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Transport of choline and its relationship to the expression of the organic cation transporters in a rat brain microvessel endothelial cell line (RBE4)

Anne Friedrich [a], Ronald L. George [a], Christy C. Bridges [a], Puttur D. Prasad [b], Vadivel Ganapathy [a,*]

[a] *Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, USA*
[b] *Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912, USA*

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Abstract

The present study was undertaken to elucidate the functional characteristics of choline uptake and deduce the relationship between choline uptake and the expression of organic cation transporters in the rat brain microvessel endothelial cell line RBE4. Confluent RBE4 cells were found to express a high affinity choline uptake system. The system is Na^+-independent and shows a Michaelis–Menten constant of approx. 20 μM for choline. The choline analogue hemicholinium-3 inhibits choline uptake in these cells with an inhibition constant of approx. 50 μM. The uptake system is also susceptible for inhibition by various organic cations, including 1-methyl-4-phenylpyridinium, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, clonidine, procainamide, and tetramethylammonium. The prototypical organic cation tetraethylammonium shows very little affinity for the choline uptake system in these cells. The inhibition of choline uptake by hemicholinium-3 is competitive. Northern analysis and RT-PCR show that these cells do not express the organic cation transporters OCT2 and OCT3. These cells do express, however, low levels of OCT1, but the functional characteristics of choline uptake in these cells are very different from the known properties of choline uptake via OCT1. The Na^+-coupled high affinity choline transporter CHT1 is not expressed in these cells as evidenced by RT-PCR. This corroborates the Na^+-independent nature of choline uptake in these cells. It is concluded that RBE4 cells express an organic cation transporter that is responsible for choline uptake in these cells and that this transporter is not identical to any of the organic cation transporters thus far identified at the molecular level in mammalian cells.

Keywords: Choline uptake; Hemicholinium-3; Organic cation transporter; Blood–brain barrier; Rat

1. Introduction

Choline is an organic cation that plays a critical role in the structure and function of biological membranes in all cells as a component of the membrane phospholipid phosphatidylcholine. In the brain, choline plays an additional role as a precursor for the synthesis of the neurotransmitter acetylcholine [1–3]. Since the synthesis of phosphatidylcholine as well as acetylcholine occurs intracellularly, availability of choline for these synthetic processes inside the cell is controlled primarily by the transport systems located in the plasma membrane of the cell. In addi-

Abbreviations: OCT, organic cation transporter; NMDG, *N*-methyl-D-glucamine; TEA, tetraethylammonium; TMA, tetramethylammonium; MPP, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

* Corresponding author. Fax: 706-721-6608;
E-mail: vganapat@mail.mcg.edu

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tion, the entry of choline from the systemic circulation into the brain occurs via transport systems present in the blood–brain barrier. This transport process is important because brain has only a limited capacity to synthesize choline and consequently depends predominantly on the transfer of choline from the systemic circulation [4]. The endothelial cells of the microvasculature in the brain constitute a selective permeability barrier that is characteristic of the blood–brain barrier. These cells exhibit a unique phenotype with the presence of a complex network of tight junctions and the absence of fenestrations and vesicular transcytosis [5]. Transcellular transfer of solutes across the endothelial cell layer of the blood–brain barrier requires differential expression of distinct transport systems in the lumenal membrane facing the vascular lumen and the basolateral membrane facing the extracellular fluid of the brain tissue [6]. The transport of choline across the blood–brain barrier has been investigated in intact animals [7,8], with isolated brain capillaries [9–11] or purified brain capillary endothelial cells [11,12]. A more recent study has used a mouse brain capillary endothelial cell line to investigate choline transport [13]. These studies have demonstrated the presence of a carrier-mediated process for the entry of choline across the endothelial cell layer of the blood–brain barrier. The process is mediated by a Na^+-independent high affinity transporter that exhibits a Michaelis–Menten constant of 10–20 μM for choline. Even though these studies have established the functional characteristics of the choline transport process in the blood–brain barrier endothelial cells, nothing is known at present on the molecular nature of the transport protein that is responsible for the transport process.

