The Fodrin-Ankyrin Cytoskeleton of Choroid Plexus Preferentially Colocalizes with Apical Na^+K^+-ATPase Rather than with Basolateral Anion Exchanger AE2

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Abstract

A unique feature of the choroid plexus as a single-layer epithelium is its localization of Na^+K^+-ATPase at its apical (lumenal) surface. In contrast, a band 3 (AE1)-related anion exchanger protein has been localized to the basolateral surface of the choroid plexus. Both Na^+K^+-ATPase and AE1 in other tissues have been shown to bind via ankyrin to the spectrin-actin–based membrane cytoskeleton. Since linkage of integral membrane proteins to the membrane cytoskeleton is important for their restriction to specialized domains of the cell surface, we investigated the polarity of the choroid plexus membrane cytoskeleton. We developed isoform-specific antibodies to confirm the identity of choroid plexus band 3–related polypeptide as AE2. We demonstrated that ankyrin, fodrin/spectrin, actin, myosin, and α-actinin are predominantly apical in choroid plexus and preferentially colocalize with apical Na^+K^+-ATPase rather than with basolateral anion exchanger AE2. Colchicine administration did not alter the polarity of apical cytoskeletal and transport proteins or basolateral AE2 in choroid plexus, suggesting that biosynthetic targeting of these proteins is not microtubule dependent. In choroid plexus papilloma, Na^+K^+-ATPase and AE2 were decreased in amount and failed to preserve their polarized distributions. (*J. Clin. Invest.* 1994. 93:1430–1438.) Key words: choroid plexus • epithelial polarity • anion exchanger 2 • sodium-potassium adenosine triphosphatase • cytoskeleton

Introduction

The great majority of plasma membrane proteins examined to date in polarized epithelia display polarity of expression. The mechanisms of plasma membrane protein targeting remain unknown. Initial experiments focused on the search for targeting signals within the primary sequences of the polarized membrane proteins. Though operationally defined targeting signals have indeed been found in a small number of single-span membrane proteins, none has been defined to date for multiple-span membrane transport proteins. Whereas consensus targeting sequences have been identified for organellar membrane proteins, such consensus sequences have not been found for plasmalemmal proteins (1).

In addition to targeting signals within protein sequences, other targeting mechanisms have been investigated. These include heterotrimeric (2) and low molecular weight G proteins (3) as vesicle targeting and docking agents and selective retention and stabilization by association via ankyrin to the spectrin/actin-based membrane cytoskeleton (4, 5). The latter mechanism, in particular, has been invoked to explain the polarized steady state distribution of anion exchanger 1 (AE1)[1] in the basolateral membrane of renal collecting duct intercalated cells (5) and of the Na^+K^+-ATPase in the basolateral membrane of Madin-Darby canine kidney (MDCK) cells (4) and kidney tubules (6, 7). One clonal line of MDCK cells appears to deliver nascent Na^+K^+-ATPase randomly to basolateral and apical cell surfaces but selectively stabilizes the protein at the basolateral surface, apparently through binding to ankyrin (4, 8).

In most polarized epithelial cells, immunoreactive ankyrin is concentrated at the basolateral membrane (5, 9). However, several cell types express ankyrin-associated P-type ATPases in the apical membrane. For example, the apicovesicular localization of the H^+K^+-ATPase in rabbit gastric gland parietal cells appears to be accompanied by apical localization of immunoreactive ankyrin (10). Another cell type which expresses apical ankyrin is the retinal pigment epithelium, in which Na^+K^+-ATPase colocalizes to the apical surface (11). However, this double-layer epithelium is unique in that its apical surface is not lumenal but rather is in N-CAM–stabilized contact with photoreceptor cells (12). The choroid plexus, in contrast, is a simple, single-layer epithelium with a lumenal apical surface. It is the only such epithelium which expresses apical Na^+K^+-ATPase. Interestingly, the choroid plexus also secretes apically into the cerebrospinal fluid (CSF) transthyretin, ceruloplasmin, and cystatin C, all proteins which are secreted basolaterally by hepatocytes (13–15). The cDNAs which encode these secreted proteins have all been cloned from both choroid plexus and from liver and have been found not to differ in the two cell types (16). In addition, peptide map, immunochemical (17, 18), and transcript analyses (19) have indicated that choroid plexus expresses the α1 subunit of the Na^+K^+-ATPase, as well as β1 and β2 subunits. The unusual apical expression and secretion of these polypeptides, biochemically indistinguishable from their basolateral counterparts in other epithelial tissues, has led to the hypothesis that the choroid plexus has undergone a "reversal" of elements of its protein-sorting mechanism.

In addition to the Na^+K^+-ATPase, another membrane transport protein expressed in abundance in choroid plexus is

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1. *Abbreviations used in this paper:* AE1, anion exchanger 1; CSF, cerebrospinal fluid; MDCK cells, Madin-Darby canine kidney cells.

the band 3–related chloride/bicarbonate exchanger AE2 (20, 21). AE2 shares with the erythroid anion exchanger (AE1, band 3) conserved sequences within their cytoplasmic domains which have been proposed as binding sites for nonerythroid homologs of erythroid cytoskeletal proteins, including ankyrin (22, 23). The choroid plexus presents a situation in which a known ankyrin-binding protein, Na^+K^+-ATPase, is localized apically (24, 25) while a proposed ankyrin-binding protein, AE2, is localized basolaterally (21).

