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A Choline Transporter in Renal Brush-Border Membrane Vesicles: Energetics and Structural Specificity

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Summary. Choline is a quaternary ammonium compound that is normally reabsorbed by the renal proximal tubule, despite its acknowledged role as a substrate for the renal organic cation (OC) secretory pathway. The basis for choline reabsorption was examined in studies of transport in rabbit renal brush-border membrane vesicles (BBMV). Although an outwardly directed H^+ gradient (pH $6.0_{in}:7.5_{out}$) stimulated uptake of tetraethylammonium (TEA), a model substrate of the OC/H^+ exchanger in renal BBMV, it had no effect on uptake of 1 μM choline. A 5 mM *trans* concentration gradient of choline did, however, drive countertransport of both TEA and choline, although *trans* TEA had no effect on choline accumulation in BBMV. A 20 mM concentration of unlabeled choline blocked uptake of both choline and TEA by >85%, whereas 20 mM TEA blocked only TEA uptake. The kinetics of choline uptake into vesicles preloaded with 1 mM unlabeled choline appeared to involve two, saturable transport processes, one of high affinity for choline (K_t of 97 μM) and a second of low affinity (K_t of ~10 mM), the latter presumably reflecting a weak interaction of choline with the OC/H^+ exchanger. An inside-negative electrical PD stimulated the rate of uptake and supported the transient concentrative accumulation of choline in BBMV. The high affinity transporter showed a marked specificity for choline and closely related analogues. A model of the molecular determinants of substrate-transporter interaction is described. We conclude that the electrogenic high affinity pathway plays a central role in renal reabsorption of choline.

Key Words choline · TEA · organic cation · transport · proximal tubule · kidney · reabsorption · secretion · molecular modeling

Introduction

Choline is required by all cells for the synthesis of the membrane phospholipid, phosphatidylcholine. In addition, it is an essential precursor in neuronal tissues for the synthesis of the neurotransmitter, acetylcholine. Therefore, it is not surprising that the plasma concentration of choline is tightly regulated (Bligh, 1952). Transport of choline in the brain (Ducis, 1988) and in red cells (Déves & Krupka, 1979) has received considerable attention, as has choline absorption in the intestine (Sanford & Smyth, 1971; Herzberg & Lerner, 1973; Kuczler, Nahrwold & Rose, 1977; Kessler et al., 1978; Hegazy & Schwenk, 1984; Sheard & Zeisel, 1986), reflecting its role as a dietary requirement in some animals, including humans (Zeisel, 1981; Zeisel et al., 1991). However, the mechanism(s) associated with the renal transport of choline are poorly understood.

Renal tubular transport is directly involved in the regulation of the plasma concentration of choline (Acara, 1975; Besseghir, Pearce & Rennick, 1981) by means of transepithelial transport pathways that produce either net tubular secretion or reabsorption, depending on the circulating level of choline (Vander, 1962; Acara & Rennick, 1973; Acara, Roch-Ramel & Rennick, 1979; Besseghir et al., 1981). When the plasma concentration is less than 100 μM, there is a net reabsorption of choline, whereas net secretion occurs when the concentration exceeds 100 μM (Besseghir et al., 1981). The secretory pathway for organic cations (and bases; OCs) is well characterized (Ross & Holohan, 1983). Flux from the blood into the cell, across the peritubular membrane, involves a carrier-mediated process that is driven by the inside-negative membrane potential of the cell or, possibly, by mediated exchange with a pool of intracellular OCs (Holohan & Ross, 1980; Dantzler & Brokl, 1988; Montrose-Rafizadeh et al., 1989; Sokol & McKinney, 1990). Flux from proximal cells into the tubular filtrate, across the luminal brush-border membrane, involves a carrier-mediated exchange of OC for H^+ (e.g., Holohan & Ross, 1981; Ross & Holohan, 1983; Wright, 1985; Wright & Wunz, 1987*b*). The luminal OC/H^+ exchanger is believed to be the rate-limiting step in OC secretion (Schäli et al., 1983; Montrose-Rafizadeh, Roch-Ramel & Schäli, 1987), as well as the active step in secretion (Holohan & Ross, 1981; Ross & Holohan, 1983). Choline is known to interact with both

the basolateral (Dantzler & Brokl, 1988; Sokol & McKinney, 1990) and luminal OC transporters (Holohan & Ross, 1980; Wright, 1985; Dantzler & Brokl, 1988), and inhibitors of OC secretion block the renal clearance of choline when the plasma concentration exceeds 100 μM (Trimble, Acara & Rennick, 1974).

Circulating levels of choline in plasma typically do not exceed ~25 μM (Bligh, 1952; Besseghir et al., 1981). Therefore, under normal circumstances, choline is reabsorbed, not secreted, by the proximal tubule. Despite this fact, the reabsorptive steps for choline have received little attention. In the present study, we examined the transport pathways for choline in BBMVs from rabbit kidney. Choline uptake involved at least two distinct pathways, one of high affinity and one of low affinity for this OC. The low affinity pathway was the OC/H^+ exchanger. The high affinity pathway was insensitive to gradients of either H^+ or Na^+, but was stimulated by inside-negative electrical PD, and showed a marked degree of specificity for choline and closely related chemical structures. It is likely that the high affinity pathway is involved in the reabsorption of choline by the proximal tubule.

Materials and Methods

Preparation of Brush-Border Membrane Vesicles

BBMV were prepared from corticies isolated from kidneys of New Zealand White rabbits, using a Ca^{2+}-Mg^{2+} precipitation procedure modified from that of Kinsella, Holohan and Ross (1979). Compared to the initial homogenate, these membranes are routinely enriched approximately 10-fold in activity of trehalase and alkaline phosphatase, and one fold or less in activity of Na,K-ATPase and K-dependent *p*-nitrophenylphosphatase (Wright & Wunz, 1987*a*). BBMV were used within 24 hr of preparation if held on ice, or within two weeks if kept frozen in liquid nitrogen (there was no systematic drop in transport activity, compared to fresh membranes, by BBMV held in these ways). Unless otherwise noted, vesicles were suspended in 150 mM KCl, 300 mM mannitol, and 10 mM N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES) adjusted with KOH to pH 7.5.

Measurement of Choline Transport

Uptake of choline was measured using a rapid filtration procedure (Wright et al., 1983). Briefly, the transport reaction was started by rapidly mixing 5–10 μl of the BBMV suspension (35–350 mg of membrane protein) with 90–195 μl of transport buffer containing salts and radiolabeled substrate (see the figure legends for a description of the composition of intra- and extravesicular solutions in individual experiments). The transport reaction was stopped by adding 1 ml of an ice-cold "quench" solution, which typically was identical to the BBMV suspension solution, with the addition of 100 μM $HgCl_2$ (in preliminary experiments we found that the addition of $HgCl_2$ inhibited efflux of accumulated choline from BBMV). One ml of the quenched reaction mixture was filtered under vacuum through a 0.45 μm filter (type HAWP; Millipore). The trapped membrane vesicles were rinsed with 4 ml of the quench solution, and the remaining radioactivity was measured using a liquid scintillation counter (Beckman model LSC3801). All samples were corrected for variable quench. Each sample was also corrected for nonspecific binding of radioactive substrate to the filters and membranes by subtracting the number of counts remaining on filters when membranes were initially mixed with the transport buffer to which the cold quench solution had already been added. Uptakes were expressed as moles of choline accumulated per milligram of membrane protein (Bio-Rad; γ-globulin standard). Experimental incubations were performed at room temperature (21–23°C). Control experiments revealed that choline uptake was a linear function of time for 3 sec over the range of substrate concentrations and under the conditions employed in the studies of the kinetics of choline transport.