Three different transporters with ability to transport organic cations have been cloned and functionally characterized [14]. These transporters, known as organic cation transporter (OCT)1, OCT2, and OCT3, exhibit a broad and overlapping substrate specificity towards organic cations [15–21]. Choline interacts with all three transporters with varying affinity. In addition, a Na^+-dependent high affinity choline transporter, designated CHT1, has been recently cloned [22]. CHT1 is expressed exclusively in specific regions of the brain (forebrain, striatum, brain stem, and spinal cord), strongly suggesting a role for this transporter in cholinergic neurons [22]. The molecular identification of another choline transporter, called CHOT1, has been reported by Mayser et al. [23], but studies from other laboratories have shown that CHOT1 does not transport choline [24], that its expression pattern in the brain does not match the location of cholinergic neurons [25], and that CHOT1 is actually a Na^+- and Cl^--coupled creatine transporter [24,25]. The present study was undertaken to investigate the possible relationship between choline transport and expression of the organic cation transporters OCT1–3 and the Na^+-dependent choline transporter CHT1 in the endothelial cells of the blood–brain barrier. For this purpose, we used an immortalized rat brain microvessel endothelial cell line (RBE4). This cell line has been shown to display a nontransformed endothelial phenotype and retains the sensitivity to angiogenic and astroglial factors for the expression of blood–brain barrier-related enzymes [26]. The results of the present study show the following: (a) RBE4 cells express a Na^+-independent high affinity transport process for choline, (b) the transport process also interacts with various other organic cations, (c) these cells do not express the organic cation transporters OCT2 and OCT3, but express at low levels the organic cation transporter OCT1, (d) choline transport in these cells is not mediated by OCT1, and (e) the Na^+-dependent choline transporter CHT1 is not expressed in these cells.

2. Materials and methods

2.1. Materials

[^{3}H]Choline and [^{14}C]tetraethylammonium (TEA) were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Unlabeled organic cations were obtained from either Sigma (St. Louis, MO, USA) or Research Biochemicals (Natick, MA, USA). Cell culture medium, fetal bovine serum, and antibiotics were from Life Technologies (Gaithersburg, MD, USA). Rat tail collagen and basic fibroblast growth factor were obtained from Boehringer-Mannheim (Indianapolis, IN, USA).

2.2. Cell culture

The RBE4 cell line was kindly provided by Dr. Françoise S. Roux (INSERM, Paris, France). The cells were grown to confluence at 37°C in a humid atmosphere of 5% CO_2 on collagen (5 μg/well)-coated 24-well culture plates in α-minimum essential medium/Ham's F-10 (1:1), supplemented with 2 mM glutamine, 10% fetal bovine serum, 1 μg/l basic fibroblast growth factor, 300 mg/l geneticin, and 10 mg/l gentamicin. Initial seeding was done at 2×10^4 cells/well and the cultures became confluent 2 days after the seeding. Confluent cultures were used for uptake measurements.

2.3. Uptake measurements

Uptake measurements were made with confluent cells at room temperature. In most experiments, the concentration of [^{3}H]choline was 10 nM and the incubation time was 10 min. Uptake of [^{14}C]TEA was measured at the concentration of 40 μM. Culture medium was removed from the wells by aspiration and the cells were washed once with the uptake buffer. Uptake was then started by the addition of 250 μl of uptake buffer containing radiolabeled choline or TEA. After 10 min incubation, uptake was terminated by the removal of the medium from the wells, followed by the addition of 1 ml ice-cold phosphate-buffered saline. Cells were washed twice with ice-cold phosphate-buffered saline and then solubilized in 0.5 ml of 1% sodium dodecyl sulfate in 0.2 M NaOH. The cell lysate was transferred to vials for determination of radioactivity associated with the cells. The composition of the uptake buffer was 25 mM HEPES/Tris (pH 7.5), supplemented with 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. When the influence of Na^+ on the uptake process was studied, the uptake buffer was modified by replacing NaCl with equimolar concentration of *N*-methyl-D-glucamine (NMDG) chloride. One well was reserved in each culture plate for protein determination. In experiments dealing with saturation kinetics, the concentration of [^{3}H]choline was kept constant at 20 nM and unlabeled choline was added to give desired choline concentrations. The nonsaturable component of uptake was determined from the radioactivity associated with the cells when the incubation was done with 20 nM [^{3}H]choline in the presence of 5 mM unlabeled choline. Total uptake at each concentration of choline was adjusted for this nonsaturable uptake to calculate the saturable component. Data were analyzed by nonlinear regression and confirmed by linear regression. Experiments were repeated two or three times with independent cultures, each experiment done in duplicate or triplicate. Results are expressed as means ± S.E. of these replicates.

