Choline uptake across the ventricular membrane of neonate rat choroid plexus

ALICE R. VILLALOBOS,1 JUDITH T. PARMELEE,2 AND J. LARRY RENFRO1
1Department of Physiology and Neurobiology, University of Connecticut, Storrs 06269; and 2Manchester Community-Technical College, Manchester, Connecticut 06045

Villalobos, Alice R., J udith T. Parmelee, and J. Larry Renfro. Choline uptake across the ventricular membrane of neonate rat choroid plexus. Am. J. Physiol. 276 (Cell Physiol. 45): C1288–C1296, 1999.—The uptake of [3H]choline from the cerebrospinal fluid (CSF) side of the rat neonatal choroid plexus was characterized in primary cultures of the choroidal epithelium grown on solid supports. Cell-to-medium concentration ratios were ~5 at 1 min and as high as 70 at 30 min. Apical choline uptake was facilitated; the $K_m$ was ~50 µM. Several organic cations (e.g., hemicholinium-3 and N-methyl nicotinamide) inhibited uptake. The reduction or removal of external Na$^+$ or the addition of 5 mM LiCl had no effect on uptake. However, increasing external $K^+$ concentration from 3 to 30 mM depolarized ventricular membrane potential (~70 to ~15 mV) and reduced uptake to 45% of that for the control. Treatment with 1 mM ouabain or 2 mM BaCl$_2$ reduced uptake 45%, and intracellular acidification reduced uptake to ~90% of that for controls. These data indicate that the uptake of choline from CSF across the ventricular membrane of the neonatal choroidal epithelium is not directly coupled to Na$^+$ influx but is sensitive to plasma membrane electrical potential.

organic cation transport; cerebrospinal fluid-blood barrier; cholinergic metabolism

THE NEUROTRANSMITTER of cholinergic systems, ACh, is synthesized from acetyl-CoA and choline, and the availability of the latter is rate limiting in this synthesis. Although choline is liberated through the metabolism of membrane phospholipids, it is not synthesized de novo at a significant rate in the brain (34). The choline incorporated into the central nervous system (CNS) is derived primarily from peripheral metabolism and traverses the blood-brain barrier by facilitated diffusion in proportion to changes in blood choline concentration (7). Despite physiological fluctuations in blood choline levels (10–50 µM) and arteriogenous differences (~2 to ~4 µM), cerebrospinal fluid (CSF) choline concentration remains virtually constant (~6 µM; Ref. 15). According to the current model for the homeostatic regulation of choline availability in the CNS, the two prevailing mechanisms responsible for removal of excess choline from the brain are 1) high-affinity reuptake of choline by presynaptic neurons and incorporation into membrane phospholipid and 2) active efflux of choline from brain to blood across the epithelial cell layer of the choroid plexus (CP) (21). Na$^+$-coupled choline reuptake by presynaptic neurons is well charac-
terized; however, the cellular transport mechanisms and modulation of the active transport of choline across the CSF-blood barrier remain poorly understood.

Ventriculocisternal perfusion studies of adult animals have demonstrated that the net clearance of choline from ventricular CSF exceeds the rate of “washout” by bulk flow of CSF from the ventricles through the arachnoid sinuses. Furthermore, clearance of radiolabeled choline from ventricular CSF is reduced in a concentration-dependent manner by unlabeled choline or other quaternary ammoniums (see, e.g., Refs. 1, 13, and 18). In vitro studies have also demonstrated carrier-mediated accumulation of choline by isolated CP (1, 8). Together, these observations are the basis for the supposition of active mediated efflux of excess choline and other organic cations from the CNS across the CSF-blood barrier. The components of transepithelial choline transport by CP are 1) uptake from CSF across the ventricular (apical) plasma membrane, 2) intracellular transport across the cell, and 3) efflux across the vascular-side (basolateral) plasma membrane into the blood compartment. However, because of the complex organization of epithelial and vascular tissue, the small size of this tissue, and the anatomical location of the CP, particularly in mammalian species, most in vivo and in vitro techniques limit direct access to the intact epithelium. Consequently, the experimental characterization of the energetics and polarities of carriers that mediate the transport of choline and other organic cations from CSF to blood has been limited and the role of choroidal choline transport in central cholinergic homeostasis remains poorly understood.