Synthesis of Choline Analogues

Several structural analogues of choline were synthesized for use in tests of the specificity of choline transport in renal BBMV (refer to Figs. 8 through 12 for chemical structures). N,N,N-trimethyl-N-(3-hydroxypropyl)ammonium iodide was synthesized by combining 10 mmol of 3-dimethylamino-1-propanol with 20 mmol of methyl iodide in 10 ml of ethanol (EtOH) and heating at reflux for 6 hr. The reaction mixture was cooled to room temperature, filtered, and the solid was washed with cold EtOH, then dried *in vacuo* 3 hr at 110°C to yield 2.4 g (98% yield) of product: mp 198–199°C. N,N,N-trimethyl-N-*n*-propylammonium bromide was prepared as described by Howton (1947). N,N,N-triethyl-N-(2-hydroxyethyl)ammonium bromide was prepared by refluxing 50 mmol of bromoethanol with 25 mmol of triethylamine in 20 ml of EtOH for 18 hr. The solution was cooled to room temperature and poured into 50 ml of ether. The resulting precipitate was collected by filtration, washed with ether, and dried *in vacuo* to yield 4.13 g (73% yield) of a white solid: softens at 185°C; mp 191–193°C. N,N-dimethyl-N,N-di(2-hydroxyethyl) ammonium bromide was synthesized by refluxing 11 mmol of dimethylethanolamine and 22 mmol of bromoethanol for 15 hr in 10 ml EtOH. The solvent was evaporated and the oil was triturated several times with ether, then dried under vacuum to give 2.3 g (98% yield) of the product as a dark oil. N-methyl-N,N,N-tri(2-hydroxyethyl)ammonium iodide was prepared from 6.7 mmol of triethanolamine and 6.7 mmol of methyl iodide using the procedure of Werber and Shalitin (1973). A solution of the reagents in EtOH was stirred for 15 hr in EtOH then refluxed for 2 hr to provide 1.4 g (72% yield) of a viscous oil. N-ethyl-N,N,N-tri(2-hydroxyethyl)ammonium iodide was synthesized as described for N-methyl-N,N,N-tri(2-hydroxyethylammonium) iodide. After cooling the reaction mixture, a small amount of solid crystallized and was collected by filtration to yield 165 mg (8% yield) of the product: mp 133–135°C. The structure of each compound was confirmed by ^{1}H-NMR. All melting points (mp) are uncorrected.

Abbreviations of Chemical Names

N,N,N-triethyl-N-(2-hydroxyethyl)ammonium (TEHE); N,N,N-trimethyl-N-(3-hydroxypropyl)ammonium (TMHP); N,N-di-

methyl-N-(2-hydroxyethyl)ammonium (DMHE); N-(2-hydroxyethyl)ammonium (HE); N-ethyl-N,N,N-tri(2-hydroxyethyl)ammonium (ETHE); N-methyl-N,N,N-tri(2-hydroxyethyl) ammonium (MTHE); N,N-dimethyl-N,N-di(2-hydroxyethyl) ammonium (DMDHE); N,N-dimethylaminopropanone (DMAP); N,N,N-trimethyl-N-*n*-propylammonium (TMP); tetrapentylammonium (TPeA); tetraethylammonium (TEA); tetramethylammonium (TMA); hemicholinium-15 (HC-15); hemicholinium-3 (HC-3).

Development of a Working Model for the Choline Transporter Binding Site

The molecular modeling program SYBYL (Tripos Associates, St. Louis, MO) was used to generate the structures of choline and its analogues. Partial atomic charges were calculated using the AM1 program within SYBYL, then the structures were energy minimized using the Tripos force field. The main structural components of the choline transporter receptor site were modeled in the following way. Using the molecular graphics program Midas-Plus (Computer Graphics Lab, School of Pharmacy, University of California, San Francisco, CA), a carboxyl group (from aspartic acid) and a peptide bond (serine-serine) were positioned approximately as shown in Fig. 15 in relation to the minimum energy conformation of HC-3. The peptide and carboxyl residues, which were arbitrarily chosen to provide the appropriate functional groups, were N-terminated as acetamides while N-methyl groups were used to terminate the carboxylic acid ends of the amino acid chains. This model was then energy minimized using AMBER (Singh et al., 1986) and the all-atom force field (Weiner et al., 1986) keeping the position of the HC-3 atoms frozen and allowing the other residues to move. Calculations involving other choline analogues kept the amino acid residues frozen in their minimized positions and allowed only the choline analogue to move. Stereo projections of the receptor model can be viewed using a 3-D viewer stereoscope (e.g., Model CF8, Abrams Instrument, Okemos, MI).

Chemicals

[^{3}H]Choline (~88 Ci · mmol^{-1}) was purchased from New England Nuclear and [^{14}C]TEA (56 mCi · mmol^{-1}) was purchased from Wizard Laboratories (Davis, CA). All other chemicals were purchased from common sources and were the highest quality available.

Results

Time Course of Choline and TEA Transport

Uptake of TEA (17 μM) by rabbit renal BBMV was stimulated by an outwardly directed H^+ gradient (pH 6.0_{in} : 7.5_{out}) (Fig. 1*A*). Uptake at 5 sec was six times higher than that noted under the control condition (pH 7.5_{in} : 7.5_{out}), and at 60 sec TEA content in the BBMV was 3.7 times that noted at 60 min (the nominal equilibrium condition). This effect of a H^+ gradient on TEA transport, reported previously by

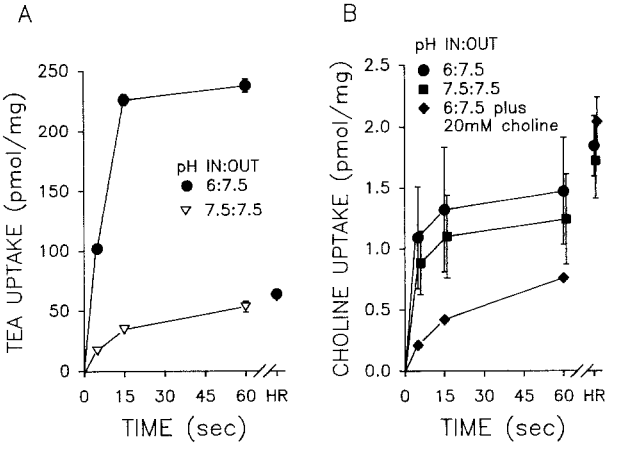

Fig. 1. Effect of pH gradients on TEA and choline uptake in BBMV. (*A*) Vesicles were pre-equilibrated in 150 mM KCl, 44 mM mannitol, 20 μM valinomycin (VAL), and 10 mM HEPES adjusted to pH 6.0 or 7.5 with KOH (HEPES-KOH). Uptakes were measured in a transport buffer with final concentrations of 150 mM KCl, 44 mM mannitol, 20 μM VAL, 10 mM HEPES-KOH (pH 7.5), and 17 μM ^{14}C-TEA. Points are the means of duplicate uptakes measured in a representative experiment. (*B*) Vesicles were pre-equilibrated in 150 mM KCl, 300 mM mannitol, and 10 mM HEPES-KOH (pH 6.0 or 7.5). Uptakes were measured in a transport buffer containing final concentrations of 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), and 1 μM choline trace-labeled with ^{3}H-choline. Points are the means (±SE) of duplicate uptakes measured in three separate membrane preparations. In two cases, 20 mM unlabeled choline was added (to block carrier-mediated choline uptake by isotope dilution), replacing 20 mM of KCl; in these cases, points are the mean of duplicate uptakes measured in two membrane preparations.

us (Wright & Wunz, 1987*b*) and others (Rafizadeh et al., 1986; Sokol & McKinney, 1990), supports the contention that TEA transport in rabbit renal BBMV involves a carrier-mediated exchange with H^+ (or, alternatively, a cotransport with OH^-). Indeed, this profile of sensitivity of transport to a pH gradient is the criterion typically used to gauge the interaction of a substrate with the renal OC/H^+ exchanger (Ross & Holohan, 1983). Thus, it was significant that an outwardly directed pH gradient had no effect on the accumulation of choline at any time point studied (Fig. 1*B*). Choline uptake into the BBMV did involve a saturable component; however, as shown in two experiments in which we included 20 mM unlabeled choline in the extravesicular solution, the 5-sec uptake of 1 μM ^{3}H-choline was reduced by 76–81%, whereas uptake at 60 min was not affected (Fig. 1*B*). Also of note, when K^+ in the extravesicular solution was replaced with Na^+, resulting in an inwardly directed Na^+ gradient, choline uptake was not affected (*data not shown*). These data suggest that mediated uptake of choline, at least from micromolar concentrations, does not involve a substantial inter-

action with the same pathway used by TEA (i.e., the OC/H^+ exchanger) or with a Na-dependent pathway.

In a separate experiment, we verified that transport of choline by BBMV involved movement of substrate into an osmotically reactive space. Accumulation of choline was measured after 60-min incubations in transport buffers containing 0.03 μM ^{3}H-choline and increasing concentrations of the impermeant solute, sucrose (all other constituents were the same as the vesicle resuspension buffer). Choline accumulation in the BBMV was inversely related to the external sucrose concentration (*data not shown*), as expected if uptake included partitioning into a space whose volume was reduced by the osmotic extraction of water. Choline uptake in the presence of 600 mM sucrose was 34% of that noted in the absence of sucrose, and the y intercept of the line describing uptake *vs.* 1/[sucrose] suggested that binding of choline to the vesicle membrane could account for as much as 29% of the total equilibrium accumulation of substrate. In several experiments we examined the effect of 20 mM choline on accumulation of ^{3}H-choline, and the results permitted two conclusions to be drawn. First, binding was presumably a nonsaturable process, because the addition of 20 mM unlabeled choline did not effect the accumulation of choline observed after 60 min of uptake (Fig. 1*B*). Second, binding was either a slow process, compared to mediated uptake, or occurred at intravesicular sites, because 20 mM choline blocked approximately 95% of total choline uptake observed at 2–3 sec (*data not shown*).

