To determine whether organic cation transporter 2 (OCT2) family members might mediate choline transport in choroid plexus (CP), the handling of choline by cloned transporters and by intact CP isolated from the adult rat was investigated. Expression of OCT1 and OCT2 in Xenopus oocytes increased hemicholinium-3-sensitive choline uptake. In contrast, OCT3 did not mediate choline transport. Estimated $K_m$ values for choline in rOCT1-, rOCT2-, and hOCT2-expressing oocytes were 346 ± 50, 441 ± 67, and 102 ± 80 μM, respectively. Membrane potential was the major driving force for choline uptake in rat and human OCT2-expressing oocytes and in intact CP in vitro. Lowering of medium pH (6 versus 7.4) was equally effective at inhibiting choline uptake in CP, suggesting that there might be a non-OCT component of choline uptake that is responsive to an H$^+$ gradient. However, choline efflux from CP was not stimulated by a trans-applied H$^+$ gradient. Choline uptake by CP was Na$^+$-independent with an estimated $K_m$ of 183 μM. Reverse transcriptase-polymerase chain reaction detected OCT2 and OCT3, but not OCT1, mRNA expression in CP. Transfection of intact CP with a rOCT2/green fluorescent protein fusion construct resulted in strong apical membrane fluorescence with no detectable signal in the basal and lateral plasma membranes. These data indicate that OCT2 mediates choline transport across the ventricular membrane of CP.

In the brain, free choline is essential for the synthesis of membrane phospholipids and the neurotransmitter acetylcholine. However, there is very little de novo synthesis of choline in the brain, and a constant supply from plasma is required (1). Movement of choline across the brain capillary endothelium changes with its concentration in plasma (physiological range of 10–50 μM), whereas the choline concentration in cerebrospinal fluid (CSF)$^3$ is tightly controlled (5–7 μM) (2–5). Thus, the balance between plasma choline levels and the net flux of brain choline is an important mechanism for maintaining brain choline homeostasis. It has been postulated that perturbations in this balance may have a role in central cholinergic dysfunctions and that impeding the transepithelial movement of choline from CSF to blood may prove to be an effective therapy (6, 7).

Ventriculocisternal perfusion studies and in vitro experiments on isolated choroid plexus (CP) show carrier-mediated uptake of choline, suggesting that the CP is actively involved in the maintenance of brain choline homeostasis through removal of excess choline from CSF (6–10). Recently, Villalobos et al. (11), using primary cultures of the choroidal epithelium, found that the characteristics of apical choline uptake in CP cells are strikingly similar to those identified for the renal basolateral organic cation (OC) transport system. Such a reversal of functional polarity is conceivable because the movement of OCs from CSF to blood requires the membrane potential-sensitive entry step to take place across the apical membrane of the cells of the CP. Therefore, we propose that the molecular basis for CP function in the regulation of free choline concentration in CSF might be the expression of organic cation transporter (OCT) family members.

Three candidate transporters, OCT1, OCT2, and OCT3, have been cloned recently from a variety of species including rat, mouse, pig, rabbit, and man (12–21). Functionally, all three transporters mediate the entry of small OCs into the cell via a potential-dependent, Na$^+$-independent, facilitative diffusion mechanism (13–15, 17, 22). They are polyspecific in that they transport and/or are inhibited by an overlapping set of substrates that includes xenobiotics, drugs, neurotransmitters, and neurotoxins (e.g. tetraethylammonium (TEA), tetramethylammonium (TMA), 1-methyl-4-phenylpyridinium, N$^3$-methyl-y-lactinotanimide (NMN), amphetamine, desipramine, quinine, dopamine, and serotonin). Some differences in substrate affinity between OCT1 and OCT2 have been identified (for review see Refs. 23 and 24), but information on OCT3 is still limited (14, 25), making comparisons between all three transporters difficult. The endeavor to identify paralog-specific substrates is further complicated by the fact that there are species differences in both the expression and specificity of these transporters as well as variation in kinetic parameters determined in different cellular systems (Xenopus oocytes versus cultured cells versus intact tissue). For example, when examined in oocytes the affinity of rat OCT1 (rOCT1) for TEA appears much greater than that of rOCT2 ($K_m = 95$ versus 393 μM, respectively), but when measured in transfected Madin-Darby canine kidney cells their affinities are virtually identical ($K_m = 38$ versus 48 μM, respectively) (15, 22, 26). Thus, despite increased understanding of the individual transporters at the molecular and mechanistic levels, correlations of the functional differences between the OCT paralogs are still tenuous.
Choroid Plexus Choline Uptake Mediated by OCT2