The objective of the present study was to characterize directly the energetics of choline uptake from CSF across the ventricular membrane of CP by using a primary culture system for the neonatal choroidal epithelium. This methodology yields differentiated, confluent epithelial monolayers that maintain morphological and functional polarization similar to that of intact tissue (33). Choroidal epithelial cells plated on an impermeable substratum with the ventricular (apical) membranes exposed at the free surface were used to characterize the coupling of apical choline transport to transmembrane ion gradients (e.g., K$^+$, Na$^+$, and H$^+$) and membrane electrical potential. The data presented here demonstrate that carrier-mediated choline uptake across the ventricular membrane of neonatal CP is electrogenic and is not directly coupled to Na$^+$ transport.

MATERIALS AND METHODS

Animals and tissue harvest. Three- to five-day-old Sprague-Dawley rats were anesthetized under hypothermic conditions...
before decapitation and removal of the brain. For each preparation, lateral, third, and fourth plexuses from a total of 30–36 neonatal rats were removed and held in chilled DMEM/Ham’s F-12 medium (DMEM/F-12) supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml).

Cell culture. Epithelial cells were isolated from CP tissue by enzymatic dispersion as described previously in detail (33). Briefly, tissue was suspended in dissociation buffer (in mM: 137 NaCl, 2.7 KCl, 0.7 Na2HPO4, 5.6 glucose, 10 HEPES; pH 7.4) with 5 U protease and 1,500 kU/ml DNAse I. The tissue-extracellular matrix was incubated at 37°C and intermittently triturated over a 20-min period. Aliquots of cells were filtered through 100-µm nylon mesh, and the filtrate was centrifuged and washed twice with DMEM/F-12. Cells were suspended in antibiotic-supplemented DMEM/F-12 with 10% Nu-Serum IV and plated for 2–3 h (37°C, 95% air-5% CO2). Unattached cells were then aspirated, centrifuged, and suspended in MEM, with d-valine substituted for l-valine, containing 10% Nu-Serum IV, triiodo-l-thyronine (1.5 µM), prostaglandin E2 (100 ng/ml), forskolin (10 µM), and epidermal growth factor (50 ng/ml). Cells were plated at a density of 4.5 × 10^5 cells/cm² in individual wells of 96-well tissue culture plates (0.32 cm²/well). Cells were maintained at 37°C in a humidified 95% air-5% CO2 atmosphere. Unattached cells were removed after 72 h postplating, and the initial plating medium was replaced with maintenance medium (i.e., DMEM/F-12 with 5% Nu-Serum IV and growth promoters). The medium was subsequently changed every 2–3 days.

Choline uptake studies. On day 7 the apical uptake of radiolabeled substrate was assayed. Cells were rinsed and preincubated with artificial CSF (aCSF; in mM: 118 NaCl, 3 KCl, 0.7 Na2HPO4, 18 NaHCO3, 2 urea, 0.8 MgCl2, 1.4 CaCl2, 12 glucose, 20 Tris-HEPES; pH 7.4) for 1 h at 37°C. To initiate uptake, the preincubation buffer was replaced with 150 µl aCSF containing 10 µM [3H]choline chloride (triplicate measurements in 2 separate culture preparations). Total uptake was assayed in triplicate for cells from at least three separate culture preparations (i.e., n = 3). Data are presented as means ± SE. Control and experimental means were compared by Student’s t-test for paired observations and were deemed to be significantly different at P < 0.05.