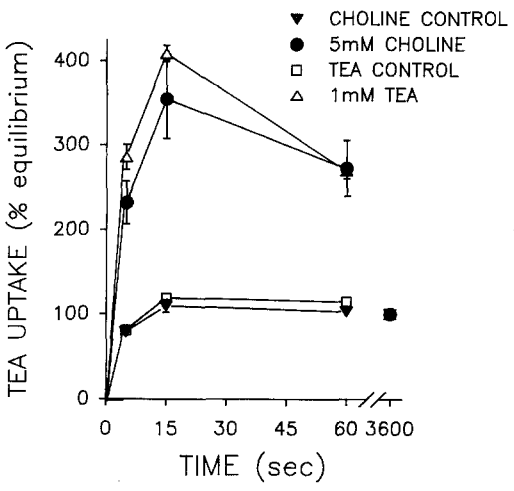

Fig. 2. Effect of *trans*-substrate concentrations on TEA uptake in BBMV. Vesicles were pre-equilibrated in 150 mM KCl and 5 mM HEPES-KOH (pH 7.5). In some cases pre-equilibration included either 5 mM choline or 1 mM TEA (as indicated in the figure). Uptake was measured in transport buffers containing 150 mM KCl, 5 mM HEPES-KOH (pH 7.5), and 25 μM ^{14}C-TEA. Unlabeled TEA or choline was added as necessary to maintain a constant extravesicular (*cis*) concentration of the preload substrate (125 μM for choline; 25 μM for TEA). Points are means (±SE) from two separate experiments.

Reciprocal Effects of *Trans*-Substrate Concentrations on Choline and Tea Transport

TEA transport in renal BBMV is accelerated by outwardly directed gradients of suitable OC's, such as TEA itself, as well as by outwardly directed gradients of H^+. As shown previously (Wright & Wunz, 1988), this reflects the carrier-mediated exchange of substrates which share a common countertransport pathway. Choline has been shown to influence the activity of OC/H^+ exchanges in renal BBMV (Holohan & Ross, 1980; Wright, 1985), so it was of interest to examine the effect of choline gradients on TEA transport and of TEA gradients on choline transport.

An outwardly directed gradient of unlabeled TEA (1 mM) increased by 3.5-fold the 5-sec accumulation of 17 μM ^{14}C-TEA and supported a 3.4-fold overshoot of ^{14}C-TEA accumulation in our BBMV (Fig. 2), in general agreement with our earlier observations (Wright & Wunz, 1988). An outwardly directed gradient of unlabeled (5 mM) choline also stimulated the 5-sec accumulation of ^{14}C-TEA (three-fold) and supported a 3.2-fold overshoot, confirming the contention that choline and TEA can share a common exchange pathway. The inverse experiment produced a different result. Whereas an outwardly directed choline gradient increased the 5-sec uptake of 125 μM ^{3}H-choline (over 20-fold), and supported an overshoot of choline accumulation (15-fold), an outwardly directed TEA gradient had no effect on choline uptake (Fig. 3).

Reciprocal *Cis* Inhibition of Choline and Tea Transport

A possible explanation of the differential effects of *trans*-substrate concentrations on TEA *vs.* choline uptake is that (at least) two separate transport pathways are involved: the TEA/H^+ (i.e., OC/H^+) exchanger, which will accept choline when present in large concentrations, thereby leading to the observed *trans*-stimulation of TEA uptake noted in Fig. 2, and a second pathway that has a relatively high affinity for choline and a low affinity for TEA and H^+, thereby explaining the failure of outwardly directed gradients of TEA and H^+ to significantly influence choline uptake (Figs. 1*B* and 3).

To provide further support for this hypothesis,

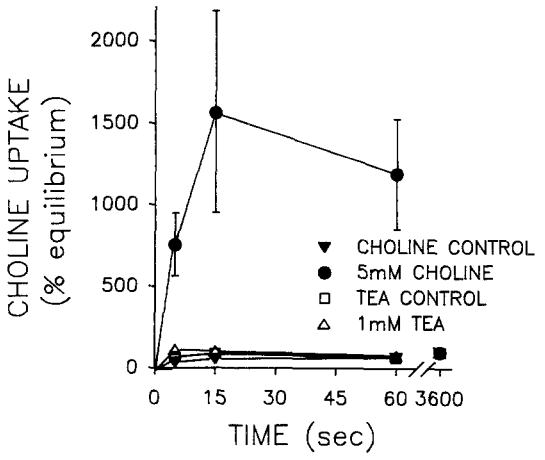

Fig. 3. Effect of *trans*-substrate concentrations on choline uptake in BBMV. Vesicles were pre-equilibrated in 150 mM KCl and 5 mM HEPES-KOH (pH 7.5). In some cases pre-equilibration included either 5 mM choline or 1 mM TEA (as indicated in the figure). Uptake was measured in transport buffers containing 150 mM KCl, 5 mM HEPES-KOH (pH 7.5), and 125 μM ^{3}H-choline. Unlabeled TEA or choline was added as necessary to maintain a constant extravesicular (*cis*) concentration of the preload substrate (125 μM for choline; 25 μM for TEA). Points are means (±SE) from two (*trans*-TEA) to five (*trans*-choline) separate experiments.

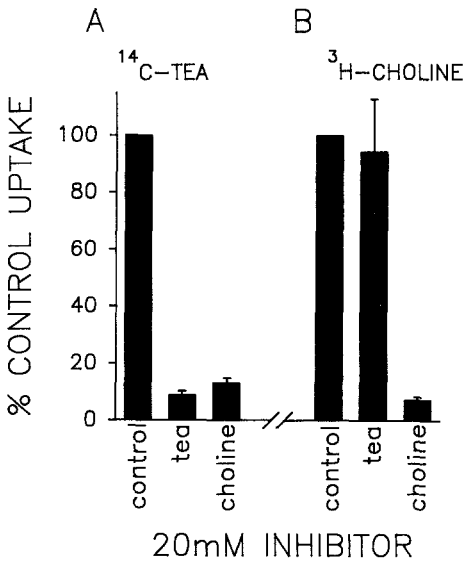

Fig. 4. Reciprocal inhibition of choline and TEA transport. Vesicles were pre-equilibrated in 150 mM KCl and 5 mM HEPES-KOH (pH 6). Two-sec uptakes were measured in buffers containing final concentrations of 150 mM KCl, 5 mM HEPES-KOH (pH 7.5), either (*A*) 50 μM ^{14}C-labeled TEA or (*B*) 1 μM ^{3}H-labeled choline, and 20 mM concentrations of either unlabeled choline or TEA. Vertical bars represent mean uptake (+SE), expressed as the percentage of uptake measured in the absence of inhibitor, from three experiments using separate membrane preparations.

we examined the effect of large *cis* concentrations of choline and TEA on the transport of these two substrates. As shown in Fig. 4*A*, uptake of 50 μM ^{14}C-TEA was blocked >90% by a 20 mM *cis* concentration of unlabeled TEA; indeed, we routinely use 20 mM TEA to provide a complete competitive block of the carrier-mediated component of TEA transport in renal BBMV (e.g., Wright & Wunz, 1987*b*, 1988). A 20 mM *cis* concentration of choline was also reasonably effective at blocking TEA transport, reducing uptake by approximately 85% (Fig. 4*A*). Uptake of choline showed a completely different profile of inhibition (Fig. 4*B*). Whereas 20 mM unlabeled choline reduced uptake of 1 μM ^{3}H-choline by >90%, 20 mM TEA had no significant inhibitory effect. These data suggest that, although choline can interact with the TEA/H$^+$ exchange pathway in renal BBMV, there is a second pathway (or set of pathways) having a substantially higher affinity for choline than does the TEA/H$^+$ exchanger.