In this paper we characterize isoform-specific antibodies to AE2 and use them to confirm AE2 as the band 3–related protein of the basolateral membrane of the choroid plexus. We localize AE2 and the Na^+K^+-ATPase to opposite surfaces of the choroid plexus epithelium. We show that immunoreactive forms of ankyrin, spectrin, and actin all are concentrated at the apical membrane, away from AE2, and together with Na^+K^+-ATPase. In spite of this "reversed polarity" of the ankyrin/spectrin cytoskeleton, the microtubule organizing center appears to be conventionally situated in the apical pole of the cell (26), as judged by the preservation only of apical microtubules after in vivo colchicine administration. Interestingly, colchicine had no effect on the steady state distribution of any of the apical proteins of the choroid plexus studied, in contrast to the significant redistribution of apical hydrolases and cytoskeletal proteins produced in the brush border of ileum (26) and the redistribution of apical gp330 in the renal proximal tubule (27). Lastly, we show that both apical Na^+K^+-ATPase and basolateral AE2 are depolarized and expressed at reduced levels in the murine choroid plexus papilloma which develops in mice transgenic for SV40 large T antigen.

Methods

Antibodies and reagents. Antipeptide antibodies to murine AE2 (28), to human ankyrin (9), and to actin, myosin, α-actinin, and tubulin (29) have been described. Antibody to sea urchin egg spectrin which cross-reacts with brain spectrin (fodrin) in many species was from D. Begg (30). Additional antibodies to human erythroid ankyrin were gifts of V. Patel (31) and C. Cohen. Rabbit polyclonal antibodies to amino-terminal dodecapeptides of rat α1 (32) and β1 (33) subunits of Na^+K^+-ATPase were raised against HPLC-purified synthetic peptides coupled with sulfo-3-maleimidobenzoic acid *N*-hydroxy succinimide ester (Pierce Chemical Co., Rockford, IL) via the COOH-terminal cysteines to keyhole limpet hemocyanin and then affinity-purified over peptide-agarose columns (28). Secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) included, for immunofluorescence, goat anti-rabbit and affinity-desorbed donkey anti-rabbit antibodies coupled to Texas red and affinity-desorbed donkey anti-mouse coupled to fluorescein. Also used were goat anti-rabbit and goat anti-mouse secondary antibodies coupled to alkaline phosphatase.

Bromochloroindolylphosphate and nitro blue tetrazolium were from Kirkegaard and Perry Laboratories, Inc. (Gaithersburg, MD). LX-112 embedding resin was from Ladd Research Industries, Inc. (Burlington, VT). Other reagents were from Sigma Chemical Co. (St. Louis, MO), Calbiochem-Novabiochem Corp. (La Jolla, CA), or Boehringer Mannheim Corp. (Indianapolis, IN).

SV40-TAg#188(8) mice transgenic for SV40 enhancer region antigen, designated Tg(SV40E)Bri7, were provided by E. Sandgren and R. Brinster (34).

Tissue preparation. Rats and mice were anesthetized and perfusion-fixed as described (35). Alternatively, animals were narcotized with CO_2, and choroid plexus was fresh-dissected into PBS or artificial CSF (36) with the aid of a dissecting microscope. The tissue was either placed directly into SDS load buffer, or fixed in 3% paraformaldehyde, quenched, and suspended in 3% agar-PBS which was allowed to solidify, and fixed for embedding (37). Transgenic mice were monitored for the development of ataxia and/or cerebellar bossing. Mice showing these physical signs were anesthetized, and choroid papilloma tumor and surrounding choroid plexus were dissected as above.

Fertilized chick eggs at embryonic day 13 were gifts of Dr. D. Wu and Dr. C. Cepko. Choroid plexus was fresh-dissected into artificial CSF and handled as above. Pig choroid plexus was obtained by dissection of heads obtained after surgery from the Tufts University School of Veterinary Medicine (Medford, MA).

Transfections. p70ZN8 (22) encoding murine AE2 protein and pL2AΔ encoding murine kidney AE1 (38) were transiently transfected into COS-1 cells using the DEAE-dextran procedure (38, 39). [^{35}S]-Methionine labeling of transfected cells and immunoprecipitations from whole transfected cell lysates were performed as described (38, 39).

Immunoblots. SDS-PAGE and electrophoretic transfer of protein to nitrocellulose were performed as described (36). Antibodies were used at concentrations ranging between 1:50 and 1:500. Competitor peptides were added to the incubations at concentrations between 4 and 25 μg/ml. Secondary antibodies coupled to alkaline phosphatase were used to localize antibody binding, and blots were developed with bromochloroindolyl phosphate and nitro blue tetrazolium. Protein was measured by the bis-cinchoninic acid assay (Pierce Chemical Co.).

Immunocytochemistry. The 3% agar blocks containing choroid plexus were postfixed at 4°C for a week, then embedded in LX-112 resin as described (37). Alternatively, small tissue fragments were quick-frozen, freeze-dried, vacuum-embedded in Epon, and processed for immunostaining as described (26). Semithin sections of nominal 0.5–1-μm thickness were cut on a Sorvall Porter-Blum microtome. Confocal microscopy of these sections (Molecular Dynamics-Sarastro, San Francisco, CA) indicated true thicknesses of up to 2.5 μm. Antibody incubations were modified from previously described procedures (36).