None-the-less, when expressed in oocytes the rat paralogs exhibit markedly different affinities for TEA with the for OCT1 being 95 μM, OCT2 being 393 μM, and OCT3 being 2.5 mM (14, 15, 22). It has also been shown that OCT1 and OCT2 are both effectively inhibited by TMA, but OCT3 is not (14, 15, 22). Guanidine could prove to be a substrate with markedly different affinities for OCT1 (Km = 172 ± 57 μM), OCT2 (Km = 1,660 ± 670 μM), and OCT3 (Km = 35 μM); however, the Km value for OCT3 was determined in transfected HeLa cells and not Xenopus oocytes (14, 27). In support of the present hypothesis that OCTs expressed in CP function in the regulation of free choline in CSF, we and others (17, 25, 30–33) have found that choline inhibits uptake mediated by rOCT2 and rOCT3 and that choline itself is transported by rOCT1 and hOCT2. However, it was also reported that choline is a good substrate for rOCT1 but is not a substrate for rOCT2 or hOCT3 (31). Additionally, there are varying reports as to whether OCT1, OCT2, and OCT3 are even expressed in brain (15–17, 25). Here, we present evidence indicating that OCT2 and OCT3 are expressed in rat CP and that choline uptake by intact rat choroid plexus has all the characteristics of OCT2-mediated transport, suggesting that this carrier plays an important role in central nervous system choline homeostasis.

EXPERIMENTAL PROCEDURES

Plasmids—Construction and transport activity of the rOCT2/green fluorescent protein (GFP) fusion and the endoplasmic reticulum (ER)-targeted GFP construct have been described elsewhere (32, 33). The rOCT3 and mOCT3 clones were the generous gift from Dr. Vadivel Ganapathy (14, 34) and hOCT2 from Dr. Kelly Bleasby (35). Xenopus Oocyte Expression Assay—Oocyte isolation procedures and uptake assay were performed as reported previously (22, 36, 37). Briefly, adult female Xenopus laevis (Xenopus One, Ann Arbor, MI) were cooled, anesthetized with tricine methanesulfonate, and decapitated. Follicle-free stage V and stage VI oocytes were isolated by treatment with collagenase A and maintained at 18 °C in Barth’s buffer containing 0.05 mg/ml gentamicin sulfate, 2.5 mM sodium pyruvate, and 5% heat-inactivated horse serum. Oocytes were allowed to recover overnight before injection. Capped cRNA for microinjection was synthesized from linearized plasmid DNA using Ambion’s mMessage mMachineaminolysis kit (Ambion, Austin, TX). Three days after injection with cRNA, oocytes were divided into experimental groups of 6–10 each and incubated at 22 °C for 30 or 60 min in oocyte Ringer 2 (OR-2) containing 75 μM [3H]choline (1 μCi/ml) or 10 μM [3H]choline (1 μCi/ml) in the absence or presence of inhibitor. For uptake studies done under short-circuiting conditions (external K+ concentration raised to 102.5 mM, a condition previously demonstrated to shorten the choline transport time (25), the membrane potential was −100 mV) were examined by confocal fluorescence microscopy (Fig. 1A) depicting the presence of functional transporters, as evidenced by a 70-fold increase in quinidine-sensitive TEA uptake (data not shown). The murine OCT3 ortholog also failed to transport choline (data not shown). Furthermore, choline uptake mediated by rOCT1 and rOCT2 was relatively insensitive to nicotinamide, an effective modulator of brain choline levels (Fig. 1B).
Time course experiments for both 100 μM and 1 mM [3H]choline indicated that choline uptake was linear for at least 90 min in OCT1- and OCT2-expressing Xenopus oocytes (data not shown); therefore, 30-min uptake was used to approximate the initial rate for kinetic measurements. Water-injected oocytes exhibited no mediated transport. cRNA-injected oocytes were incubated in buffer containing 0.05–2 mM choline, and uptake was determined (Fig. 2). Transport in the presence of 200 μM quinine was also determined at 0.05, 0.2, and 1 mM choline concentrations as a measure of the diffusive (linear) component of uptake (Fig. 2). This component was subtracted from total uptake to yield the mediated component. Double-reciprocal analysis of the mediated component yielded a mean $K_m$ (S.E.) for choline of 346 ± 50 μM for rOCT1 and 441 ± 67 μM for rOCT2 (Fig. 2). A choline $K_m$ value for hOCT2 of 102 ± 80 μM was also found (data not shown). These values are similar to respective reported values for the transporters (17, 27, 39).