Kinetics of Choline Transport

Measurements of the effect of *cis* choline concentration on the rate of choline uptake into BBMV used vesicles preloaded with 1 mM unlabeled choline as a means to increase the initial rate of uptake. Also, because membrane potential was found to influence choline transport in these BBMV (discussed below), all studies of the kinetics of choline transport were carried out under "voltage-clamp" conditions, using equal intra- and extravesicular concentrations of K$^+$ in the presence of the K$^+$ ionophore, valinomycin. As shown in Fig. 5, choline uptake was a curvilinear function of concentration over the range of 25 μM to 10 mM. An Augustinsson-Woolf-Hofstee plot of these data (*see* inset of Fig. 5) was distinctly nonlinear indicating that choline uptake into BBMV involved more than a single saturable site described by Michaelis-Menten kinetics. Two models for choline uptake were considered: (i) a single saturable carrier, with high affinity for choline, plus a nonsaturable process (i.e., diffusion or nonspecific binding); and (ii) two saturable carriers, one with high affinity and one with low affinity for choline. Both models described the data with equal efficacy. However, the results presented in Figs. 1–4 indicate that choline interacts with at least two separate transport pathways, so the two-carrier model offers the most likely explanation for the observed profile of choline uptake in BBMV:

$$J = \frac{J^1_{max}[C]}{K^1_t + [C]} + \frac{J^2_{max}[C]}{K^2_t + [C]} \quad (1)$$

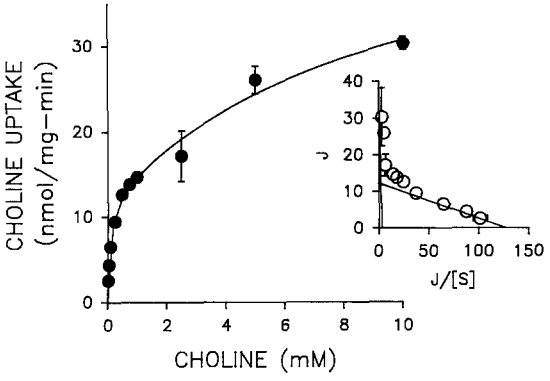

Fig. 5. The effect of increasing *cis* choline concentration on the rate of choline transport into BBMV. Vesicles were pre-equilibrated in 149 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), 20 μM VAL and 1 mM choline (to establish a *trans*-stimulation condition). Three-sec uptakes were measured in a transport buffer with final concentrations of 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), 20 μM VAL, a trace-label of ^{3}H-choline plus unlabeled choline to achieve total substrate concentrations ranging from 25 μM to 10 mM. Points are means (±SE) of uptakes measured in triplicate in three separate membrane preparations. The curve was fit to the data using a nonlinear regression algorithm (Enzfitter, BioSoft), assuming the presence of two saturable choline transport pathways ($K_t^1 = 97 \pm 40.4$ μM; $J_{max}^1 = 12 \pm 2.0$ nmol · mg^{-1} · min^{-1}; $K_t^2 = 10 \pm 8.0$ mM; $J_{max}^2 = 38 \pm 13.1$ nmol · mg^{-1} · min^{-1}; *see* text). The inset is a Woolf-Augustinsson-Hofstee plot of these data; the lines are drawn according to the kinetic constants for the two pathways calculated from the nonlinear regression algorithm.

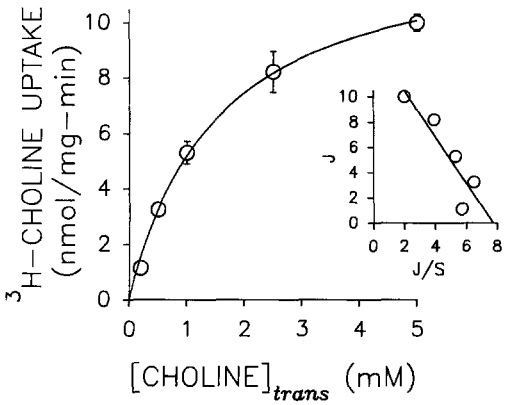

Fig. 6. The effect of increasing *trans* concentration of choline on the rate of choline transport into BBMV. Vesicles were pre-equilibrated in 145–150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), and from 0 to 5 mM unlabeled choline. Three-sec uptakes were measured in transport buffers containing final concentrations of 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), and a total concentration of choline (i.e., ^{3}H-choline and unlabeled choline carried over from the membrane suspension) of 125 μM. Each point is the mean (±SE) of uptakes, measured in triplicate, in BBMV from three separate membrane preparations. Uptakes presented here represent choline accumulation in excess of that noted in the absence of intravesicular choline (i.e., zero *trans* condition). Zero *trans* uptake was 2.5 ± 0.29 nmol · mg^{-1} · min^{-1}. The line describes a Michaelis-Menten-type relationship (*see* text) and was fit to the data using a nonlinear regression algorithm ($K_{t\text{-}trans} = 1.54 \pm 0.08$ mM; $J_{max}^* = 13.2 \pm 0.59$ nmol · mg^{-1} · min^{-1}). The inset is a Woolf-Augustinsson-Hofstee plot of these data.

where J is the rate of total choline transport from an external substrate concentration of $[C]$; J_{max} is the maximal rate of transport *via* the indicated pathway (the superscripts 1 and 2 refer to the "high affinity" and "low affinity" choline transport pathways, respectively), and K_t is the external choline concentration resulting in half-maximal choline transport *via* the indicated pathway. Pathway 2 ($K_t^2 = 10 \pm 8.0$ mM; $J_{max}^2 = 38 \pm 13.1$ nmol · mg^{-1} · min^{-1}) is likely to be the OC/H$^+$ exchanger, with which choline appeared to have an interaction when present at high concentration. However, the kinetic characteristics of pathway 1 ($K_t^1 = 97 \pm 40.4$ μM; $J_{max}^1 = 12 \pm 2.0$ nmol · mg^{-1} · min^{-1}) indicate that it was the dominant mode of choline uptake from concentrations of 1 mM or less.

Intravesicular choline acted as an energetic activator of choline uptake (Fig. 3) and, therefore, must bind at the cytoplasmic face of the choline transporter (*see* Turner, 1983). The concentration dependency of this interaction was examined by gauging the ability of increasing *trans* concentrations of choline to stimulate the uptake of a fixed concentration of ^{3}H-choline. As shown in Fig. 6, the stimulatory effect of intravesicular choline on uptake of 125 μM choline was a saturable function of the *trans*-substrate concentration over the range of concentrations examined. The relationship between the *trans*-choline concentration and uptake could be described by the Michaelis-Menten equation:

$$J^* = \frac{J_{max}^* [C]_{trans}}{K_{t\text{-}trans} + [C]_{trans}} \quad (2)$$

where J^* is the rate of uptake of 125 μM ^{3}H-choline (minus the uptake measured in the absence of intravesicular choline), J_{max}^* is the maximal rate of uptake of 125 μM ^{3}H-choline, $[C]_{trans}$ is the intravesicular concentration of choline, and $K_{t\text{-}trans}$ is an apparent half-saturation constant for the interaction of choline at the *trans*-aspect of the choline transporter. In three separate experiments, the average value for J_{max}^* was 13.2 ± 0.59 nmol · mg^{-1} · min^{-1}, with a $K_{t\text{-}trans}$ of 1.54 ± 0.08 mM. It is appropriate to emphasize that these apparent kinetic constants probably reflect a saturable choline flux through the two separate pathways noted above (i.e., the high and low affinity pathways), although uptake of 125

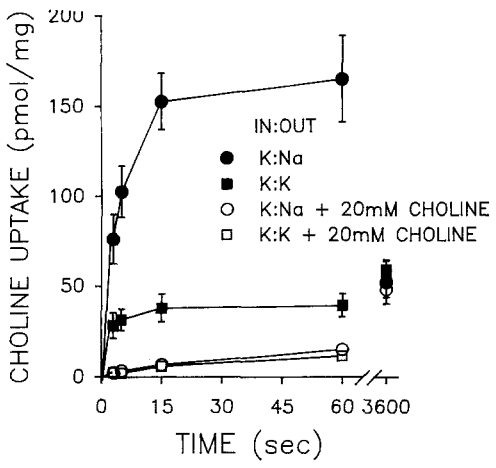

Fig. 7. Effect of an inside-negative PD on choline uptake into BBMV. Vesicles were pre-equilibrated in 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), and 20 μM VAL. Uptakes were measured in a transport buffer containing either 150 mM KCl or NaCl (as indicated), 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), and 25 μM choline trace-labeled with ^{3}H-choline. In some cases 20 mM unlabeled choline chloride was added (replacing an equal concentration of KCl) to block carrier-mediated choline uptake. Points are means (±SE) of uptakes measured in duplicate in three separate membrane preparations.

μM choline was likely to have been dominated by the high affinity pathway.

Effect of Membrane Potential on Choline Transport

As a quaternary ammonium compound, choline is positively charged. Therefore, we examined the effect of an inside-negative electrical PD on the uptake of choline by renal BBMV. Vesicles were pre-equilibrated in a solution containing 150 mM K^+ and the K^+-ionophore, valinomycin, and uptakes were measured in transport buffers containing either 150 mM K^+ (resulting in a nominal Nernstian PD of 0 mV) or 150 mM Na^+ (a nominal Nernstian PD at time zero of 60 mV, inside negative). As shown in Fig. 7, the inside-negative PD stimulated the initial (i.e., 3-sec) rate of choline uptake 2.7-fold over the control rate and supported a 3.2-fold overshoot at 60 sec. Membrane potential appears to have influenced a carrier-mediated process, rather than simple diffusion, because the addition of 20 mM unlabeled choline to the transport buffer blocked the stimulatory effect of the inside-negative PD by >95%.