Transfected COS cells were plated onto 12-mm coverslips, which were fixed in 3% paraformaldehyde, quenched, washed, mounted, and processed as previously described (39).

Results

Confirmation that the band 3–related protein of choroid plexus is AE2. AE2 mRNA has been detected in choroid plexus papilloma (22) and normal choroid plexus, and the cDNA has been

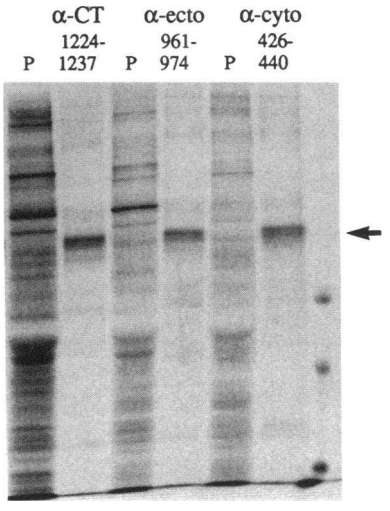

Figure 1. Immunoprecipitation of AE2 by three anti-AE2 peptide antibodies (as noted) from lysates of AE2 cDNA-transfected COS cells. The predominant AE2 species is the 145-kD endoplasmic reticulum form (*arrow*). The more highly processed Golgi form, barely visible at ~ 160 kD, was not prominent in these cells. Crude preimmune sera (*P*) did not precipitate AE2 from transfected cell lysates. ^{14}C-Labeled molecular size standards in right-most lane are (from bottom) 45, 68, 97, and (above *arrow*) 200 kD.

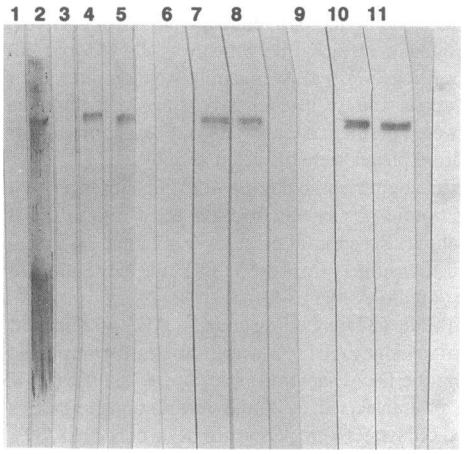

Figure 2. Specificity of immunoblot detection of AE2 in rat choroid plexus by three anti-AE2 peptide antibodies: Preimmune serum (lane *1*), crude anti-424–440 (lane *2*), affinity-purified anti-424–440 in the presence of peptide antigen (lane *3*), nonspecific peptide (lane *4*), or no peptide (lane *5*); anti-961–974 in the presence of peptide antigen (lane *6*), nonspecific peptide (lane *7*), or no peptide (lane *8*); anti-1224–1237 in the presence of peptide antigen (lane *9*), nonspecific peptide (lane *10*), or no peptide (lane *11*). Choroid plexus harvested directly into SDS displayed only one mature AE2 band of 165-kD M_r without degradation. Prestained size standards in the rightmost lane above and below the AE2 band are 208 and 97 kD.

cloned from choroid plexus. Furthermore, a band 3–related polypeptide has been detected using a cross-reactive antibody to the carboxy-terminal peptide of AE1 (20). To establish definitively the identity of this protein of the choroid plexus, we developed antibodies to AE2 peptides and tested their specificity first on COS cells transiently transfected with AE2 cDNA and then on choroid plexus. Fig. 1 shows the results of immunoprecipitation with affinity-purified immune or preimmune sera from detergent lysates of [^{35}S]methionine–labeled transfected COS cells. The core-glycosylated AE2 polypeptide of 145 kD (*arrow*) was the predominant biosynthetic product of the cells, with only traces of the 165-kD mature glycosylated AE2 (38). The AE2 polypeptides were precipitated by all three antipeptide antibodies but not by their corresponding crude preimmune sera. Whereas anti-1224–1237 precipitated kidney AE1 transiently expressed in COS cells, anti-961–974 and anti-426–440, directed against unique AE2 sequences, did not recognize AE1. Similarly, antibodies directed against unique AE1 sequences (36) and AE3 sequences (in preparation) did not recognize AE2 (not shown). Each of the three anti-AE2 antibodies also detected transiently overexpressed AE2 in COS cells by immunocytochemical staining (not shown).

The three antipeptide AE2 antibodies were next used to search for AE2 in choroid plexus. Fig. 2 demonstrates specific immunodetection of mature glycosylated AE2 polypeptide in rat choroid plexus. The 165-kD M_r of choroid plexus AE2 was of the same size as in gastric mucosa (28) and as the (barely visible) upper band of AE2-transfected COS cells (Fig. 1). The AE2 band detected by each of the three antibodies was completely abolished by inclusion of peptide antigen, but not nonspecific peptide, in the antibody incubation mix.