Choline uptake mediated by hOCT2 was further characterized to determine whether its driving force and transport characteristics were like those documented for rOCT2 (22). hOCT2-mediated TEA uptake (Fig. 3) was markedly reduced by 200 μM quinine, 5 mM NMN, 1 mM choline, and 200 μM tetrapentylammonium as established previously for rOCT2 (22).2 Choline uptake via hOCT2 exhibited a similar inhibition profile, with 1 mM TEA replacing choline as an inhibitor (Fig. 3). Additionally, choline uptake mediated by the rat and human OCT2 orthologs was significantly reduced by short-circuiting the oocyte membrane potential (102.5 mM K$^+$ + 10 μM valinomycin), as demonstrated previously for TEA transport mediated by rOCT2 (Fig. 4) (22).

Expression of OCTs in Adult Rat CP—To determine whether OCT1, OCT2, and/or OCT3 are expressed in CP, mRNA or total RNA was isolated, in independent preparations, from plexus tissue and used as template for reverse transcription. Subsequent PCRs were performed with rOCT1-, rOCT2-, and rOCT3-specific primers using 1 μl of the CP-RT reactions as template. PCR products were detected for rOCT2 (569 bp) and rOCT3 (841 bp) providing direct evidence that these genes are expressed in CP and may play a role in choline clearance from CSF (Fig. 5). No PCR product was detected for rOCT1 (962 bp) in CP. Identical results were obtained for each CP preparation. Positive control reactions with adult rat kidney yielded the expected products for all three genes (Fig. 5). The rOCT2 choroid plexus RT-PCR product was sequenced and found to be identical to rOCT2 cloned from rat kidney (18, 20).

Choline Transport in Adult Rat CP—We next examined whether the driving forces for choline uptake by intact CP in vitro were the same as those utilized by these OCT family members (Fig. 6). Isolated CP supported substantial choline uptake that was completely inhibited by quinine (Fig. 6A). This uptake was largely potential-sensitive as demonstrated by the reduction in the tissue/medium ratio from 14 to 5 when the...
The concentration of potassium in the transport buffer was raised to 102.5 mM (Fig. 6A). CP choline transport was also markedly pH-sensitive, being reduced to background level by lowering buffer pH from 7.4 to 6.0 (Fig. 6B). To determine whether this pH-dependent decrease was due to OC/H+ exchange, we examined [3H]choline efflux from preloaded CP tissue (Fig. 7). Excess external H+ failed to trans-stimulate choline exit, even at >20-fold increased external H+ (pH 6.0 versus pH 7.4). As a positive control, the effect of external TMA on choline efflux was examined because TMA is known to trans-stimulate rOCT2-mediated efflux (22). Choline efflux was significantly trans-stimulated by TMA, whereas excess H+ failed to have an effect (Fig. 7). Uptake of choline by intact CP was also unaffected by reduced Na+/H+ concentration, which significantly reduced uptake of both proline and methotrexate (Fig. 8).

[3H]Choline uptake by intact CP was linear for at least 15 min (data not shown), and 5-min uptakes were used to approximate initial rates. CP were incubated in aCSF containing 18–450 μM choline, and uptake was measured (data not shown). The diffusional component was estimated by the slope of the line defined by uptake at 1.5, 5, 10, and 15 mM choline. Mediated transport was determined by subtracting the calculated value for diffusion at each concentration from the mean total uptake value. Double-reciprocal analysis of the mediated component of uptake yielded an estimated Km for choline of 183 μM (Fig. 9).

Morphology of Adult Rat CP and Subcellular Distribution of rOCT2—The fluorescent plasma membrane marker, FM 4-64, was used to visualize the spatial relationship between the plasma membranes of the CP and the underlying capillary bed (Fig. 10). Transmitted light images show the complex structure of plexus tissue (Fig. 10, A and C). The tissue is composed of “finger-like” capillary projections surrounded by a single layer of cells that protrude into the cerebrospinal fluid-filled ventricles of the brain. The geometry is such that the apical membrane of the cells is bathed by the CSF, and the basal membrane is oriented toward the underlying capillary. The corresponding fluorescence micrographs show that, after a 15-min incubation with 10 μM FM 4-64, the cells of the choroidal epithelium were surrounded by a wall of fluorescence (Fig. 10, B and D). The apical (brush border) membrane appears as a broad band in contact with the medium, and the basal membrane is much narrower in nature and is associated with the
capillary membrane. The nuclei and the capillary space were unlabeled.