Structural Specificity of Choline Transport

Several series of choline analogues were tested for their ability to inhibit choline accumulation in renal BBMV. The apparent affinity of the electrogenic choline transporter (K_t of ~100 μM) is approximately 100-times that of the OC/H^+ exchanger (K_t of ~10 mM). Consequently, the use of a concentration of labeled substrate (25 μM) that was much less than the apparent K_t of either transporter assured that the bulk of the choline transport (>90%) occurred *via* the high affinity, electrogenic pathway. We used 1 mM concentrations of the analogues to maximize their interaction with the transport receptor. Choline accumulation measured in the presence of 20 mM unlabeled choline averaged less than 5% of that measured in its absence, suggesting that virtually all of the choline accumulation occurred *via* a carrier-mediated pathway(s).

As shown in Fig. 8, changes in the alkyl substituents on the quaternary nitrogen of choline had a profound effect on inhibitory effectiveness of choline analogues. HE was a poor inhibitor of labeled choline uptake, whereas DMHE and unlabeled choline both blocked transport by >85%. Displacing the hydroxyl group from the quaternary nitrogen by one additional carbon (TMHP) still permitted an effective interaction with the transporter, but replacing the methyl residues around the quaternary nitrogen with ethyl groups (TEHE) completely eliminated the inhibitory interaction with the receptor. When one of the three methyl residues of choline was replaced with a hydroxyethyl residue, there was still a marked inhibition of choline transport (Fig. 9, DMDHE), whereas replacement of two of the methyl residues with hydroxyethyl residues effectively eliminated inhibitory interactions (Fig. 9, MTHE, ETHE).

The importance of the hydroxyl residue for receptor recognition is apparent from the inhibitory interactions presented in Fig. 10. Replacement of the hydroxyl group with either a methyl (TMP), carbonyl (DMAP), or carboxyl residue (betaine) virtually eliminated an interaction of inhibitor with the transporter. Likewise, the tetraalkylammonium compounds (Fig. 11) had little effect on the transporter, with neither TMA nor TEA reducing transport of choline. TPeA did reduce choline transport (by 60%), but that effect was likely to be noncompetitive in nature (*see* Naujokaitis, Fisher & Rabinovitz, 1982).

Finally, both HC-3 and HC-15, inhibitors of high affinity choline transport in a number of systems (Lerner, 1989), were effective inhibitors of choline transport in renal BBMV (Fig. 12), although HC-3 was substantially more potent than HC-15. Indeed, the apparent K_i for HC-3 as an inhibitor of choline uptake was 80 ± 11.8 μM ($n = 3$), which was approximately equivalent to the K_t^1 for choline uptake *via* the high affinity pathway.

We also examined the effects of several other

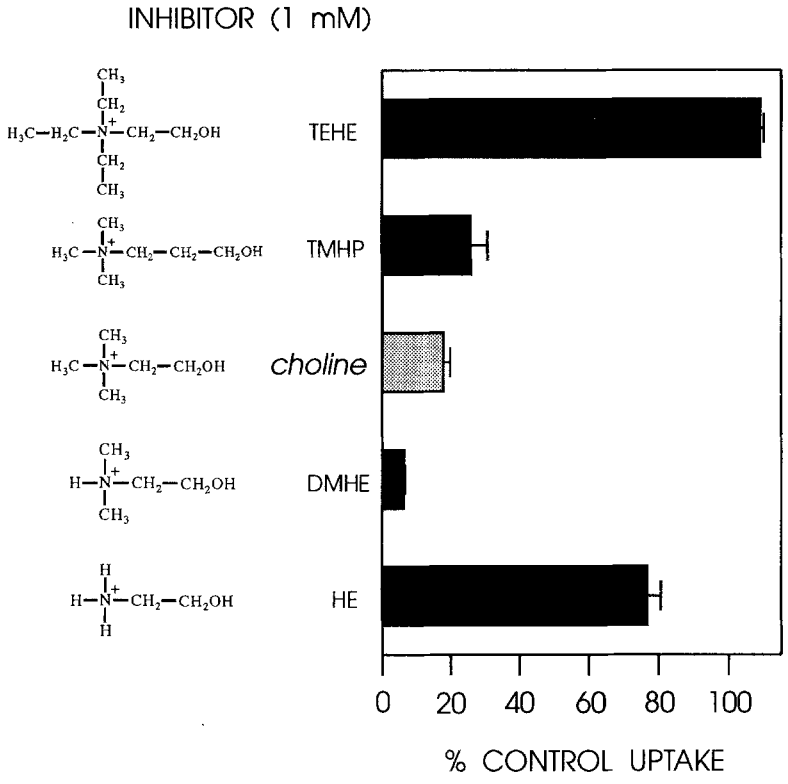

Fig. 8. Inhibition of choline uptake into BBMV by *cis* concentrations of choline analogues; effect of different alkyl substituents on the quaternary nitrogen. Vesicles were pre-equilibrated in 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), 20 μM VAL, and 1 mM unlabeled choline. Two- or three-sec uptakes were measured in transport buffers containing final concentrations of 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), a total concentration of choline of 25 μM, labeled with ^{3}H-choline, and a 1 mM concentration of choline or a structural analogue of choline. Each horizontal bar represents the uptake of choline (+SE), as a percentage of control measured in the absence of inhibitor, measured in triplicate in BBMV from two or more separate membrane preparations. Control uptake for the three experiments was 21.6 ± 3.5 pmol · mg^{-1} · sec^{-1}. The structural formula of each inhibitor is presented to the left of the horizontal bars. Abbreviations used and sample size: N,N,N-triethyl-N-(2-hydroxyethyl)ammonium (TEHE; n = 2); N,N,N-trimethyl-N-(3-hydroxypropyl)ammonium (TMHP; n = 3), N,N-dimethyl-N-(2-hydroxyethyl)ammonium (DMHE; n = 2); N-(2-hydroxyethyl)ammonium (HE; n = 2); choline (n = 6).

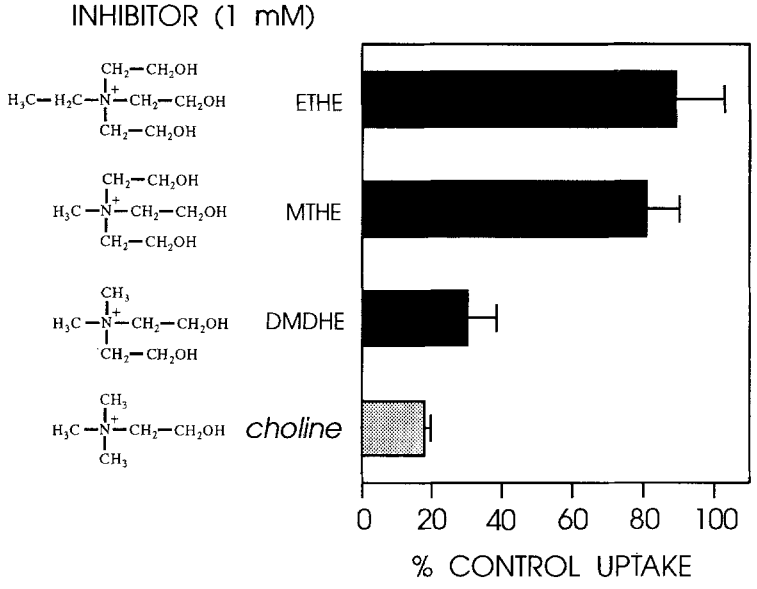

Fig. 9. Inhibition of choline uptake into BBMV by *cis* concentrations of choline analogues; effect of hydroxyethyl substituents on the quaternary nitrogen. Conditions as in Fig. 8. Abbreviations used and sample size: N-ethyl-N,N,N-tri(2-hydroxyethyl)ammonium (ETHE; n = 3); N-methyl-N,N,N-tri (2-hydroxyethyl)ammonium (MTHE; n = 3); N,N-dimethyl-N,N-di (2-hydroxyethyl)ammonium (DMDHE; n = 3); choline (n = 6).

OCs on choline transport in renal cortical BBMV. One mM thiamine reduces choline transport in guinea pig placenta by >50% (Sweiry & Yudilevich, 1985), whereas it reduced choline transport in renal BBMV by 25% (±10.7%; n = 2). N^1-methylnicotinamide (NMN) has been suggested to be both reabsorbed, as well as secreted (Besseghir et al., 1981). However, in a single experiment, we found no effect of 1 mM NMN on the uptake of choline in renal BBMV (*data not shown*).