When these antibodies were used for immunofluorescence localization of AE2 (Fig. 3, *upper panels*), they each detected basolateral staining of the choroid plexus epithelial cells. Immu-

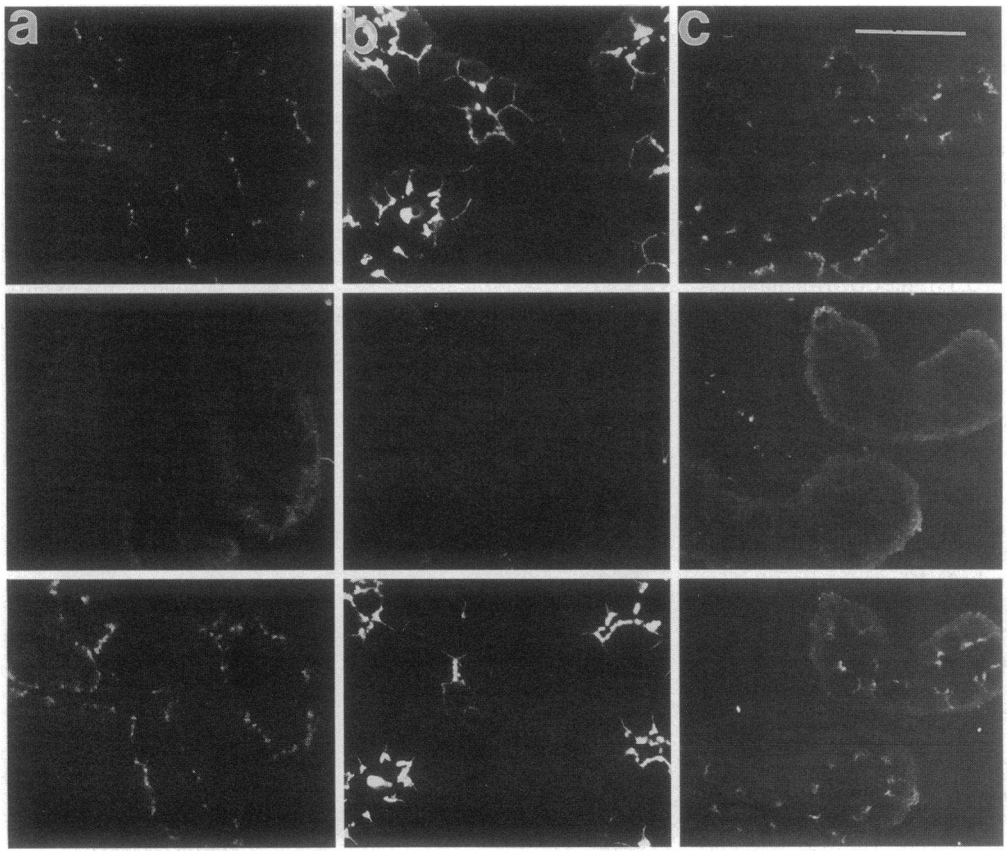

Figure 3. Specificity of immunocytochemical detection of AE2 in rat choroid plexus by three anti-AE2 peptide antibodies. Each column represents sequential semithin sections immunostained with antibody alone (*top row*) or in the presence of peptide antigen (*middle row*) or nonspecific peptide (*bottom row*). *a*, anti-424–440; *b*, anti-1224–1237; *c*, anti-961–974. Antibodies to all epitopes detected basolateral membrane staining. Bar = 30 μm.

nostaining was abolished by inclusion of peptide antigen (*middle panels*) but not nonspecific peptide (*lower panels*) in the antibody incubation. Sequential sections incubated individually with the three antibodies immunostained the basolateral membranes of identical cells (not shown). Furthermore, antipeptide antibodies raised against two additional peptide epitopes of AE2 produced similar staining patterns (not shown). In contrast, immunostaining of choroid plexus with isoform-specific antibody to AE1 and to AE3 was negative (not shown). This colocalization of antigen-specific immunostaining by distinct antibodies against multiple peptide epitopes of AE2 confirmed the identity of the band 3–related protein of choroid plexus as AE2.

The anti-1224–1237 antibody (Fig. 3, column *b*) consistently produced the strongest immunocytochemical signal among the antibodies tested, whether used to detect AE2 in Epon sections of choroid plexus or gastric mucosa (28) or in transfected COS cells fixed on coverslips. Basal membrane staining was generally heavier than was lateral staining with all the antibodies. In the lateral membranes of choroid plexus epithelial cells, immunostaining was denser at the basal than at the apical poles.

Fig. 4 documents that all three antipeptide antibodies also detected mouse choroid plexus AE2 in an antigen-specific fashion (lanes *1–6*). In addition, all three antibodies cross-reacted with AE2 in choroid plexus from pig (lanes *7–9*). Only two of the antibodies recognized AE2 in choroid plexus from chick embryo (lanes *10–12*). Though AE2 cDNA sequences from pig and chick have not been reported, this immuno–cross-reactivity suggests considerable sequence conservation among mammals and birds in the recognized epitopes. The cross-reactivity is lowest for anti-961–974, which recognizes the poorly conserved large ectoplasmic loop between putative membrane spans 5 and 6 of AE2. In this region of AE2, sequence diver-