2.4. Northern blot analysis

The expression of mRNAs for OCT1, OCT2, and OCT3 in RBE4 cells was analyzed by Northern blot. Poly(A)$^+$ RNA was isolated from RBE4 cells using a commercially available mRNA isolation kit (Invitrogen, San Diego, CA, USA). Poly(A)$^+$ RNA from rat kidney was used as a positive control for OCT1 and OCT2 expression and poly(A)$^+$ RNA from rat placenta was used as a positive control for OCT3 expression. mRNA was size-fractionated on a denaturing formaldehyde-agarose gel and probed with ^{32}P-labeled cDNAs specific for the three organic cation transporters (OCT1–3). OCT1 and OCT2 cDNAs were isolated from a rat liver cDNA library and their identities confirmed by sequencing. The nucleotide sequences of these cDNAs were identical to the sequences reported in the literature [15,16]. OCT3 cDNA was isolated from a rat placental cDNA library [18]. The blots were also probed with ^{32}P-labeled β-actin cDNA as an internal control for the presence of RNA in each lane. The probes were labeled with [α-^{32}P]dCTP by random priming using the ready-to-go oligolabeling kit from Amersham-Pharmacia Biotech (Piscataway, NJ, USA). Hybridization and washing were carried out under high stringency conditions.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR using poly(A)$^+$ RNA isolated from RBE4 cells was done with primer pairs specific for rat OCT1, OCT2, OCT3, and CHT1. Poly(A)$^+$ RNA from rat kidney was used as the positive control for all three OCTs. Poly(A)$^+$ RNA from rat brain stem was used as the positive control for CHT1. The sense

and antisense primers for OCT1 were 5′-TGCAGACAGGTTTGGCCGTAA-3′ and 5′-TCGAGGCCGCTATTGGGTAGA-3′ and the size of the expected product was 722 bp. The sense and antisense primers for OCT2 were 5′-GCTGGTTAATTGGCTACATCC-3′ and 5′-TTCTTGGCCTCTGCATATTC-3′ and the size of the expected product was 960 bp. The sense and antisense primers for OCT3 were 5′-CCACCATCGTCAGCCAGTTT-3′ and 5′-ACACGACACCCCTGCCACTA-3′ and the size of the expected product was 851 bp. The sense and antisense primers for CHT1 were 5′-GAACGCAGCGAAGCCATCATAGTTG-3′ and 5′-CCACAGAGTAGAGCCCTCCCACGAG-3′ and the size of the expected product was 445 bp.

3. Results

3.1. Functional characteristics of choline uptake in RBE4 cells

Fig. 1 describes the time course of the uptake of choline in RBE4 cells in the presence and absence of Na^+. The uptake was not stimulated by the presence of a transmembrane Na^+ gradient. In fact, the uptake was slightly higher in the absence of Na^+. The uptake was linear at least up to 10 min. We then assessed the influence of extracellular pH on the uptake process, both in the presence and in the absence of Na^+ (Fig. 2A). The uptake was found to be inhibited significantly (approx. 30%) when the extracellular pH was changed from 7.5 to 5.5. This inhibition caused by acid pH was seen in the presence as well as absence of Na^+. The uptake was, however, not influ-

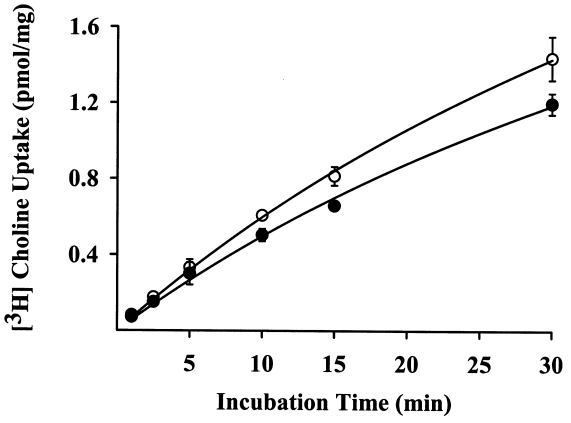

Fig. 1. Time course of choline uptake in RBE4 cells. Uptake of [^{3}H]choline (10 nM) was measured in confluent RBE4 cells for various time periods (1–30 min) in the presence of either NaCl (●) or NMDG chloride (○). The pH of the uptake buffer was 7.5. Results are means ± S.E. for four independent determinations.