To examine the subcellular localization of rOCT2, intact adult rat CP were isolated in vitro and transfected with GFP constructs. CP transfected with cytoplasmic GFP exhibited a diffuse fluorescence that extended throughout the cytoplasm and permeated the nucleus (Fig. 11A). CP transfected with the ER membrane localization marker, ER-GFP, clearly showed a reticulate staining pattern restricted to the cytoplasm and surrounding the nucleus, typical of endoplasmic reticulum labeling (Fig. 11B). Note that with ER-GFP there is no labeling of the plasma membrane and no signal in the nucleus. In contrast, rOCT2-GFP was clearly targeted to the apical plasma membrane and was excluded from the nucleus and basal region of CP cells (Fig. 11, C and D).

**DISCUSSION**

The blood-brain barrier (brain capillary endothelium) and the blood-CSF barrier (epithelia of the choroid plexus, arachnoid membrane, and circumventricular organs) effectively isolate the brain from the systemic circulation. Tight junctions between the cells limit the penetration of solutes and the epithelia regulate the composition of the extracellular fluid.
of the brain (interstitial fluid plus CSF). Since these barriers also limit passive efflux from the brain, specialized excretory systems are required to prevent buildup of potentially toxic compounds, including neurotransmitters and their metabolites (e.g. choline). Therefore, identification of the transporters present in these epithelia and the properties that govern their function is fundamental to understanding the maintenance of brain homeostasis and the basis for disorders involving its perturbation. Toward that end, we investigated the mechanism of transport and localization of OCT2 in the CP of the adult rat. Demonstration of inhibitable choline transport by OCT2, detection of OCT2 mRNA in CP, characterization of the mechanism of choline uptake in intact CP, and localization of OCT2 in the apical membrane of CP cells, taken together, suggest OCT2 may play a critical role in the maintenance of brain choline homeostasis (Figs. 1, 4–8, and 11). Recent investigations of the brain capillary endothelium have shown that the mechanism controlling the entry of choline, neurotransmitters, and therapeutics into the brain has functional properties similar to those exhibited by OCT1 and OCT2, possibly indicating OCT2 controls the net flux of brain choline in its entirety (42, 43).

To support the vectorial movement of solutes like choline, barrier epithelia such as the kidney, brain capillary endothelium, and CP establish polarity of structure and function. In kidney tubule cells basolateral uptake of positively charged organic cations is driven by the potential difference across the membrane and apical exit involves a proton-coupled exchanger, i.e. the OC transport system utilizes carriers responsive to different driving forces for uptake and secretion of substrate (for review see Refs. 44 and 45). Choline transport across the apical membrane of primary cultures of choroidal epithelium from neonatal rats was markedly reduced by membrane depolarization (11). We demonstrate that choline uptake across the apical membrane of intact rat CP in vitro is also membrane potential-driven (Fig. 6). These data are consistent with the polarity of the choroidal epithelium for its role in the clearance of OCs from CSF to blood, such that the potential-sensitive entry step occurs across the ventricular (apical) surface of the choroid plexus.

**FIG. 7.** Effect of trans-H⁺ on choline efflux from isolated adult rat choroid plexus. Freshly isolated plexus tissue was pre-loaded by 60 min of incubation at 37 °C in aCSF containing 50 μM [3H]choline. The plexus tissue was then rapidly rinsed and incubated at 22 °C in aCSF, pH 7.4, or aCSF adjusted to pH 6.0. Duplicate medium samples were taken at the times indicated. Choline efflux from CP was unaffected by excess trans-H⁺, yet significantly stimulated by TMA, confirming choline transport across the apical membrane does not occur via an OC/H⁻ exchanger. Data are presented as % of initial cell content, and values are mean ± S.E. After the initial trans-H⁺ test, the experiment was repeated with a TMA trans-stimulation control (i.e. n = 6 CP/treatment for pH 7.4 and 6.0; n = 3 CP/treatment for TMA). * denotes p < 0.05.