RESULTS

Time-dependent accumulation of choline across the apical membrane. The apical uptake of 10 µM [3H]choline by cells grown on solid substratum was time dependent through 180 min (Fig. 1). The uptake was linear through 30 min [sample coefficient of correlation (r) = 0.9918]. At the earliest sample (1 min) total cellular choline uptake was 481 ± 5.5 pmol/mg protein; the mean cell-to-medium concentration ratio was ~5. Even at cell-to-medium concentration ratios of 395, steady state was not reached (by 180 min). The total cellular accumulation of choline could reflect both concentrative transport across the apical membrane into the cytosolic compartment and intracellular sequestration of substrate, as has been shown for other organic cations in this culture system (24). Although choline metabolism was not directly examined in this culture system, others have shown that in CP the metabolic conversion of choline is minimal (see, e.g., Ref. 18).
Kinetics of apical choline uptake. The kinetic parameters of the initial rate of choline uptake across the apical membranes of cultured cells were determined by tracer displacement (23). The 5-min uptake of 5 µM \(^{3}H\) choline in the presence of 0–5 mM unlabeled choline was assayed (Fig. 2). On the basis of the assumption that unlabeled choline competitively inhibits the uptake of \(^{3}H\) choline, the initial rate of \(^{3}H\) choline uptake (\(V\)) was expressed as a function of the external concentration of unlabeled choline (CHOL): 
\[
V = (\frac{V_{\text{max}} \times [^{3}H\text{CHOL}]}{K_{m} + [^{3}H\text{CHOL}]}),
\]
where \(V_{\text{max}}\) is the maximal rate of uptake, \([^{3}H\text{CHOL}]\) is the concentration of \(^{3}H\text{choline}\), and \(K_{m}\) is the Michaelis-Menten constant for choline. As the external concentration of unlabeled choline increased, total cellular \(^{3}H\text{choline}\) uptake progressively decreased, asymptotically approaching zero. Apical \(^{3}H\text{choline}\) uptake was inhibited 98% by 5 mM unlabeled choline. Thus a saturable carrier mediates uptake. The calculated mean \(V_{\text{max}}\) ± SE was 4,671 ± 841 pmol mg protein\(^{-1}\) 5 min\(^{-1}\); the mean \(K_{m}\) for choline ± SE was 49 ± 5 µM (n = 4). With consideration of technical limitations, it appears that a nonsaturable process such as simple diffusion or the nonspecific high-affinity binding of a radioligand accounts for a minimal fraction of total apical \(^{3}H\text{choline}\) uptake.

Cis-inhibitory effects of organic cations on apical choline uptake. To assess the general specificity of the apical choline carrier, the uptake of \(^{3}H\text{choline}\) (10 µM) at 30 min in the presence of several endobiotic and xenobiotic organic cations was assayed (Fig. 3). Increasing unlabeled choline to 5 mM inhibited uptake 95% (3,387 ± 277 vs. 128 ± 19 pmol mg protein\(^{-1}\) 30 min\(^{-1}\); 1 mM choline was nearly as effective. At comparable test concentrations, two other prototypic organic cations, N\(^{1}\)-methyl nicotinamide (NMN) and tetraethylammonium (TEA), reduced uptake 20–50%. At 1 mM, tetrapentylammonium (TPeA) inhibited uptake ~95%. Hemicholinium-3 (HC-3) and quinacrine inhibited uptake ~90%.

Trans effects of organic cations on apical choline uptake. Cis inhibition of apical choline uptake by various agents suggested that uptake was carrier mediated. This possibility was further examined by assaying the mediated uptake of 10 µM \(^{3}H\text{choline}\) (i.e., in the absence of HC-3 and in the presence of 500 µM HC-3) by cells preincubated with unlabeled choline, TEA, or NMN (Fig. 4). Mediated choline uptake was stimulated ~40% after preincubation with each test compound. For example, in cells preloaded with unlabeled choline, the uptake of \(^{3}H\text{choline}\) increased from 3,547 ± 164 to 5,517 ± 117 pmol mg protein\(^{-1}\).

Na\(^{+}\) dependence of apical choline uptake. The energetic coupling of choline uptake across the ventricular plasma membrane to gradients of inorganic ions was examined. The 30-min apical uptake of \(^{3}H\text{choline}\) (10 µM) in response to changes of extracellular concentrations of Na\(^{+}\), K\(^{+}\), and H\(^{+}\) was monitored. Studies of the isolated adult CP have suggested that tissue accumulation and CSF clearance of choline may be Na\(^{+}\) dependent (8, 18, 19). The Na\(^{+}\) dependence of choline uptake in CP was examined directly by observing a cultured choroidal epithelium incubated with various external concentrations of Na\(^{+}\) (i.e., isosmotic replacement of Na\(^{+}\) with mannitol; Fig. 5). The reduction of external Na\(^{+}\) from 140 to 20 mM or the complete removal of external Na\(^{+}\) failed to alter uptake. The addition of 5 mM LiCl in the presence of high external...
Fig. 4. Trans effects of organic cations on apical choline uptake by primary cultures of choroidal epithelium plated on an impermeable support and preincubated at 37°C for 60 min in artificial CSF without test compound or with 10 mM unlabeled choline chloride, 5 mM TEA bromide, or 10 mM NMN chloride. After a triple rinse with chilled artificial CSF, cells were incubated at 37°C for 30 min with artificial CSF containing 10 µM [3H]choline chloride and 0 or 500 µM HC-3. Uptake is expressed as mean percentage ± SE of uptake in absence of HC-3 by cells preincubated without test compound (n = 3). *Significantly different from control (P < 0.05). Regardless of preincubation treatment, HC-3 significantly inhibited [3H]choline uptake (P < 0.05).

