stimulation Effect on Choline Uptake** Figure 3 shows the *trans*-stimulation effect of unlabeled choline and tetraethylammonium on [3 H]choline uptake by brush-border membrane vesicles. The uptake of [3 H]choline was markedly stimulated by preloading the membrane vesicles with unlabeled choline. On the contrary, the stimulatory effect was not observed when the membrane vesicles were preloaded with unlabeled tetraethylammonium. Thus, although tetraethylammonium and choline can share a common transport system (H^+ /organic cation antiport system), the contribution of this transport system to choline uptake must be small in renal brush-border membrane.

Driving Force of Choline Uptake by Brush-Border Membrane Vesicles To characterize the choline transport system in renal brush-border membrane, we examined the effect of an inward Na^+ gradient and an interior-negative membrane potential on choline uptake. As shown in Fig. 4, choline uptake by brush-border membrane vesicles did not change in the presence of the inward Na^+ gradient ($[K^+]_{in} = [Na^+]_{out} = 100$ mM) compared with that in the absence of Na^+ gradient ($[K^+]_{in} = [K^+]_{out} = 100$ mM). Interior-negative membrane potential generated by K^+ -valinomycin, however, markedly stimulated the choline transport, and transient accumulation of choline against a concentration gradient was observed (overshoot phenomenon). Under physiological conditions the cell interior-negative membrane potential is generated, thus it is likely that choline is transported from lumen to cell *via* a carrier-mediated process driven by cell interior-negative membrane potential across the renal brush-border membrane.

Concentration Dependence of Choline Uptake by Brush-Border Membrane Vesicles Concentration dependence of choline uptake was examined in the presence of an interior-negative membrane potential to analyze the kinetics

Fig. 4. Effect of Inward Na^+ Gradient and Interior-Negative Membrane Potential on Choline Uptake by Brush-Border Membrane Vesicles

Membrane vesicles were suspended in 100 mM mannitol, 10 mM Hepes (pH 7.5), and 100 mM K-gluconate. The aliquots (20 μ l) were incubated with the substrate mixture (180 μ l) containing 100 mM mannitol, 10 mM Hepes (pH 7.5), 100 mM K-gluconate (○) or 100 mM Na-gluconate (△, ●), and 55.6 μ M [3 H]choline, with (●) or without (○, △) valinomycin (7.0–11.1 μ g/mg of protein). Each point represents the mean $\pm$ S.E. of three separate experiments performed in two to three determinations.

of choline transport in renal brush-border membrane. Choline uptake in the presence of an interior-negative membrane potential was linear up to 5 s (data not shown). Therefore, 5 s incubation was employed to measure the initial uptake rate. As shown in Fig. 5A, the relationship between concentration and rate of total uptake was nonlinear, which is evidence for saturability. Using nonlinear least squares regression analysis,³¹⁾ kinetic parameters were calculated from the following equation:

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

where V is the initial uptake rate, $[S]$ is the initial concentration, V_{max} is the maximum uptake rate by the carrier-mediated process, K_m is the Michaelis constant, and K_d is the coefficient of simple diffusion.³²⁾ The nonsaturable component is estimated as $K_d[S]$ (the dashed line in Fig. 5A). The values of apparent K_m , V_{max} and K_d were 0.77 ± 0.12 mM, 12.5 ± 0.9 nmol/mg of protein per min and 0.76 ± 0.16 nmol/mg of protein per min per mM, respectively (each value represents the mean $\pm$ S.E. of three separate experiments). The Eadie-Hofstee plot of the data after correction for the nonsaturable component showed a linear relationship (Fig. 5B), which indicates the existence of one mode of choline transport system under this experimental condition.

Effect of PCMBs Pretreatment on Choline Uptake by Brush-Border Membrane Vesicles The effect of PCMBs, a sulfhydryl reagent, on tetraethylammonium and choline uptake was examined. As shown in Fig. 6A, tetraethylammonium uptake was markedly inhibited by the pretreatment of brush-border membrane vesicles with PCMBs, as previously described.¹¹⁾ On the other hand, choline uptake up to 30 s was not affected by the same pretreatment (Fig. 6B). A decrease in choline uptake was observed thereafter; however, this may be due to the

Fig. 5. Concentration Dependence of Choline Uptake by Brush-Border Membrane Vesicles

(A) The uptake of choline for 5 s at concentrations between 0.05 and 10 mM. Membrane vesicles were suspended in 100 mM mannitol, 10 mM Hepes (pH 7.5), and 100 mM K-gluconate. The aliquots (20 μ l) were incubated with substrate mixture (180 μ l) containing 100 mM mannitol, 10 mM Hepes (pH 7.5), 100 mM Na-gluconate, valinomycin (8.2–9.4 μ g/mg of protein), and [3 H]choline. Each point represents the mean $\pm$ S.E. of three separate experiments performed in three determinations. (B) Eadie-Hofstee plot of choline uptake, after correction for the nonsaturable component.