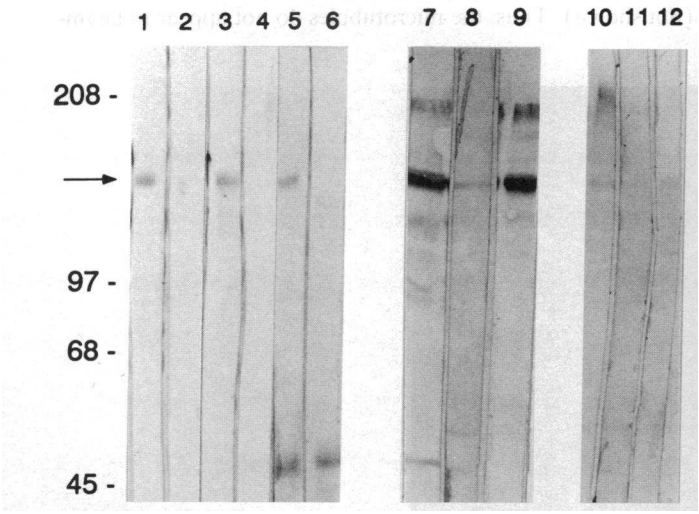

Figure 4. Immunoblot reactivity across species by anti-AE2 peptide antibodies (*arrow*). Mouse choroid plexus AE2 detected by anti-1224–1237 (lane *1*), anti-961–974 (lane *3*), and anti-424–440 (lane *5*), or in the presence of the respective peptide antigens (lanes *2, 4,* and *6*); Pig choroid plexus AE2 detected by anti-1224–1237 (lane *7*), anti-961–974 (lane *8*), and anti-424–440 (lane *9*); Chick choroid plexus AE2 detected by anti-1224–1237 (lane *10*) and anti-424–440 (lane *12*). Anti-961–974 (lane *11*) failed to cross-react with chick AE2. M_r of prestained size standards indicated at left.

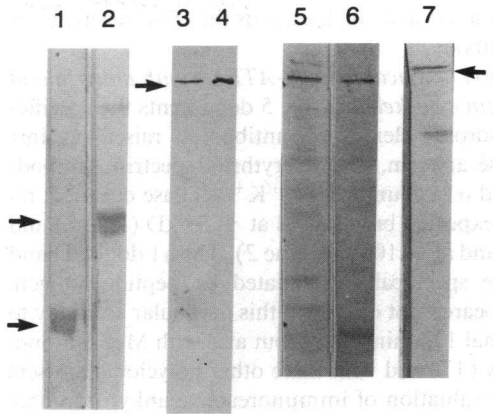

Figure 5. Immunoblot characterization of choroid plexus Na^+K^+-ATPase $\beta 1$ (lane *1*) and $\alpha 1$ subunits (lane *2*); ankyrin from choroid plexus (lane *3*) and from whole blood (lane *4*); ankyrin from unperfused choroid plexus (lane *5*) and perfused, blood cell–free choroid plexus (lane *6*); and choroid plexus fodrin (lane *7*).

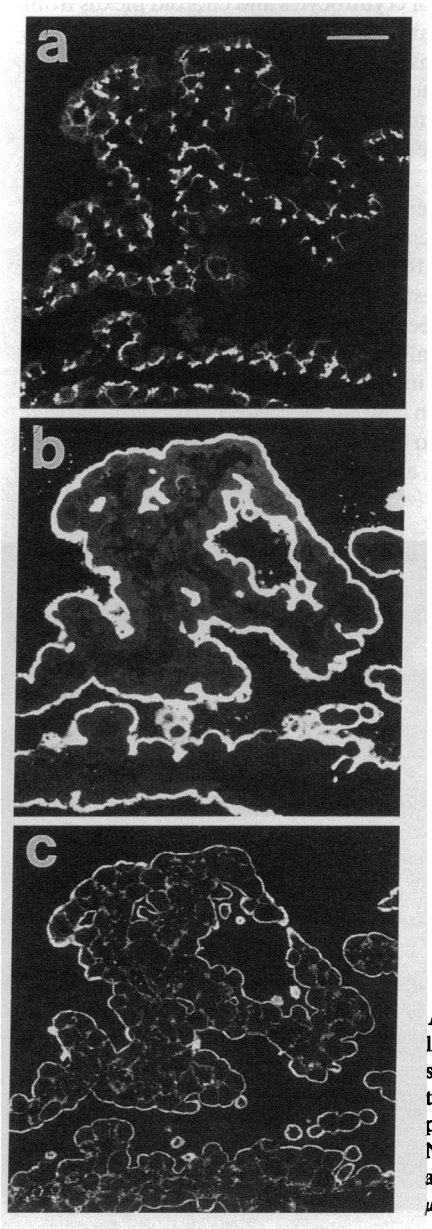

Figure 6. Immunolocalization in sequential semithin LX-112 sections of rat choroid plexus of AE2 (*a*), $\alpha 1$ Na^+K^+-ATPase (*b*), and fodrin (*c*). Bar = 30 μm.

gence between mouse and chick exceeds the cross-reactivity of anti-mouse antibody.