**FIG. 8.** Effect of sodium on choline uptake in intact choroid plexus. Freshly isolated plexus tissue was preincubated for 30 min at 37 °C in either standard aCSF (129 mM Na⁺) or low Na⁺ aCSF (26 mM Na⁺) before being transferred to experimental media containing 50 μM [3H]choline, 10 μM [3H]proline, or 1 μM [3H]methotrexate. Choline uptake (5 min) was unaffected under the low Na⁺ conditions despite significant reductions in proline and methotrexate uptake. This indicates that apical CP choline uptake is not directly coupled to the Na⁺ gradient (i.e. is not mediated by a Na⁺/choline exchanger). The experiment was repeated twice, and the data presented are mean values ± S.E. (n = 3 CP/treatment) from a single experiment. * denotes p < 0.05, and ** denotes p < 0.01.

**FIG. 9.** Kinetic analysis of choline uptake in adult rat choroid plexus. Intact CP were incubated in aCSF containing 18–450 μM choline, and 5-min uptake was measured (data not shown). The diffusional component was estimated by the slope of the line defined by uptake at 1.5, 5, 10, and 15 μM choline. Mediated transport was determined by subtracting the calculated value for diffusion at each concentration from the mean total uptake value. Double-reciprocal analysis of the mediated component of uptake yielded an estimated Kₘ for choline of 183 μM. The experiment was repeated twice, and the raw uptake data were analyzed as mean values with 3–6 CP/concentration.
cells. A similar reversal of function between kidney and CP has been reported previously (38) for organic anion transport. Correspondingly, we have shown that the major driving force for rOCT2- and hOCT2-mediated choline uptake is the membrane potential (Fig. 4).

Apical CP choline uptake was observed to be pH-sensitive as well (Fig. 6B), suggesting that a choline/H+ exchange mechanism may also be involved. However, externally applied (i.e. trans) H+ failed to stimulate choline efflux, whereas trans-applied OC (TMA) significantly stimulated efflux, indicating that an OC/H+ exchanger does not mediate choline transport across the apical membrane of adult rat CP (Fig. 7). It was also possible that the high and low affinity choline transporters present in cholinergic neurons could play a role in apical CP...
Choline transport. Previous work (9, 10, 46, 47) on intact adult CP (from rat, rabbit, and bullfrog) indicated that apical CP choline transport is coupled to the Na⁺ gradient at some level, perhaps through a direct coupling to the Na⁺ gradient (i.e. Na⁺/choline cotransport) as observed for the high affinity choline transporter in cholinergic neurons (48–51). Therefore, the effect of Na⁺ on choline uptake in CP was examined by lowering external Na⁺ from 129 to 26 mM (Fig. 8). Low Na⁺ conditions produced a significant reduction in the uptake of proline, which is mediated by a Na⁺/proline cotransporter (52, 53), and of methotrexate, an organic anion handled by transporters (54, 55) dependent upon a counterion gradient that is maintained by a Na⁺/dicarboxylate cotransporter (56, 57). However, lowering of Na⁺ was without effect on CP choline uptake, demonstrating choline uptake across the apical CP membrane is not directly coupled to Na⁺ influx (Fig. 8) and, therefore, does not involve the high affinity transporter from cholinergic neurons. These results are in agreement with similar experiments conducted in primary choroidal epithelial cultures (11). Choline uptake in primary cultures was inhibited by HC-3 (11) demonstrating that the HC-3-insensitive low affinity transporter is also not expressed in CP (51). Correspondingly, rOCT2 is also HC-3-sensitive (Fig. 1A). Taken together, all of these properties are consistent with OCT2-mediated apical choline uptake from CSF.

Accordingly, OCT2 message should be found in the CP. However, the literature on OCT expression in brain is contradictory. OCT3, but neither OCT1 nor OCT2, has been detected in brain by Northern blot (15, 17, 25). PCR analysis of brain expression is equally confusing, with results indicating the following: (i) rOCT2, but not rOCT1, is expressed (16); (ii) rOCT3, but not rOCT1 or rOCT2, is expressed (25); (iii) both hOCT1 and hOCT2 are expressed (17). Perhaps these contradictions are a result of where and at what levels each of the various paralogs are expressed in the brain, and detection is dependent upon how the tissue is collected for message isolation (e.g. pieces of cortex versus whole brain). Regardless, by using RT-PCR we readily detected rOCT2 and rOCT3, but not rOCT1, message in two independent preparations from adult rat CP (Fig. 5). This finding suggests that OCT3 could also play a role in CP choline transport. However, choline was found to be a poor inhibitor of rOCT3-mediated uptake, even when present at 5,000-fold excess (14, 25), and was shown not to be a substrate for rOCT3 (Fig. 1A), mOCT3 (data not shown), or hOCT3 (31). Thus, despite detection of OCT3 message in rCP, this transporter should not mediate ventricular choline transport.