Na⁺ concentration (135 mM) also had no effect. These data indicated that apical choline uptake is not directly coupled to Na⁺ influx across the ventricular membrane. K⁺ dependence of apical choline uptake. Other in vitro studies have suggested that choline transport by CP is K⁺ dependent (see, e.g., Refs. 8 and 18). To examine directly the K⁺ dependence of ventricular choline uptake by CP, apical [3H]choline uptake was assayed in the presence of various external concentrations of K⁺, as well as of ouabain, a Na⁺-K⁺-ATPase inhibitor, and Ba²⁺, a nonspecific K⁺ channel blocker (Fig. 6). A 10-fold increase in external K⁺ concentration (3 vs. 30 mM KCl) significantly depolarized the intracellular potential (−70 mV ± 5.1 mV, n = 7 cells, vs. −15 mV ± 4.8 mV, n = 6; P < 0.0001) and reduced choline uptake to −45% of control. In the presence of 3 mM K⁺, treatment of cells with ouabain (1 mM) or Ba²⁺ (2 mM BaCl₂) reduced choline uptake −50 and −35%, respectively.

Effects of intracellular acidification on apical choline uptake. The mediated transport of organic cations, including quaternary ammoniums, by H⁺-coupled exchange has been well characterized for the renal proximal tubule (25). Transport of the organic base cimetidine was shown to be pH sensitive in CP (35). The possibility that ventricular uptake of choline was mediated by H⁺ exchange was tested with choroidal cells subjected to NH₄Cl pulse acidification. In the control condition cells were preincubated for 15 min with standard aCSF and then incubated for 30 min in aCSF containing [3H]choline. To acidify the intracellular pH, cells were preincubated with 30 mM NH₄Cl in HCO₃⁻-free CSF and then incubated in Na⁺- and HCO₃⁻-free CSF containing [3H]choline and no NH₄Cl. Na⁺ was isosmotically replaced with mannitol for these experiments. The effectiveness of this technique to reduce intracellular pH in this culture system was
previously demonstrated with 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM, which is converted intracellularly to the free acid, BCECF, a fluorescent pH indicator. A 30 mM NH₄Cl pulse and the subsequent removal of NH₄Cl and external Na⁺ reduce intracellular pH by ~1 (32). Intracellular acidification reduced mediated apical choline uptake ~90% (Fig. 7). A preliminary experiment assayed choline uptake in cells that were incubated with control aCSF (containing Na⁺ and HCO₃⁻) after the NH₄Cl pulse and, thereby, allowed to regulate intracellular pH (26). Total choline uptake was the same as that in cells never subjected to the NH₄Cl pulse (control: 5,375 ± 945 pmol·mg protein⁻¹·30 min⁻¹; recovered: 6,026 ± 379 pmol·mg protein⁻¹·30 min⁻¹; triplicate measurements in a single culture preparation). Furthermore, uptake was equally inhibited by 500 µM HC-3 (control: 658 ± 52 pmol·mg protein⁻¹·30 min⁻¹; recovered: 543 ± 24 pmol·mg protein⁻¹·30 min⁻¹). These preliminary results suggest that decreased choline uptake after intracellular acidification was not the result of cellular deterioration.

**DISCUSSION**

The present direct characterization of the first step in the transepithelial absorption of choline across the neonate CSF-blood barrier, i.e., uptake of choline across the ventricular (apical) membrane of the choroidal epithelium, showed that choline is accumulated by a saturable, electrogenic transport process. Unlike the mediated reuptake of choline by presynaptic cholinergic neurons, choline uptake is not directly coupled to Na⁺ influx across the apical membrane of CP. Instead, uptake is energetically driven by the K⁺ diffusion potential across the apical membrane, as indicated by the decreased choline uptake after either direct or pharmacological perturbations of the K⁺ gradient. Thus the K⁺ dependence of apical choline uptake may not involve a direct physical coupling to the K⁺ flux but rather an energetic coupling of uptake to the negative intracellular potential dictated predominantly by the electrodiffusive efflux of K⁺ across the plasma membrane.