Colocalization of apical Na^+K^+-ATPase with ankyrin and the spectrin/actin cytoskeleton. Fig. 5 documents the specificity in rat choroid plexus of antibodies raised against Na^+K^+-ATPase, ankyrin, and nonerythroid spectrin. Antibodies to rat $\beta 1$ and $\alpha 1$ subunits of Na^+K^+-ATPase detected, respectively, the expected broad band at ~ 55 kD (lane *1*) and the narrower band at ~ 100 kD (lane *2*). The $\alpha 1$ doublet band intensities were specifically attenuated by peptide antigen. Both bands appeared not only with this particular antibody to the NH_2-terminal 12 amino acids, but also with McK1 monoclonal antibody (17) and with three other polyclonal antisera (not shown). Evaluation of immunoreactive ankyrin in choroid plexus was potentially problematic, since choroid plexus is a highly vascular tissue, and contaminating erythrocytes could provide a positive immunoblot signal. Ankyrin from erythrocytes (lane *3*) and from choroid plexus (lane *4*) comigrated at ~ 220 kD. Semithin sections of choroid plexus from the perfused rats were devoid of erythrocytes (not shown). Therefore, ankyrin immunoblots of erythrocytes and choroid plexus from paired, nonperfused (lane *5*), and perfused rats (lane *6*) were compared, and demonstrated that choroid plexus epithelial cells expressed endogenous ankyrin or an immunoreactive isoform. Antibody to sea urchin spectrin which has broad reactivity across phyla (30) detected a principal band of ~ 240–260 kD (lane *7*).

These antibodies were applied as immunocytochemical reagents to sequential semithin sections of rat choroid plexus (Figs. 6–8). Unlike the basolateral localization of AE2, Na^+K^+-ATPase was restricted to the apical cell surface. Immunoreactive forms of spectrin (fodrin), ankyrin, and actin were also concentrated apically and colocalized with Na^+K^+-ATPase (Figs. 7 and 8). As in other transporting epithelial cells, the distribution of spectrin and actin was not strictly polarized. Both proteins were also observed along the basolateral aspects of these epithelial cells, albeit with apparently lower concentrations than apically. However, unlike the situation in other simple transporting epithelial cells with a free, nonattached apical surface, Na^+K^+-ATPase was clearly restricted to the apical membrane and absent from the basolateral surface. Ankyrin immunostaining, always less prominent than spectrin staining, was minimal or absent at the basolateral membrane. Similar observations were obtained with sections of human choroid plexus (not shown).

This striking reversal of the distribution of the Na^+K^+-ATPase–ankyrin complex (and of the expression of other basolateral proteins, see Introduction) in the choroid plexus raised the question of whether microtubules might be causally involved in generation or maintenance of this phenomenon. Recent studies on microtubules in the intestinal epithelium and kidney tubules indicated a role of microtubules in the vectorial transport of membrane proteins to the apical membrane domain (26, 27, 40). Disruption of microtubules in these polarized epithelial cells caused redistribution of several apical membrane proteins to the basolateral cell surface or to intracellular vacuoles, whereas the basolateral localization of the Na^+K^+-ATPase remained unaffected in these cells (26 and Alper, S. L., unpublished observations). As shown in Fig. 9 *A*, microtubules were labeled throughout the plexus epithelium, with a slight apical orientation. 6 h after intraperitoneal administration of colchicine (Fig. 9 *B*), most of the intracellular immunostaining of tubulin had disappeared, and only the apical staining remained at reduced levels. This indicates that the organizational center for microtubules in choroid plexus is located beneath the apical cell surface, as is the case in the intestinal epithelium (26) and in MDCK cells (41). In contrast to apical membrane proteins in intestinal and kidney epithelia, the distribution of Na^+K^+-ATPase within the apical membrane of the choroid plexus was not affected by colchicine treatment. Also unaffected were the subcellular distributions of spectrin (fodrin), actin, and ankyrin. Basolateral AE2 of the choroid plexus was similarly unperturbed in its distribution (not shown). Thus, the microtubules do not appear to be im-

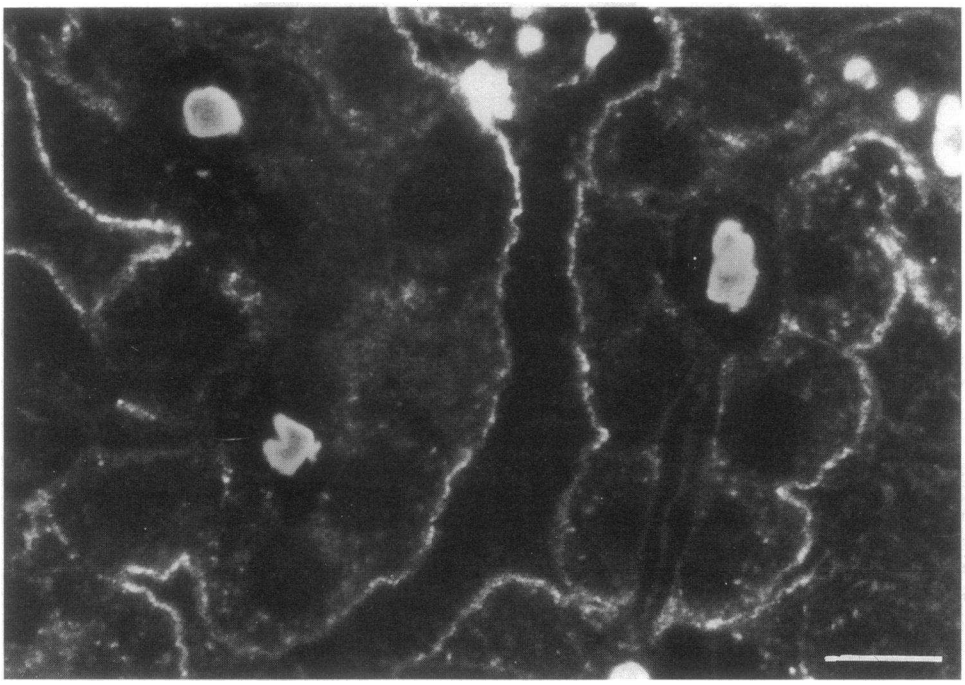

Figure 7. Immunocytochemical localization of ankyrin in rat choroid plexus by antibody raised against human erythroid ankyrin. The 6-μm frozen section shows ankyrin immunoreactivity not only at the apical epithelial cell membrane but also in membranes of erythrocytes of the plexus capillaries. Bar = 10 μm.