Two additional transporters with moderate sequence homology and similar predicted topology to these OCT family members, OCTN1 and OCTN2, have also been identified in human and rat (58–61). Detection of their expression in rCP by RT-PCR raises the possibility that they, too, may function in choline transport (data not shown). However, choline does not interact with rOCTN1 and is a poor inhibitor of TEA uptake by rOCTN2 (58, 59). Studies with the human orthologs found that choline present at as much as a 40,000-fold excess produced only weak inhibition of OC transport by either transporter, again indicating choline does not interact with these carriers (59, 60, 62, 63). Furthermore, OC uptake mediated by these transporters is pH-dependent and membrane potential-insensitive, properties that do not correspond mechanistically with the properties governing CP choline uptake, but rather indicates that they function as OC/H⁺ exchangers (58–63).

Functionally, OCT2 is a potential-driven transporter and, thus, for renal OC secretion mechanism requires that OCT2 should thus be in the basolateral membrane of the proximal tubule cells (22, 26). Indeed, OCT2 has been directly observed in the basolateral membrane of cultured renal cells and intact renal tubules (32, 64). To address the issue of OCT2 membrane localization, we recently developed an alternative approach to transporter localization using GFP fusion constructs. The proper targeting of cytoplasmic, endoplasmic reticulum, and mitochondrial GFP variants in cultured renal cell monolayers and isolated renal proximal tubules has been shown, demonstrating that these GFP constructs accurately reflect the subcellular localization of the native proteins (32, 37). Madin-Darby canine kidney cells stably transfected with the rOCT2-GFP construct showed strong basal and lateral membrane localization that correlated with increased specific, potential-driven, basal TEA uptake (32). Transfection experiments with renal proximal tubules provided direct evidence of rOCT2-GFP targeting to the basal and lateral membranes of an intact, polarized renal epithelium, with no evidence for luminal membrane localization (32). In marked contrast to these findings in renal epithelia, rOCT2-GFP was specifically targeted to the apical membrane of intact CP transfected in vitro (Fig. 11). This reversal of membrane targeting in CP (as compared with kidney) is in agreement with the functional data obtained for CP (Figs. 6–8). We recently reported similar findings for the organic anion transporter rROAT1 using GFP methodology, rROAT1-GFP localized to the basolateral membrane in renal proximal tubule (37), and the apical membrane in adult rat CP (38). Thus, rOCT2 function, expression, and localization are all consistent with the conclusion that OCT2 plays an active role in brain choline homeostasis.

The presence of OCT2 in the apical membrane of CP may explain, at the molecular level, long standing physiological observations on the mechanism of CP choline transport. For example, despite the fact that NMN does not readily cross the blood-brain barrier (65), it was observed to inhibit effectively the efflux of choline from CSF (8, 9). Subsequently, it was demonstrated that NMN’s parent compound, nicotinamide, is readily taken up into the brain (66, 67) and that subcutaneous administration of nicotinamide leads to increased choline levels in the brain (7). This latter observation was proposed to be due to the inhibition of ventriculocisternal choline transport systems by the conversion of nicotinamide to NMN (7). In support of this hypothesis, the formation of NMN from nicotinamide by an enzyme present in rat brain cytosol has been demonstrated (5). Thus, the lack of effect of nicotinamide on OCT2-mediated choline transport (Fig. 1B) and the substantial inhibition of this transporter by NMN (Fig. 3) (22) correlates with these observations on CP choline flux. Studies showing that the coadministration of nicotinamide with choline (at doses corresponding to choline levels encountered in the diet) leads to greatly increased brain choline levels underscore the possibility that inhibition of OCT2-mediated choline transport could be an effective therapy for central cholinergic dysfunction (6).

In summary, we have demonstrated that the properties of apical choline uptake in CP correspond with those established for OCT2. Furthermore, we have detected expression of OCT2 message in CP and observed apical membrane localization of OCT2-GFP in intact CP. Together, these results suggest that OCT2 mediates apical CP choline uptake from the CSF.

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REFERENCES