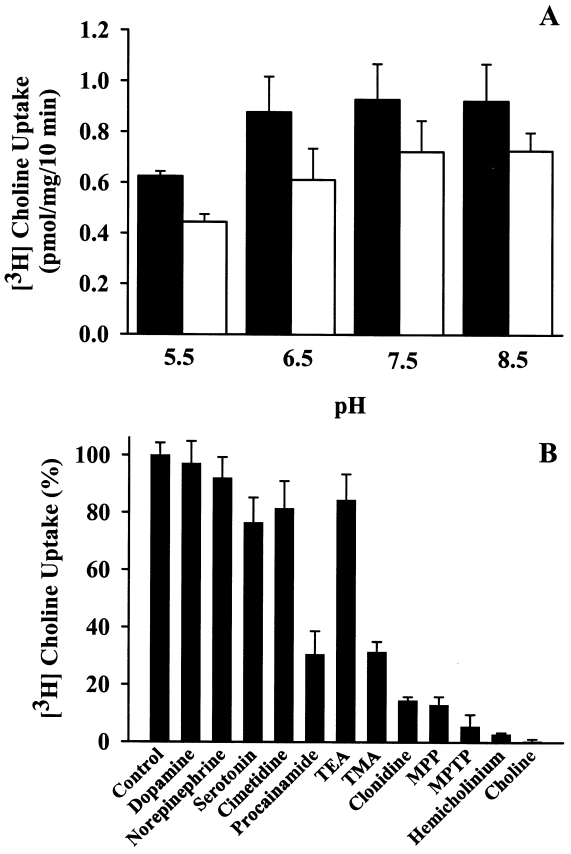

Fig. 2. (A) Influence of pH on choline uptake. Uptake of [^{3}H]choline (10 nM) was measured in confluent RBE4 cells with a 10 min incubation in the presence of either NaCl (open bars) or NMDG chloride (closed bars) at varying pH. Uptake buffers of varying pH were prepared by mixing appropriately the following two buffers: 25 mM MES/Tris (pH 5.5) and 25 Tris/HEPES (pH 8.5). Both buffers contained 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, and either 140 mM NaCl or 140 mM NMDG chloride. Results are means ± S.E. for six independent determinations. (B) Inhibition of [^{3}H]choline uptake by various organic cations. Uptake of [^{3}H]choline (10 nM) was measured in confluent RBE4 cells with a 10 min incubation in the presence of NaCl at pH 7.5 either in the absence (control) or presence of 2 mM organic cations. Unlabeled choline was also used as a competitor of [^{3}H]choline uptake. Results (means ± S.E. for six independent determinations) are given as percent of control uptake measured in the absence of inhibitors (0.58 ± 0.01 pmol/mg of protein/10 min).

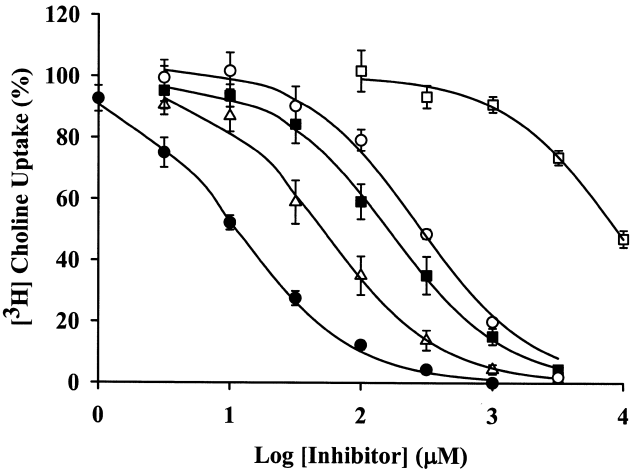

Fig. 3. Dose–response relationship for the inhibition of [^{3}H]choline uptake by various organic cations. Uptake of [^{3}H]choline (10 nM) was measured in confluent RBE4 cells with a 10 min incubation in the presence of NaCl at pH 7.5 either in the absence (control) or presence of increasing concentrations of various organic cations. Results (means ± S.E. for six independent determinations) are given as percent of control uptake measured in the absence of inhibitors (0.71 ± 0.06 pmol/mg of protein/10 min). ●, unlabeled choline; △, hemicholinium-3; ■, MPP; ○, MPTP; □, TEA.

enced when the pH was changed from 7.5 to 8.5. Based on these data, subsequent uptake measurements were made with a 10 min incubation in the presence of Na^+ at pH 7.5.

We investigated the substrate selectivity of the uptake process that is responsible for choline uptake in these cells. This was done by assessing the ability of various organic cations (2 mM) to inhibit the uptake of [^{3}H]choline (10 nM). Several organic cations were found to cause marked inhibition of choline uptake (Fig. 2B). The inhibitors included tetramethylammonium (TMA), procainamide, clonidine, 1-methyl-4-phenylpyridinium (MPP), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and hemicholinium-3. The order of inhibitory potency was as follows: hemicholinium-3 > MPTP > MPP > clonidine > procainamide = TMA. The inhibition observed with these compounds was in the range of 70–95%. As expected, unlabeled choline competed with [^{3}H]choline for the uptake process, causing 100% inhibition. Interestingly, TEA caused only about 15% inhibition. Cimetidine and serotonin were also slightly inhibitory. Dopamine and norepinephrine did not cause any significant inhibition. Fig. 3 shows the dose–response relationship for the inhibition of [^{3}H]choline uptake caused by TEA, MPP, MPTP, hemicholinium-3, and unlabeled choline. Unlabeled choline inhibited the uptake of [^{3}H]choline with an IC_{50} value (i.e., concentration at which the inhibition was 50%) of 12 ± 1 μM. The choline analogue hemicholinium-3 inhibited the uptake with an IC_{50} value of 53 ± 5 μM. The IC_{50} values for MPP and MPTP were 169 ± 10 μM and 273 ± 34 μM. TEA