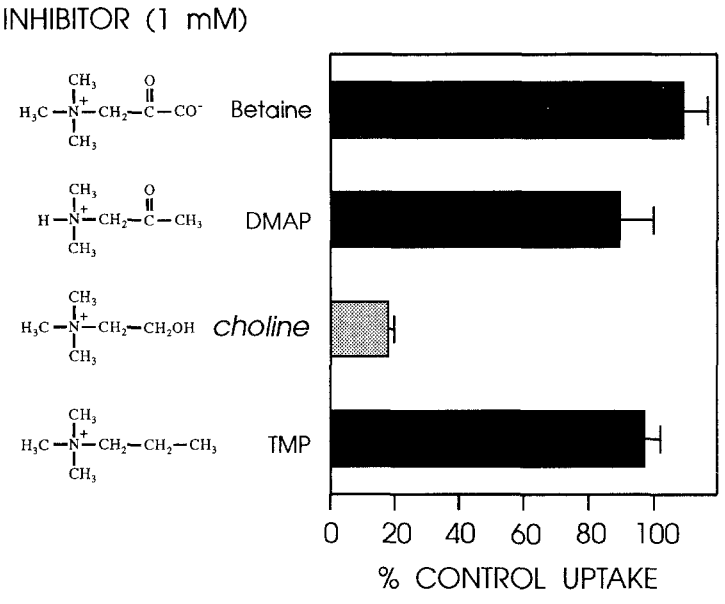

Fig. 10. Inhibition of choline uptake into BBMV by *cis* concentrations of choline analogues; effect of modification of the hydroxyl residue. Conditions as in Fig. 8. Abbreviations used and sample size: N,N-dimethylaminopropanone (DMAP; $n = 2$); N,N,N-trimethyl-N-*n*-propylammonium (TMP; $n = 3$); betaine ($n = 3$); choline ($n = 6$).

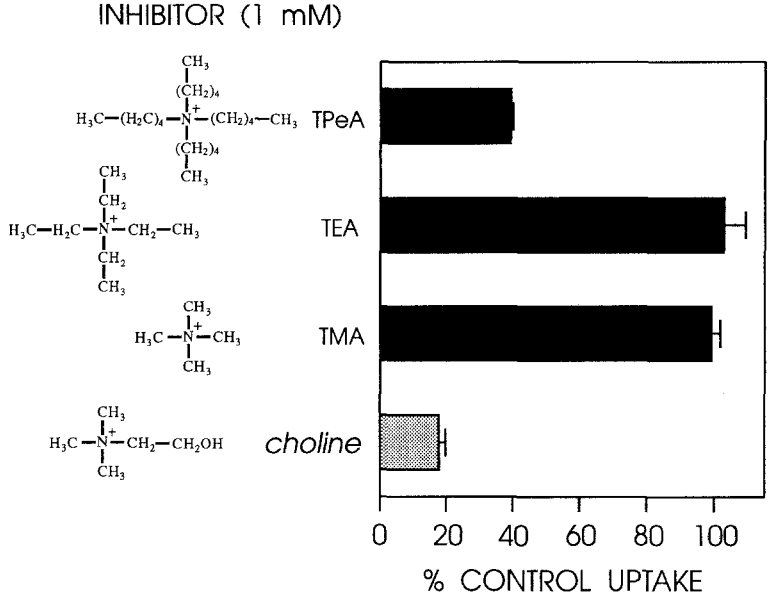

Fig. 11. Inhibition of choline uptake into BBMV by *cis* concentrations of choline analogues; effect of tetraalkyl quaternary ammonium compounds. Conditions as in Fig. 8. Abbreviations used and sample size: tetrapentylammonium (TPeA; $n = 2$); tetraethylammonium (TEA; $n = 3$); tetramethylammonium (TMA; $n = 3$); choline ($n = 6$).

A *cis* inhibitor of transport need not be transported itself; it can simply bind to the transport receptor and thereby form a nontranslocatable transport complex. TMHP and DMHE (*see* Fig. 8) were both effective at producing a *trans*-stimulation of choline uptake in BBMV, with the former compound supporting a transient overshoot of choline accumulation (Fig. 13). Therefore, it is likely that both of these compounds can, in addition to binding to the choline transport receptor, serve as effective substrates for transport. Neither compound, however, was as effective as choline itself at producing a *trans*-stimulation of choline uptake (Fig. 13). HC-3, despite its inhibitory effectiveness, was completely ineffective as a *trans*-stimulator of choline uptake (Fig. 13). It is possible, therefore, that HC-3 binds to, but

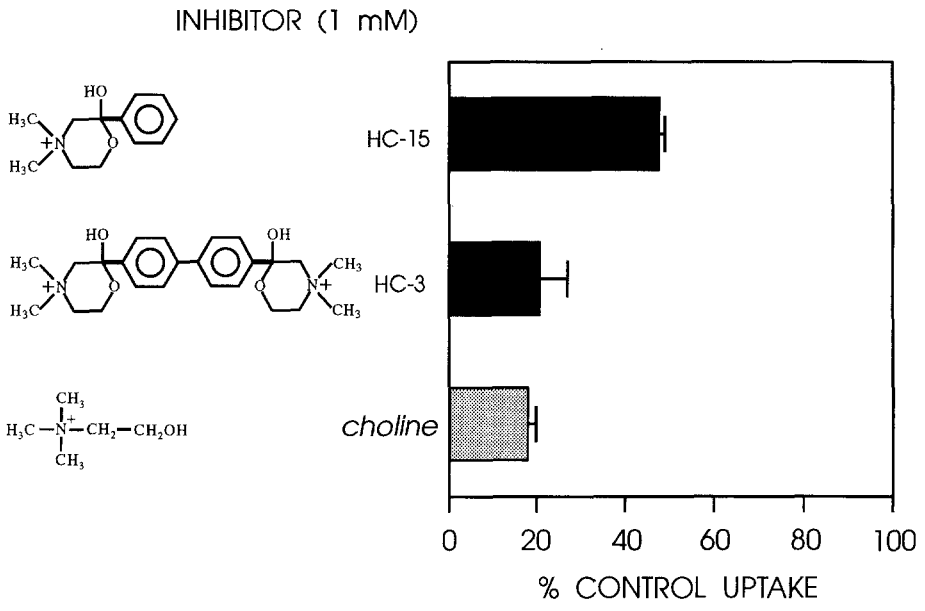

Fig. 12. Inhibition of choline uptake into BBMV by *cis* concentrations of choline analogues; effect of hemicholinium analogs. Conditions as in Fig. 8. Abbreviations used and sample size: hemicholinium-15 (HC-15; $n = 2$); hemicholinium-3 (HC-3; $n = 3$); choline ($n = 6$).

is ineffectively transported by, the choline transporter of renal BBMV.

Discussion

Characteristics of Brush-Border Choline Transport

The present set of experiments identified two transport pathways accessible to choline in rabbit renal BBMV: the OC/H^+ exchanger and a second, novel pathway that had a high degree of specificity for choline and closely related structures. The OC/H^+ exchanger had a low affinity for choline, compared to the second pathway, which accounted for the failure of a H^+ gradient to stimulate choline uptake (Fig. 1*B*). The large (5 mM) concentration of choline employed in the *trans*-stimulation experiment (Fig. 2) was, however, adequate to activate the OC/H^+ exchanger, thereby resulting in the observed countertransport of TEA. The high affinity pathway was not influenced by gradients of either H^+ or Na^+. Instead, uphill accumulation of choline into BBMV was supported by an inside-negative membrane potential (Fig. 7) and by *trans*-concentration gradients of a small family of choline-like molecules (Fig. 13).

The high affinity choline transporter in renal brush borders is markedly different, in terms of its ion dependency and kinetic characteristics, from choline transporters described in other tissues. Whereas Na^+ had no effect on choline transport in renal BBMV, the choline transport in the brain involves cotransport with Na^+ (Kuhar & Murrin, 1978). Likewise, several studies have reported a Na sensitivity for absorptive choline transport in intestine (Herzberg & Lerner, 1973; Hegazy & Schwenk, 1984), although other studies have not (Kuczler et al., 1977; Kessler et al., 1978). Transport of choline in erythrocytes is not influenced by Na^+ but, instead, involves simple facilitated diffusion (*see* Krupka, 1990). However, as with transport in the brain, the red cell transporter has a much higher affinity for choline than does the "high affinity" transporter of renal brush borders: the K_t for choline transport in renal BBMV was 97 μM (Fig. 5) compared to the Michaelis constants that range from 10 μM to <1 μM in erythrocytes (Déves & Krupka, 1979) and synaptosomes, respectively (Lerner, 1989). Thus, our use of term "high affinity" with respect to renal choline transport involves an implicit comparison to the OC/H^+ exchanger and its lower apparent affinity for choline.