Mediated uptake of choline across the ventricular membrane of cultured choroidal epithelium. Ventriculocisternal clearance studies have demonstrated that choline absorption across the CSF-blood barrier is an active process (1, 18). In vitro choline accumulation by CP isolated from several species also indicates a saturatable process(es) (see, e.g., Refs. 1 and 8). In the present study, choline accumulated across the apical membrane of a cultured neonatal CP epithelium in a time-dependent manner and the total cell-to-medium concentration ratios at all time points exceeded unity, suggesting a metabolically dependent process (Fig. 1). Developmental studies of isolated CP from rabbits have demonstrated that the capacity to accumulate choline increases approximately fourfold as the animal matures (20). However, the present 30-min cell-to-medium choline ratios for neonate rat CP at the same external choline concentration (10 µM) are comparable to tissues-to-medium ratios reported for isolated CP of adult rats (19).

These present data indicate that the initial uptake of choline from CSF into the choroidal epithelial cell is a carrier-mediated membrane transport process (Figs. 2 and 3). Previous studies indicated that the transport capacity for choline in CP increases with age (20) and may vary among species (19). However, the Kₘ of the rat neonatal CP apical carrier for choline (~50 µM) closely approximates the reported Kₘ for adult choline clearance from CSF in vivo (~16 µM; Ref. 18) and that for choline accumulation by the adult rabbit CP in vitro (~40 µM; Ref. 1). On the basis of these collective kinetic data on CSF clearance and tissue accumulation, choline is transported with greater affinity across the CSF-blood barrier than it is across the blood-brain barrier (Kₘ = 225–445 µM; Ref. 7). Although the affinity of this CP choline carrier is much lower than that of the choline carrier in cholinergic neurons (Kₘ ~ 1–3 µM; Ref. 12), it is similar to that of the choline carrier in the apical membranes of the renal proximal tubule (Kₘ = 100 µM; Ref. 36) and small intestine (Kₘ ~ 150 µM; Ref. 27). More importantly, these kinetic data support the proposed role of CP in central cholinergic homeostasis. The rate of the carrier-mediated removal of choline from CSF would be most responsive to changes in CSF choline concentration within a range whose upper limit is less than the experimentally determined Kₘ. The physiological range of CSF choline concentration is 3–10 µM (15). Thus it appears that the
CP choline transporters, working in series or in parallel, have affinities appropriately set to respond to normal or pathophysiological increases in CSF choline concentrations.

Prior evaluation of the specificity of choline transport by CP has been limited. NMN, an endogenous quaternary ammonium that is transported across the CSF-blood barrier and that inhibits the transport of organic cations and bases by CP (see, e.g., Refs. 28, 30, and 33), also inhibits CSF clearance of choline and its accumulation by isolated CP in a concentration-dependent manner (1, 16, 18). In a cultured choroidal epithelium, NMN also inhibited apical choline uptake in a concentration-dependent manner (Fig. 3). However, hexamethonium, another cationic substrate transported by CP (28, 30), did not alter uptake, even at concentrations 3 orders of magnitude greater than that of radiolabeled choline (see Fig. 3). Nevertheless, choline uptake was inhibited by several other organic cations that are known substrates of carriers for choline and other organic cations in neuronal and epithelial tissues, including the present CP culture system. Uptake was inhibited by HC-3, a high-affinity inhibitor of choline transport by presynaptic neurons and the renal proximal tubule, liver, intestine, and placenta (12, 17, 27, 31, 36), as was the clearance of choline from ventricular CSF (9). Tetraalkylammoniums TEA and TPEA also reduced choroidal uptake of choline; their relative inhibitory potencies at 1 mM (TPEA > TEA) qualitatively paralleled those observed for transport by apical membranes of the renal proximal tubule (36). However, the reduction of uptake of 10 μM choline across the intact choroidal epithelial cell by these tetraalkylammoniums at 1 mM may be due in part to reduced K+ channel conductance (see, e.g., Ref. 4). Quinacrine, which shares a common apical carrier with TEA in CP (24), was among the most potent inhibitors tested; however, other possible effects of this agent have not been characterized. Collectively, these kinetic and inhibitor data indicate that mediated choline uptake across the ventricular membrane of a cultured choroidal epithelium is partially mediated by an organic cation carrier with a substrate specificity profile qualitatively consistent with that described previously for choline transport in intact CP and other epithelial tissues.