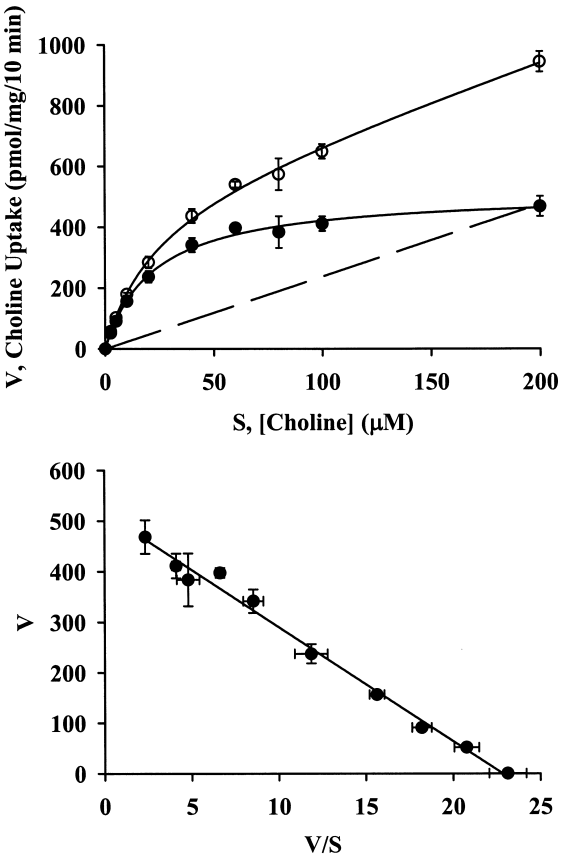

Fig. 4. Saturation kinetics of choline uptake. Uptake of choline was measured in confluent RBE4 cells with a 10 min incubation in the presence of NaCl at pH 7.5. The concentration of [^{3}H]choline was kept constant at 20 nM and unlabeled choline was added to give the indicated choline concentrations. Nonsaturable uptake was calculated from the uptake of radioactivity in the presence of an excess amount (5 mM) of unlabeled choline. (Upper panel) Choline uptake versus choline concentration. The saturable component of uptake (●) was calculated by subtracting the nonsaturable component (broken line) from the total uptake (○) at each concentration of choline. (Lower panel) Eadie–Hofstee plot of the saturable component of uptake. V, uptake rate; V/S, uptake rate/choline concentration. Results represent means ± S.E. for three independent determinations.

was the least potent inhibitor among these compounds with an IC_{50} value of 8.8 ± 0.9 mM.

We then analyzed the saturation kinetics of choline uptake over the concentration range of 2.5–200 μM. Fig. 4 (top) describes the total uptake and the nonsaturable component at each concentration of choline. The saturable component represented >80% of total uptake at choline concentrations 2.5–50 μM. Even at the highest concentration of choline employed in this experiment (200 μM), the nonsaturable component represented only about 50% of total uptake. The difference between the total uptake and the nonsaturable component showed saturation with increasing concentrations of choline. Fig. 4 (bottom) shows the linear transformation of the data for the saturable component. The Michaelis–Menten constant (K_t) for the saturable uptake was 23 ± 1 μM and the maximal velocity (V_{max}) was 516 ± 11 pmol/mg of protein/10 min.

The inhibition of choline uptake by hemicholinium-3 was competitive. Fig. 5 shows the Dixon plot of the inhibition. In this experiment, the uptake of choline was measured at two different concentrations of choline (1 and 5 μM). The concentration of hemicholinium-3 was varied over the range of 25–250 μM. The Dixon plots (i.e., 1/uptake rate versus hemicholinium-3 concentration) were linear at both concentrations of choline and the lines intersected above the *X*-axis, indicating the competitive nature of the inhibition. The K_i value (i.e., inhibition constant) was 65 μM.