Fig. 6. Effect of PCMBs Pretreatment on Tetraethylammonium (A) and Choline (B) Uptake by Brush-Border Membrane Vesicles

Brush-border membrane was pretreated with PCMBs (0.1 mM, pH 7.5) at 0°C for 5 min, and then washed. (A) The uptake of tetraethylammonium was measured in the presence of an outward H^+ gradient as in the legend for Fig. 1. Each point represents the mean $\pm$ S.E. of three determinations from a typical experiment. (B) The uptake of choline was measured in the presence of an interior-negative membrane potential as in the legend for Fig. 4. Each point represents the mean $\pm$ S.E. of six determinations from two separate experiments. Control ($\circ$); and PCMBs pretreatment ($\bullet$).

increased Na^+ permeability by PCMBs pretreatment^{11,33}) which, in turn, facilitates the dissipation of the interior-negative membrane potential.

Effect of Various Compounds on Choline and Tetraethylammonium Uptake To examine the substrate specificity of the choline transport system in renal brush-border membrane, the initial rates of choline uptake were measured in the presence of various compounds (5 mM) known to inhibit the choline transport in other cells and tissues. As shown in Table I, hemicholinium-3, choline and 2-dimethylaminoethanol were potent inhibitors of [3 H]choline uptake by brush-border membrane vesicles. Thiamine and 2-aminoethanol also inhibited the choline uptake significantly ($p < 0.05$).

Effect of these compounds on tetraethylammonium uptake was also examined in the presence of an outward H^+ gradient. All the compounds except choline intensely inhibited the tetraethylammonium uptake. Thus, these compounds can also interact with the H^+ /organic cation

TABLE I. Effect of Various Compounds on Choline and Tetraethylammonium Uptake by Brush-Border Membrane Vesicles

Addition	[3 H]Choline uptake (% of control)	[14 C]TEA uptake (% of control)
Choline	16.3 $\pm$ 0.4	75.2 $\pm$ 0.7
HC-3	10.7 $\pm$ 0.8	10.4 $\pm$ 0.7
2-DMAE	30.2 $\pm$ 1.5	17.0 $\pm$ 0.6
Thiamine	55.2 $\pm$ 1.9	4.1 $\pm$ 0.4
2-AE	81.4 $\pm$ 3.3	24.5 $\pm$ 2.1

Choline uptake for 10 s was measured in the presence of an interior-negative membrane potential as in the legend for Fig. 4, and tetraethylammonium (TEA) uptake for 10 s was measured in the presence of an outward H^+ gradient as in the legend for Fig. 1. The compounds were added at the final concentration of 5 mM. HC-3, hemicholinium-3; 2-DMAE, 2-dimethylaminoethanol; 2-AE, 2-aminoethanol. Each value represents the mean $\pm$ S.E. of six determinations from two separate experiments.

antiport system, and the affinity for this system seems higher than that for the choline transport system.

Discussion

The present results demonstrate that choline can be transported by a carrier-mediated system distinct from H^+ /organic cation antiport system in rat renal brush-border membrane. First, the initial rate of choline uptake by brush-border membrane vesicles was not affected by the pretreatment of the vesicles with a sulfhydryl reagent, PCMBS. Second, the interior-negative membrane potential generated by K^+ -valinomycin induced marked stimulation of choline uptake against a concentration gradient. These findings suggest the presence of a novel choline transport system different from the electroneutral H^+ /organic cation antiport system in renal brush-border membrane. On one hand, choline uptake was slightly, but significantly stimulated in the presence of an outward H^+ gradient. In addition, tetraethylammonium uptake was *cis*-inhibited and *trans*-stimulated by unlabeled choline, although the effects were less pronounced than those produced by unlabeled tetraethylammonium. Thus, choline can also interact with the H^+ /organic cation antiport system, but the affinity of choline for the antiporter should be much lower than that of tetraethylammonium.