K+ dependence of ventricular choline uptake. Several Na+-independent electrogenic transport systems for choline in nonneuronal tissues have been characterized. For example, choline transport across the apical membranes of the renal proximal tubule, hepatocyte, and placenta is mediated by Na+-independent electrogenic facilitated diffusion (17, 31, 36). The electrodiffusion of K+ across the apical membrane ultimately dictates the negative membrane potential of the choroidal epithelial cell (37, 39), and studies of isolated CP from adult amphibians demonstrate that pharmacological manipulation of either the K+ gradient or K+ conductance across the ventricular membrane depolarizes the membrane potential. A 10-fold increase in ventricular K+ concentration depolarizes membrane potential (39). The addition of ouabain to the ventricular compartment reduces CP tissue K+ content and increases ventricular (CSF) K+ concentration (14, 29, 39), as well as depolarizing membrane potential (38, 39). In the present CP culture system, increased external K+ (3–30 mM KCl) or treatment with ouabain reduced apical choline uptake to 45–50% of control (Fig. 6), and by direct measurement it was confirmed that increases in external K+ at the membrane depolarized intracellular potential. In addition, choline uptake was significantly reduced by Ba2+, a nonspecific K+ channel blocker shown to not only inhibit K+ efflux from isolated CP but also to induce marked depolarization of ventricular membrane potential (14, 39). On the basis of single-channel recordings from excised apical membranes of amphibian CP, Ba2+-induced depolarization may involve decreased K+ conductance through...
Ca\textsuperscript{2+}-activated channels as a result of reduced channel-open probability and increased closed time (4). In the renal proximal tubule, treatment with Ba\textsuperscript{2+} also results in the depolarization of membrane potential and decreased basolateral organic cation uptake, an electrogenic facilitated diffusion mechanism (25). Thus the marked reductions of apical choline uptake after increased external K\textsuperscript{+} and pretreatment with either ouabain or Ba\textsuperscript{2+} are consistent with the energetic coupling of choline transport to the K\textsuperscript{+} diffusion potential across the ventricular pole of the neonatal choroidal epithelial cell.

Apical choline uptake in the cultured choroidal epithelium was also reduced by TEA and TPeA (Fig. 3), tetraalkylammoniums that directly block K\textsuperscript{+} channels (see, e.g., Ref. 5). At 1 mM, TEA modestly inhibited choroidal apical choline uptake (20%). At similar or higher concentrations, TEA does not alter choline transport in microvillus membranes isolated from other epithelia (see, e.g., Refs. 17 and 36). However, inhibition of uptake in the intact choroidal cell may involve the concurrent block of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels. Indeed, in patches from the apical membrane of amphibian CP, TEA at the extracellular or cytoplasmic face reduces single-channel current and channel-open probability, albeit with greater potency at the extracellular face (4). In membrane patches, the affinity of the binding site for TEA at the external opening of the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels is relatively high [dissociation constant (K\textsubscript{d}) = 230 \textmu M; Ref. 4]; however, in intact epithelial cells the affinity for extracellular TEA is greatly reduced (22). A similar decreased sensitivity to TEA of K\textsuperscript{+} channels in membrane patches vs. those in intact tissue is also observed in colonic smooth muscle (5). In contrast to TEA, TPeA reduced choline uptake ~95%. In apical membranes of the renal proximal tubule at similar substrate and inhibitor concentrations, TPeA inhibits choline transport ~60%; Wright et al. (36) have suggested that this may involve interactions of TPeA at sites other than the substrate-binding site of the choline transporter. Extracellular TPeA is a potent inhibitor of maxi-K\textsuperscript{+} channels in colonic smooth muscle (K\textsubscript{d} = 12 \textmu M; Ref. 5). Thus, in the choroidal epithelium, decreased choline uptake in the presence of TPeA could involve reduced K\textsuperscript{+} channel activity and the subsequent depolarization of membrane potential, as well as a direct inhibitory interaction with the choline carrier.