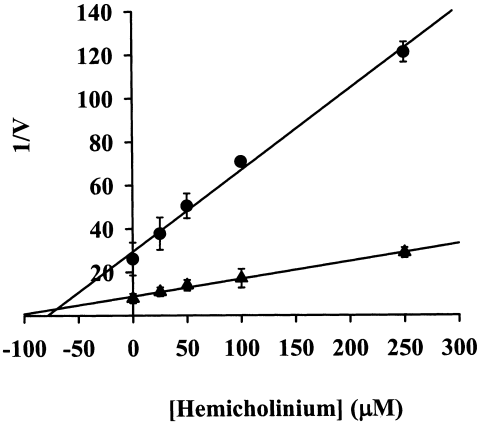

Fig. 5. Kinetic analysis of choline uptake by hemicholinium-3. Uptake of choline at two different concentrations (1 and 5 μM) was measured in confluent RBE4 cells with a 10 min incubation in the presence of NaCl at pH 7.5. The concentration of [^{3}H]choline was kept constant at 20 nM in both cases. Uptake was measured either in the absence or presence of increasing concentrations of hemicholinium-3. Results are given as Dixon plots (1/choline uptake in nmol/mg of protein/10 min versus hemicholinium-3 concentration in μM) with 1 μM (●) and 5 μM (▲) choline. Results represent means ± S.E. for six independent determinations.

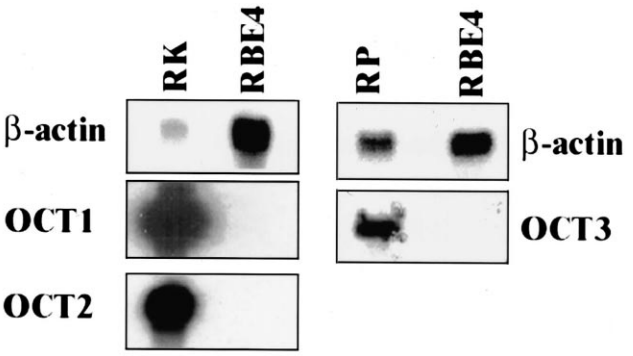

Fig. 6. Northern blot analysis of the expression of organic cation transporters in RBE4 cells. Poly(A)$^+$ RNA, isolated from RBE4 cells, rat kidney and rat placenta, was size-fractionated on a denaturing formaldehyde-agarose gel and probed by sequential hybridization under high stringency conditions with ^{32}P-labeled cDNAs specific for rat OCT1, OCT2, OCT3, and β-actin. Rat kidney (RK) was used as a positive control for OCT1 and OCT2 and rat placenta (RP) was used as a positive control for OCT3.

3.2. *Analysis of the expression of organic cation transporters in RBE4 cells*

We investigated the expression in RBE4 cells of the three organic cation transporters (OCT1–3) identified thus far in mammalian cells. This was done first by Northern analysis and then by RT-PCR. Fig. 6 describes the data from Northern analysis. These studies showed no evidence of expression of any of the three OCTs in RBE4 cells. The rat kidney was positive for OCT1 and OCT2 and the rat placenta was positive for OCT3 under identical conditions. These two tissues were selected as positive controls based on the known abundance of mRNA for each of the three OCTs [15,16,18]. OCT1 and OCT2 are expressed abundantly in rat kidney and OCT3 is expressed abundantly in rat placenta. However, β-actin mRNA was detectable in the lane containing the poly(A)$^+$ RNA from RBE4 cells. Fig. 7 shows the RT-PCR data. The experimental conditions used for