The characteristics of choline transport in renal cortical brush-border membranes noted here are comparable to those reported recently for choline transport in collecting duct cells isolated from rat renal inner medulla (Bevan & Kinne, 1990). These similarities include an independence from external Na^+, a sensitivity to membrane potential, and a Michaelis constant on the order of 100 μM. In addition,

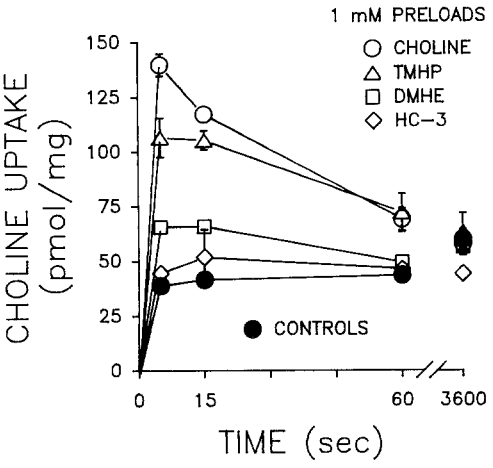

Fig. 13. Effect on choline uptake into BBMV of *trans* concentrations of several choline analogues. Vesicles were pre-equilibrated in 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), 20 μM VAL, and a 1 mM concentration of analogue (the control condition had no analogue). Uptake was measured in transport buffers containing final concentrations of 150 mM KCl, 300 mM mannitol, 10 mM HEPES-KOH (pH 7.5), 20 μM VAL, 25 μM analogue (carried over from the membrane suspension), and 0.03 μM ^{3}H-choline. For the control experiments in which vesicles were not preloaded with analogue, the transport buffers included 25 μM analogue to maintain a constant *cis*-substrate condition. Points are the mean (±SE) of uptakes measured in triplicate in one membrane preparation. The experiment was repeated in a second preparation with similar results.

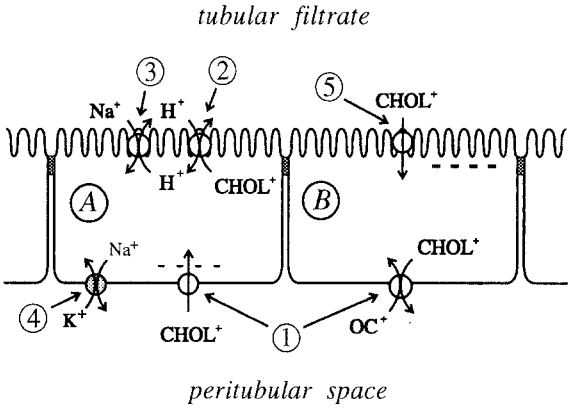

Fig. 14. Model for bidirectional choline transport in rabbit renal proximal tubules. Cell *A* emphasizes transepithelial secretion of choline; cell *B* emphasizes transepithelial reabsorption of choline. *See* text for discussion.

the profile of apparent specificity of the medullary choline transporter is very similar to that of cortical BBMV, with maximum inhibition produced by N,N-dimethyl-N-(2-hydroxyethyl)ammonium, intermediate inhibition produced by N-(2-hydroxyethyl)ammonium and thiamine, and minimal effects produced by betaine and TEA.

The half-saturation constants of the *cis vs. trans* aspects of the choline transporter appeared to be quite different (100 μM *vs.* 1.5 mM). The K_t of an exchange process may be influenced by the presence of *trans* substrate (*see* Krupka, 1989), which precludes an absolute comparison of the values reported above. Nevertheless, the extremes of apparent affinity observed at the two faces of the membrane are suggestive of a kinetic asymmetry that may play a role in the preferential reabsorption of choline under physiological conditions.

Basis of Bidirectional Choline Flux in the Proximal Tubule

The concentration of choline in plasma appears to be tightly regulated by the kidney (Acara & Rennick, 1973; Acara, 1975), with the principal regulatory site being the proximal tubule (Acara et al., 1979). The presence in the proximal tubule of bidirectional choline transport has been recognized since Vander's work in the early 1960s (Vander, 1962). When plasma concentrations of choline are less than ~100 μM, a reabsorptive pathway predominates, whereas plasma concentrations greater than 100 μM result in a net secretory flux (Acara et al., 1979; Besseghir et al., 1981). Plasma choline concentration in rabbits, rats, and humans is <25 μM (Bligh, 1952; Besseghir et al., 1981); consequently, the typical physiological situation is one associated with net choline reabsorption.

How choline can be reabsorbed under some circumstances and secreted under others has not been explained. Most OCs (and organic bases), including TEA, are secreted at all concentrations tested (Acara et al., 1979; Rennick, 1981), and the transepithelial mechanisms associated with this secretory flux have been well characterized (refer to Fig. 14). Movement of OCs into proximal cells involves a carrier-mediated step at the basolateral membrane (system "*1*" in Fig. 14). Studies using basolateral membrane vesicles (BLMV) from kidneys of rabbit (Wright & Wunz 1987*b*; Jung, Kim & Lee, 1989; Montrose-Rafizadeh et al., 1989; Sokol & McKinney, 1990), rat (Takano et al., 1984), and dog (Holohan & Ross, 1980) suggest that this process involves either electrogenic facilitated diffusion or the countertransport of an intracellular substrate for the extracellular OC. Studies with intact rabbit proximal tubules confirm that peritubular OC transport is carrier mediated and may be capable, in the intact cell, of moving an OC against its electrochemical gradient (Schäli et al., 1983; Tarloff & Brand, 1986). Significantly, choline is an effective substrate for the peri-

tubular OC transporter, capable of rapid exchange with TEA (Dantzler & Brokl, 1988; Sokol & McKinney,1990).

The luminal step in OC secretion, first described by Holohan and Ross (1981; *see also* Ross & Holohan,1983), involves the carrier-mediated exchange of an OC for H^+ (system "2" in Fig. 14) and appears to be rate limiting in secretion (Schäli et al., 1983; Montrose-Rafizadeh et al., 1987). The inwardly directed H^+ gradient across the luminal membrane, generated, at least in part, by the activity of the Na/H^+ exchanger (system "3" in Fig. 14), supports the secondary active secretion of OCs; the Na,K-ATPase ultimately drives these processes (system "4" in Fig. 14). Choline can serve as a substrate for the OC/H^+ exchanger; i.e., it blocks OC/H^+ exchange (Fig. 4*A*; Wright, 1985) and choline gradients can support uphill transport of OCs (Fig. 2; Holohan & Ross, 1980). However, the interaction between choline and the OC/H^+ exchanger is relatively weak which was reflected by the apparent K_t of 10 mM for choline transport *via* the OC/H^+ exchanger (Fig. 5).

Although it is likely that the secretion of choline arises from its interaction with the peritubular and luminal transporters for OCs described above, the mechanism(s) associated with the reabsorption of choline, the normal physiological occurrence, has received little attention. Holohan and Ross (1980) suggested that bidirectional net transport of OCs could arise *via* a single pathway if there were an appropriate set of kinetic asymmetries for the relevant substrates. Acara et al. (1979) suggested that the rapid intracellular conversion of choline to betaine in proximal cells would represent a metabolic sink that could produce an effective means to clear choline from tubular fluid, although this mechanism would not involve a net transepithelial transport and still requires a means for choline to enter the proximal cell from the tubular fluid.

The present set of results, which documents the presence of separate, high affinity and low affinity transporters for choline in rabbit BBMV, provides a means to explain the concentration-dependent, bidirectional flux of choline in the kidney. The high affinity pathway (system "5" in Fig. 14), with its K_t of 100 μM, would dominate *trans*-luminal choline flux in the concentration range from which net reabsorption is typically noted (Acara et al., 1979; Besseghir et al., 1981). Furthermore, its electrogenic mode of action (Fig. 7) would allow choline to cross the luminal membrane in response to the inside-negative membrane potential of proximal cells. Exit from the cell could occur *via* the peritubular OC transporter, which is known to exchange cytoplasmic choline for extracellular TEA (Dantzler & Brokl, 1988; Sokol & McKinney, 1990). When the plasma concentration exceeds 100 μM, cytoplasmic choline concentration would rise to levels far in excess of that due to uptake from the plasma *via* the basolateral OC transporter. Consequently, the low affinity pathway of the luminal membrane (i.e., the OC/H^+ exchanger), with its apparent K_t of 10 mM, would increasingly play a part in defining the net transepithelial flux of choline. As the comparatively large transport capacity of the OC/H^+ exchanger is accessed by choline, a net secretory flux would result.

Structural Specificity of the Choline Reabsorptive Pathway

The low affinity choline transport pathway, i.e., the OC/H^+ exchanger, accepts a very broad range of chemical structures (e.g., *see* McKinney, 1987). In contrast, the high affinity pathway described here showed a marked selectivity for choline and a small set of structurally similar molecules, including N,N-dimethyl-N,N-di(2-hydroxyethyl)ammonium, N,N-dimethyl-N-(2-hydroxyethyl)ammonium, N,N,N-trimethyl-N-(3-hydroxypropyl)ammonium, and hemicholinium-3 (Figs. 8–12). Several compounds known to interact with the OC/H^+ transporter had little inhibitory influence on the high affinity choline pathway, including TEA and NMN. Likewise, guanidine, a substrate for a separate proton exchange process in rabbit renal brush borders (Miyamoto et al., 1989), had no effect on choline transport *via* the high affinity pathway.