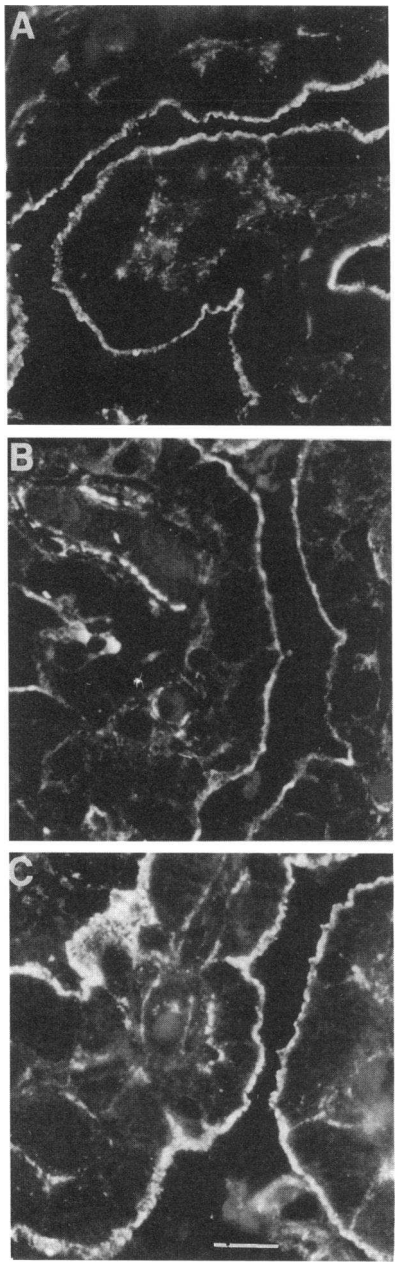

Figure 8. Nonsequential 6-μm frozen sections of rat choroid plexus immunostained with antibodies to actin (*A*), myosin (*B*), and α-actinin (*C*). All panels show predominantly apical staining. Bar = 20 μm.

portant for generation and/or maintenance of the polarized distribution of Na^+K^+-ATPase and of AE2 in the choroid plexus.

Another structure that has been suggested to be involved in the control of the exocytotic incorporation of membrane proteins into the apical plasma membrane is the apical terminal web (26, 42). The major constituents of this web are the actin cross-linking proteins spectrin (fodrin), α-actinin, and myosin (43). As shown in Figs. 6–8, all four cytoskeletal proteins are enriched underneath the apical plasma membrane. This indicates that the choroid plexus contains a well-developed apical terminal web which obviously does not constitute an effective barrier to the access of Na^+K^+-ATPase–containing exocytotic vesicles to the apical plasma membrane.

We examined the fate of apical and basolateral intrinsic membrane transport proteins in another setting in which polarity is frequently disrupted, namely malignancy. We took advantage of the propensity of mice transgenic for the SV40 large T antigen driven by its own promoter to develop choroid plexus papilloma between 3 and 5 mo of age (35). In an earlier study, the papilloma expressed substantial amounts of AE2 mRNA (22). However, when semithin sections were examined for AE2 immunostaining, AE2 was absent from most visual fields (Fig. 10, *b* and *c*), and when (rarely) present was expressed circumferentially in the morphologically depolarized tumor cells (Fig. 10 *a*). Cross-reactive AE1 in erythrocytes was brightly stained (Fig. 10, *a–c*). Na^+K^+-ATPase α1 subunit was similarly depolarized but in most visual fields was still well above the threshold for immunocytochemical detection (Fig. 10, *d–f*).

Discussion

The abundant expression of basolateral AE2 and apical Na^+K^+-ATPase in choroid plexus epithelium suggests that both play a prominent role in the secretion of CSF. The apically situated ATPase probably contributes to the decreased CSF $[K^+]$. Basolateral AE2 (or AE1) in other tissues (28, 36, 44) is generally associated with transepithelial transport of acid-base equivalents. Since the principal function of the choroid plexus is the transepithelial transport of NaCl, $NaHCO_3$, and water, along with nutrients and secreted proteins, it seems reasonable to hypothesize the presence in choroid plexus of basolateral Na/H exchange as well. Since our attempts to detect Na^+/H^+ exchanger 1 by immunoblot and immunocytochemistry were unsuccessful, we suggest the presence of an alternate Na^+/H^+ exchanger isoform, though other basolateral Na^+ entryways may also exist. Further studies on CSF elaboration would be facilitated by development of polarized choroid plexus cells in primary culture and eventually in permanent culture.