Organic cation/H\textsuperscript{+} exchange mechanisms mediate the transport of organic cations in several epithelial tissues, including the renal proximal tubule, liver, and intestine (25). Investigation of mediated transport of organic cations by H\textsuperscript{+} exchange in CP has been limited. Nonetheless, as shown in ATP-depleted slices and isolated apical membrane vesicles of CP, accumulation of the xenobiotic cimetidine is stimulated after intracellular acidification or imposition of an outwardly directed H\textsuperscript{+} gradient, respectively. Such stimulation was taken as an indication of H\textsuperscript{+}-driven exchange for the organic base (35). Likewise, in cultured neonatal CP, intracellular acidification markedly stimulates mediated apical uptake of the organic cation TEA (32). In contrast, apical choline uptake was reduced 90% after intracellular acidification (Fig. 7). These data indicate that choline uptake is not mediated by a proton exchange mechanism but may indeed be a pH-sensitive process. On the basis of the observed correlation of changes in intracellular pH with changes in K\textsuperscript{+} conductance and membrane potential in CP and other tissues, these data are consistent with energetic coupling of choline uptake to membrane potential. In isolated hepatocytes intracellular acidification induced by the NH\textsubscript{4}Cl pulse technique is associated with depolarization of the resting membrane potential (2). Furthermore, in isolated hepatocytes and isolated CP, intracellular acidification reduces the K\textsuperscript{+} permeability of the plasma membrane and intracellular alkalinization has the converse effect (2, 38). It has been suggested that the depolarization of membrane potential and reduced K\textsuperscript{+} permeability are due in part to reduced activity of K\textsuperscript{+} channels. In excised patches of the apical membrane of amphibian CP, acidification of the cytoplasmic side decreases the open probability of both maxi-K\textsuperscript{+} channels and Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (4, 6). Indeed, for maxi-K\textsuperscript{+} channels, cytoplasmic acidification (pH 7.4 to 6.4) decreases open time and increases closed time by factors of 10 and 2, respectively, and reduces voltage-dependent activation (6).

In the present study, total cellular choline uptake did not reach equilibrium, even at 180 min. Others have reported similar delays in the equilibration of choline and other organic cations in isolated CP (8, 30), as well as tissue-to-medium ratios that exceed predicted values for electrical equilibrium (20, 30). Indeed, for this CP system with a membrane potential of ~70 mV, the predicted cell-to-medium choline ratio would be 15, and 30-min values ranged from 40 to 70. Thus facilitated electrodiffusive uptake of choline across the apical membrane alone could not account for this unexpectedly high accumulation. Metabolic transformation of substrate could increase the total radioactivity within the cell over time. However, uptake by isolated CP apparently involves little synthesis of ACh because of low levels of choline acetyltransferase activity (11), and unlike renal or hepatic clearance of choline from plasma, which involves metabolic conversion to betaine (3), the clearance of exogenous choline from ventricular CSF involves no metabolism of radiolabeled substrate (18). Total cellular accumulation of choline may instead reflect concentative uptake across the apical membrane into the cytosol and maintenance of the choline electrochemical diffusion potential by subsequent intracellular sequestration of substrate, as has been shown for the organic cations quinacrine and TEA in this culture system (24). The sequestration of substrate from the cytosol into an intracellular compartment is most likely metabolism dependent and could very well contribute to the high tissue-to-medium ratios for organic cations observed in this and other experimental CP systems. The possible role of the intracellular compartmentation of substrate in the net transepithelial-
Choline transport by choroid plexus

C1295

Lial transport of choline and other organic ions across the CSF-blood barrier remains to be elucidated.

In conclusion, choline uptake across the ventricular plasma membrane of primary monolayer cultures of the neonatal CP epithelium is a mediated process directly dependent on the plasma membrane electrical potential and factors that may alter that potential. Uptake is not directly coupled to the Na$^+$ flux across the membrane. However, the energetic coupling of choline uptake to Na$^+$ transport at the apical pole of the CSF-blood barrier may change as the animal develops. Better characterization of the mechanisms of choline transport across this brain barrier in the adult may yield greater insight into any such developmental changes.

The authors acknowledge the expert assistance of Dr. Joseph Lo Turco and Eric Charych in measuring the intracellular electrical potential.

This work was supported by National Science Foundation Grants IBN 904070 and IBN 9808616, National Institute of Environmental Health Sciences Grant ES 07163–07, and National Institute of Neurological Disorders and Stroke Grant F32 NS 10475.

Address for reprint requests and other correspondence: Dr. A. R. Villalobos, Dept. of Physiology and Neurobiology, Univ. of Connecticut, Box U-156, 3107 Horsebarn Hill Rd., Storrs, CT 06269-4156 (E-mail: villalobos@oracle.pnb.uconn.edu).

Received 28 September 1998; accepted in final form 1 March 1999.

REFERENCES