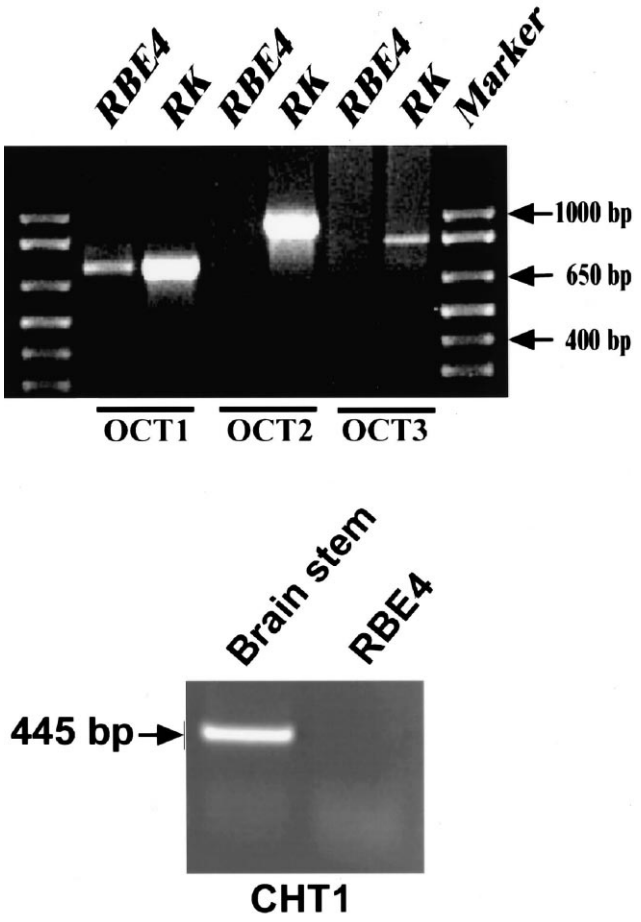

Fig. 7. RT-PCR. Poly(A)$^+$ RNA samples from RBE4 cells and rat kidney (RK) were used for RT-PCR with primer pairs specific for rat OCT1, rat OCT2, and rat OCT3. The RT-PCR products were size-fractionated with molecular size markers run in parallel. For RT-PCR with primer pairs specific for rat CHT1, poly(A)$^+$ RNA from rat brain stem served as the positive control.

RT-PCR were validated by demonstrating the RT-PCR products of expected sizes for OCT1, OCT2, and OCT3 with rat kidney mRNA. As expected, OCT1 and OCT2 were expressed abundantly in rat kidney. The expression of OCT3 in rat kidney was lower than that of OCT1 and OCT2, again corroborating the earlier findings [15,16,18]. Under similar conditions, RT-PCR was negative for OCT2 and OCT3 with RBE4 cell mRNA, showing lack of expression of these two organic cation transporters in these cells. However, the expression of OCT1 was detectable in these cells. Thus, even though Northern analysis indicated that OCT1 was not expressed in RBE4 cells, the more sensitive RT-PCR was able to detect the presence of OCT1 mRNA in these cells. The level of expression of OCT1 in RBE4 cells was, however, much lower than that in rat kidney. Taken collectively, these data demonstrate that RBE4 cells do not express OCT2 and OCT3, but express low levels of OCT1. The RT-PCR data also show that RBE4 cells do not express the Na^+-dependent choline transporter CHT1 (Fig. 7). Poly(A)$^+$ RNA from rat brain stem, used as the positive control for CHT1 expression, yielded the expected RT-PCR product for CHT1. The lack of expression of CHT1 in RBE4 cells agrees with the present findings that there is no detectable Na^+-dependent choline uptake in these cells.

Since OCT1 mRNA was detectable in RBE4 cells, it became necessary to assess if OCT1-specific transport function was detectable in these cells. OCT1 has been shown to transport choline and TEA [17]. The Michaelis–Menten constants for the transport of choline and TEA through OCT1 are approx. 600 μM and approx. 100 μM, respectively. Therefore, we first measured the uptake of choline at 500 μM, an appropriate concentration for interaction of choline with OCT1, and assessed if the uptake of choline at this concentration was inhibitable by 2 mM TEA. These experiments showed that choline uptake under these conditions was not inhibited by TEA (choline uptake in the absence of TEA, 2.53 ± 0.18 nmol/mg of protein/10 min; choline uptake in the presence of TEA, 2.51 ± 0.16 nmol/mg of protein/10 min). Similarly, we measured the uptake of TEA at 40 μM and assessed if the uptake of TEA at this concentration was inhibitable by 5 mM choline. These studies showed that TEA uptake under these conditions was not inhibited by choline (TEA uptake in the absence of choline, 33.33 ± 3.7 pmol/mg of protein/10 min; TEA uptake in the presence of choline, 31.0 ± 6.5 pmol/mg of protein/10 min). We conclude from these data that even though OCT1 mRNA is detectable at low levels in RBE4 cells, there is no detectable transport function in these cells that can be ascribed specifically to OCT1.