Na^+ dependence of choline transport appears to differ with tissues. Na^+ -independent transport of choline was reported in intestinal tissue from guinea pig,³⁴⁾ brush-border membrane vesicles from rabbit intestine³⁵⁾ and guinea pig placenta.³⁶⁾ Recently, Na^+ -independent choline transport was also demonstrated in the inner medullary collecting duct cells from rat kidney.³⁷⁾ On the other hand, Na^+ -dependent transport was reported in chicken intestine,³⁸⁾ rat kidney cortical slices,³⁹⁾ and cultured bovine aortic endothelial cells.⁴⁰⁾ The present results showed that choline transport in rat renal brush-border membrane vesicles was not affected by an inward Na^+ gradient, but was stimulated by an interior-negative membrane potential. These characteristics of choline transport were similar to those in collecting duct cells isolated from rat renal inner medulla.³⁷⁾

The kinetic parameters of choline uptake by brush-border membrane vesicles in the presence of interior-negative membrane potential were determined; the apparent K_m and V_{max} values were 0.77 mM and 12.5 nmol/mg of protein per min, respectively. The reported apparent K_m values are 110 μM in chicken intestine,³⁸⁾ 600 μM in guinea pig jejunum,³⁴⁾ 83 μM in brush-border membrane vesicles from rabbit small intestine,³⁵⁾ 120–130 μM in guinea pig placenta,³⁶⁾ 3 μM in cultured bovine aortic endothelial cells,⁴⁰⁾ and 80 μM in rat inner medullary collecting duct cells.³⁷⁾ Thus, the K_m value of choline transport in the renal brush-border membrane may be higher than those of other choline transport systems.

Hemicholinium-3 and 2-dimethylaminoethanol were potent inhibitors of choline uptake by renal brush-border membrane vesicles. Thiamine and 2-aminoethanol were also effective inhibitors. Thus, the substrate specificity of the choline transport system in the renal brush-border membrane should be similar to those of choline transport systems in other cells and tissues.^{36–38,40)}

In previous reports, we¹¹⁾ and others¹⁸⁾ have demonstrated that sulfhydryl groups are essential for H^+ /organic cation antiport in the renal brush-border membrane and act as binding sites for organic cations such as tetra-

ethylammonium. Therefore, in order to further compare the transport systems for choline and tetraethylammonium, the effect of PCMBS on choline uptake was examined. In contrast to tetraethylammonium uptake, the initial rate of choline uptake was not inhibited by the pretreatment of brush-border membrane vesicles with PCMBS. The present findings indicate that sulfhydryl groups contribute little to potential-sensitive choline transport in the renal brush-border membrane.

During the preparation of this manuscript, Wright *et al.*⁴¹⁾ independently reported the characteristics of choline transport in the rabbit renal brush-border membrane. Our results are compatible with theirs concerning the general characteristics of choline transport such as driving force and substrate specificity. However, discrepancies between the two studies exist. First, they reported that choline uptake was not stimulated in the presence of an outward H^+ gradient and was not inhibited by unlabeled tetraethylammonium. In our study, however, an outward H^+ gradient stimulated the choline uptake slightly but significantly, and this stimulation was suppressed by unlabeled tetraethylammonium. Secondly, they determined the kinetic parameters of choline transport under *trans*-stimulated condition (using membrane vesicles preloaded with unlabeled choline) and showed the existence of a high affinity transport system for choline. We determined the kinetic parameters of choline transport in the presence of an interior-negative membrane potential (a driving force for choline uptake). The K_m value of choline transport in the rat renal brush-border membrane obtained here seems relatively higher than that in the rabbit. Finally, they stated that the addition of $HgCl_2$ inhibited the choline efflux, while we demonstrated the lack of inhibitory effect of PCMBS pretreatment on choline transport. The reason for these discrepancies is not clear at this stage.

The plasma concentration of choline is regulated by the kidney by means of tubular transport and metabolism.^{21–25)} In a micropuncture study in the rat, Acara *et al.*²⁴⁾ demonstrated that a progressive reabsorption of choline along the proximal convoluted tubule occurred at a low plasma concentration of choline below 325 μM , and net secretion of choline occurred when plasma choline levels were elevated above 325 μM . Moreover, they discussed that the metabolism of choline to betaine in the kidney was the major route for the renal clearance of choline up to plasma levels of approximately 325 μM , at which point the choline oxidase system began to saturate. Therefore, at a low plasma concentration, choline should be transported from lumen to cell by a carrier-mediated system which is stimulated by the interior-negative membrane potential and then rapidly metabolized to betaine within the cells. At a high plasma concentration, the intracellular concentration of choline should be increased due to the saturation of metabolism, which in turn would stimulate choline transport from cell to lumen *via* the H^+ /organic cation antiport system.

In conclusion, the present findings suggest that choline can be actively transported by a carrier-mediated system distinct from the H^+ /organic cation antiport system in the renal brush-border membrane and that choline transport is driven by a cell interior-negative membrane potential. This system may play an important role in the tubular reabsorption of choline.

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