Given the degree of inhibition of choline transport displayed by the compounds studied, a set of structural elements was deduced that allow interaction of a substrate with the transport receptor: (i) a positively charged nitrogenous nucleus, (ii) a hydroxyl group separated from the nitrogenous nucleus by two to three methylene carbons, and (iii) a minimum of two methyl groups attached to the nitrogen. The poor inhibitors of choline transport deviated from this set of minimum requirements in one or more ways. N,N,N-triethyl-N-(2-hydroxyethyl)ammonium has ethyl, rather than methyl groups about the nitrogen, indicating a steric limit within the negative "pocket" of the receptor. Similarly, N-methyl-N,N,N-tri(2-hydroxyethyl)ammonium and N-ethyl-N,N,N-tri(2-hydroxyethyl)ammonium have two ethanol substituents about the nitrogen (in addition to the required hydroxyethyl group). It should be noted, however, that the failure of these compounds to inhibit uptake could have arisen from physical, rather than simply steric properties of the extra ethanol groups. N,N,N-Trimethyl-N-*n*-

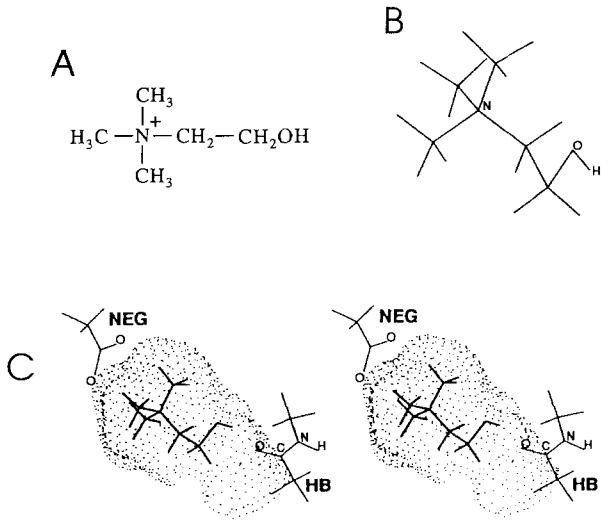

Fig. 15. Model of the high affinity renal choline transport receptor. (*A*) Structural formula of choline. (*B*) Three-dimensional wire model of choline's structure. Note the position of the quaternary nitrogen and the hydroxyl residue. (*C*) Stereo view of a model of the choline transport receptor showing the proposed orientation of choline within the receptor and its interactions with a negative (*NEG*) site and a hydrogen bonding (*HB*) site. *See* text for discussion.

propylammonium and tetramethylammonium (Figs. 10 and 11, respectively), which have the required number of methyl groups, lack a hydroxyl group. Tetraethylammonium lacks the requisite hydroxyl group and has ethyl, rather than methyl substituents about the quaternary nucleus. The inhibitory effectiveness of tetrapentylammonium (Fig. 11) would appear to represent a deviation from the predicted influence of large alkyl substituents on the nitrogenous nucleus. However, tetrapentylammonium also inhibits choline transport in murine leukemia cells, but the interaction is noncompetitive in nature (Naujokaitis et al., 1982), and therefore, probably reflects an interaction at a site removed from the transport receptor. Finally, the failure of betaine, N,N-dimethylaminopropanone, and N,N,N-trimethyl-N-*n*-propylammonium (Fig. 10) to inhibit choline transport suggests that there is a strict requirement for a hydroxyl group.

Figure 15 presents a stereo view of a model for the high affinity choline transport receptor. The stippled region represents the surface of the transport protein around the choline receptor site derived from the steric limits of substrate-receptor interactions noted above. The receptor consists of a negative (NEG) site, presumably a carboxyl function, and a hydrogen bonding (HB) site, presumably the carbonyl oxygen of an amide (e.g., amide residue of arginine, glutamine, or a peptide bond). The molecular mechanics calculations suggest that the choline hydroxyl hydrogen must lie about 1.8 Å from the HB site. The carboxyl oxygens of the NEG site, which form an ionic bond with the quaternary nucleus of choline, are each separated by ~3.8 Å from the choline nitrogen atom. It is useful to consider the proposed interaction of hemicholinium-3 with this hypothetical transport receptor. The conformation of hemicholinium-3 is superimposable on that of choline within the proposed receptor. The space occupied by the other atoms of the hemiketal ring of hemicholinium-3 will extend away from the surface of the receptor, and the remaining three ring structures will extend out into the aqueous medium. Recall that the ring-restricted conformation of hemicholinium-3 was as effective at inhibiting the uptake of ^{3}H-choline as was unlabeled choline itself ($K_{i\text{-HC-3}} \approx K_{t\text{-choline}}$). This suggests that the binding site "prefers" this conformation of choline rather than the extended configuration, hence the assumption concerning the conformation of bound substrate. This also provides a basis for how the other active analogues fit into the receptor. The structure of each of these can be rotated to provide the optimum bond lengths, as derived from the choline/HC-3 interaction, between substrate and the NEG and HB sites of the receptor. The lack of inhibitory activity of betaine and N,N-dimethylaminopropanone suggest that the HB site of the receptor must be a HB acceptor functionality (i.e., carbonyl oxygen) which forms a HB with the hydroxyl hydrogen, rather than a HB donor such as an amine or hydroxyl group which would interact with the oxygen atom of choline. The NEG site of the receptor is very specific for (at least) two methyl groups attached to the nitrogen atom. N-Methyl-N,N,N-tri(2-hydroxyethyl)ammonium and N-ethyl-N,N,N-tri(2-hydroxyethyl) ammonium (Fig. 9) each have substituents on the quaternary nitrogen which exceed the steric limits imposed by the model's receptor site. N,N-Dimethyl-N,N-di(2-hydroxyethyl)ammonium, however, was a reasonably effective inhibitor (Fig. 8), which supports the contention that one larger group on the nitrogen can be accommodated by simply extending away from the receptor out into the aqueous medium. Protonated ethanolamine, N-(2-hydroxyethyl)ammonium or HE (Fig. 8), which has no methyl groups attached to the nitrogen, did not inhibit choline transport. A molecular model of ethanolamine in the hypothetical receptor can be constructed which provides reasonable interactions with the NEG and HB sites of the receptor. However, these interactions come at the expense of other van der Waal's interactions with the receptor pocket due to a difference in the location of the molecular

surface of ethanolamine in the receptor compared to that of choline. Finally, the model permits an explanation of the relatively poor inhibitory effect of hemicholinium-15. Although it has the minimum structural features that permit binding to the receptor, it lacks the hydrophilic moiety found in hemicholinium-3 which extends well into the aqueous environment. Instead, it has a strongly lipophilic residue extending into the aqueous environment which may tend to weaken the substrate-receptor interaction.

The structural specificity of choline transporters in other tissues has received considerable attention (*see* Lerner, 1989). Although it is not the purpose of the present report to review this broad subject, it is relevant to make a few comparisons of the apparent specificity of the renal choline transporter and those of some selected tissues. A unifying observation is the inhibitory effectiveness of hemicholinium-3, which blocks choline transport in the kidney (Fig. 13; *see also* Trimble et al., 1974) and in every other tissue that has been tested (e.g., Lerner, 1989). The transporter with characteristics most similar to those of the renal choline carrier is that of human erythrocytes (*see* Krupka, 1990). In addition to being independent of Na^+, in marked contrast to the high affinity choline transporter in brain (Ducis, 1988), the specificity of the red cell carrier is very similar to that seen here for the renal carrier. In both cases, the transport receptors appear to have subsites for the three methyl substituents on the quaternary nitrogen; substitutions on one of these sites are permitted, but restrictions on the size of the other substituents are severe. Finally, it is worth noting the striking dissimilarity between the specificity of the renal transporter and that found in the placenta; neither thiamine nor N-(2-hydroxyethyl)ammonium was particularly effective at inhibiting choline transport in renal BBMV, yet both are potent blockers of choline transport in perfused guinea pig placenta (Sweiry & Yudilevich,1985).

In summary, the normal, reabsorptive flux of choline in the rabbit proximal tubule probably stems from the activity of a high affinity transporter for choline in the luminal, brush-border membrane. This transporter is electrogenic, thereby permitting choline to enter the cell in response to the inside-negative electrical PD across the luminal membrane. The electroneutral exchange of choline for another, endogenous OC presumably completes the transepithelial flux. Secretion of choline, which occurs when plasma choline concentrations exceed 100 μM, involves the OC-secretory transporters, which include an electrogenic, facilitated diffusion process which can also exchange one OC for another (this is the process that we suggest can be involved in exchange of choline for another OC, noted above); and the OC/H^+ exchanger in the luminal membrane.

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