4. Discussion

The functional characteristics of choline uptake in

RBE4 cells deduced from the present study can be summarized as follows: (a) RBE4 cells express a saturable transport system for the uptake of choline, (b) the transport system is Na^+-independent and exhibits high affinity for choline with a K_t value of 10–20 μM, (c) the choline analogue hemicholinium-3 competes with choline with a K_i value of 50–65 μM, (d) the transport system recognizes several other organic cations including MPP, MPTP, clonidine, procainamide, and TMA as substrates, (e) monoamines (e.g., dopamine, serotonin, and norepinephrine) exhibit little or no affinity for the transport system, and (f) TEA, a prototypical organic cation that is used widely in studies involving the transport of organic cations, interacts with the transport system with very low affinity (IC_{50} value, approx. 10 mM). Based on the kinetic data, we speculate that a single transport system is responsible for choline uptake in these cells. It is, however, possible, though unlikely, that the observed choline uptake is mediated by more than one transport system that are kinetically indistinguishable. The kinetic characteristics of the choline transport system in RBE4 cells are very similar to those reported recently by Sawada et al. [13] in the mouse brain capillary endothelial cell line MBEC4. Choline uptake in MBEC4 cells was also found to be Na^+-independent and saturable with a K_t value for choline of approx. 20 μM. However, there are notable differences between RBE4 cells and MBEC4 cells in the potencies with which various organic cations compete with choline for the uptake process. Hemicholinium-3 inhibits choline uptake in MBEC4 cells with an IC_{50} value of approx. 700 μM which is an order of magnitude higher than the IC_{50} value found in RBE4 cells (approx. 50 μM). Similarly, procainamide at a concentration of 2 mM inhibited choline uptake in RBE4 cells by more than 50%, indicating that the IC_{50} value for the inhibition is much lower than 2 mM. In contrast, the IC_{50} value for the inhibition of choline uptake by procainamide in MBEC4 cells is considerably much higher (approx. 7 mM) than in RBE4 cells. The inhibition data with monoamines are, however, comparable in both cell types. It is surprising that while the kinetic parameters of choline uptake are similar between RBE4 cells and MBEC4 cells, the inhibitory potencies for various organic cations are significantly different between these two cell types. The reasons for these discrepancies are not known.

The primary purpose of the present study was to investigate the relationship between choline uptake and the expression of organic cation transporters in RBE4 cells. This is the first study in the literature to address this issue. Among the three different organic cation transporters thus far identified in mammalian cells at the molecular level, OCT1 and OCT2 transport choline [17]. The transport process is Na^+-independent and has a K_t value of approx. 0.6 mM in the case of OCT1 and approx. 0.2 mM in the case of OCT2. OCT3 does not recognize choline as a substrate [18,20,21]. RBE4 cells do not express OCT2 and OCT3, but contain detectable levels of OCT1 mRNA, indicating a low but significant expression of this transporter. Even though OCT1 mRNA is expressed at low, but detectable, levels in RBE4 cells, there is no detectable transport function in these cells that can be ascribed specifically to OCT1. Choline transport reported in the present study does not appear to be mediated by OCT1. The affinity of rat OCT1 for choline is about 30 times lower than found in RBE4 cells (K_t for rat OCT1, 600 μM; K_t in RBE4 cells, 20 μM). Furthermore, rat OCT1 transports TEA with a K_t value of approx. 100 μM whereas the choline transport system identified in RBE4 cells interacts with TEA with very low affinity. The IC_{50} value for TEA to inhibit choline uptake in these cells is about 80 times higher than the K_t value for TEA for transport via rat OCT1. Similarly, MPP is transported by rat OCT1 with very high affinity (K_t, approx. 10 μM), but the IC_{50} value for MPP to inhibit choline uptake in RBE4 cells is approx. 170 μM. These data clearly show that even though RBE4 cells express low levels of OCT1, the high affinity Na^+-independent choline uptake detected in these cells does not occur via OCT1. This suggests that a hitherto unidentified organic cation transporter is responsible for choline uptake in RBE4 cells.

There are at least two distinct high affinity transport systems for choline in the brain, both being sensitive to inhibition by hemicholinium-3 [27,28]. These two systems are distinguished primarily by their dependence on Na^+. One of the systems is energized by a transmembrane Na^+ gradient and exhibits very high affinity for choline (K_t, approx. 1 μM) and

hemicholinium-3 (K_i, <10 μM). The other is Na^+-independent and exhibits a K_t value of approx. 20 μM for choline and a K_i value of approx. 50 μM for hemicholinium-3. The Na^+-dependent choline transporter, designated CHT1, has been recently cloned [22]. The functional characteristics of choline uptake in RBE4 cells strongly suggest that the Na^+-dependent choline transporter is not expressed in these cells. This is supported by RT-PCR data that show lack of expression of CHT1 mRNA in these cells. The properties of the Na^+-independent choline transporter described in the brain bears marked similarity to those of the choline uptake process in RBE4 cells. A similar transport system has been described not only in the brain but also in various epithelial cells derived from nonneuronal tissues [22,29–31]. Therefore, it appears that the expression of the Na^+-independent high affinity choline transport system may not be restricted to the central nervous system. This system is likely to play a critical role in the delivery of choline in various cell types of neuronal as well as nonneuronal origin. The expression of this transport system in RBE4 cells indicates that this transporter also participates in the delivery of choline to the brain across the blood–brain barrier. However, the molecular identity of this transporter remains unknown.

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