The localization of ankyrin to the apical membrane away from AE2 suggests that the ankyrin isoforms detectable with antibodies to erythroid ankyrin do not bind to AE2. The same conclusion issues from the localization of AE2 to the basolateral membrane (28) and immunoreactive ankyrin to the apicovesicular membrane (10) of the parietal cell. Attempts to localize the ankyrin-binding site on AE1 have concluded that the binding interface extends over > 100 amino acids and may also involve the far amino terminus (45). Thus, the several short regions of highly conserved sequence within the amino-terminal cytoplasmic domains of AE1, AE2, and AE3 (which do not include the very divergent far amino-terminal sequences) probably do not comprise a common binding site for a single ankyrin isoform. It remains possible that alternate ankyrin isoforms such as ANK2 or ANK3 (45) may interact with AE2, but such an ankyrin might not bind to the α1 subunit of the Na^+K^+-ATPase. Choroid plexus ankyrin cDNA sequences have not been reported.

The degree to which the choroid plexus is truly an epithelium of reversed polarity is unresolved. Clearly, some aspects of polarized secretion and membrane protein insertion are reversed compared with renal and intestinal epithelial cells. Though apical Na^+K^+-ATPase in one clone of MDCK cells (4, 8) is enzymatically inactive, this appears not to be true of the apical ATPase of the choroid plexus. Apical sphingolipids in MDCK cells have been postulated to inactivate that ATPase, but the apical lipid composition of choroid plexus has not been reported. The retinal pigment epithelial cell also colocalizes Na^+K^+-ATPase and ankyrin to its apical membrane, but that

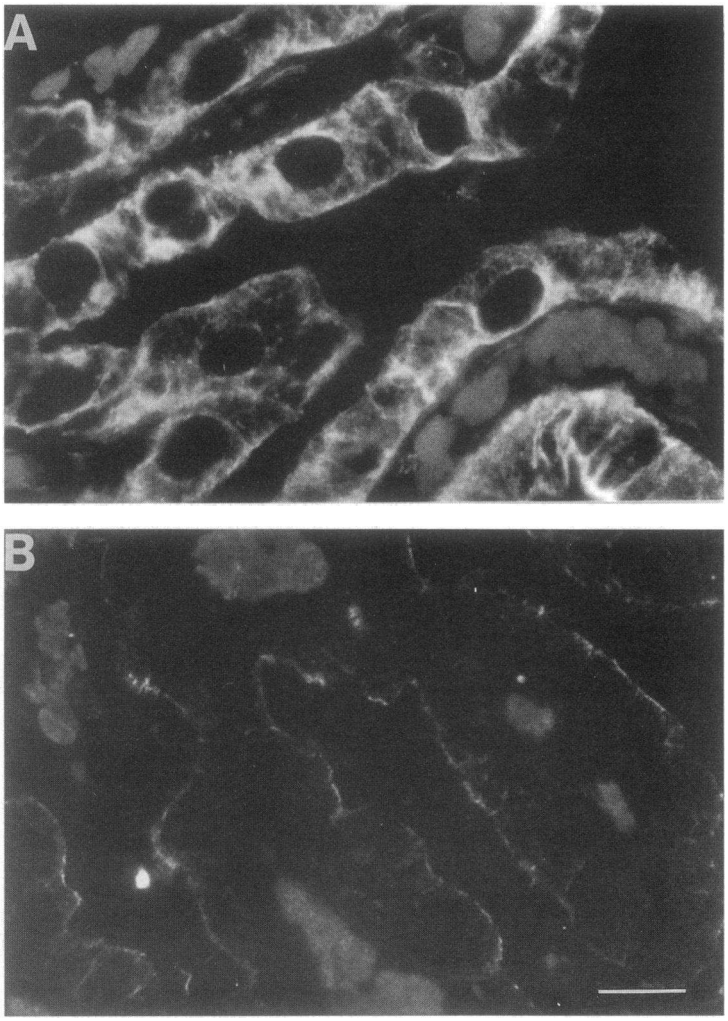

Figure 9. Rat choroid plexus immunostained with antitubulin antibody before (*A*) or 5 h after (*B*) administration of intraperitoneal colchicine as described (23). *B* shows retention in the epithelial cells only of some apical membrane–associated tubulin immunoreactivity. Bar = 20 μm.

steady state distribution is dependent on apical surface contact with the photoreceptor cells which overlie it in situ. Other indices of polarity, such as viral budding, have yet to be reported for choroid plexus.

One possible mechanism responsible for the partial reversal of polarity in choroid plexus could be a reversal of the orientation and polarity of the microtubule system. In enterocytes, proximal tubular epithelium of the kidney, and MDCK cells, microtubules have been shown to be important for vectorial delivery of membrane proteins to the apical cell surface (26, 27, 40, 41). The structural basis for vectorial transport from the Golgi apparatus to the apical plasma membrane is provided in part by the uniform orientation and alignment of microtubules. These have their organizing centers underneath the apical terminal web (subterminal space). As shown in this study, this general pattern of microtubule orientation appears also to be present in the choroid plexus epithelium. However, disruption of the microtubule system by colchicine did not have any detectable effect on the apical position of Na^+K^+-ATPase or on the basolateral localization of AE2. This indicates that microtubules are probably not involved in the partial reversal of polarity in these cells. In intestine and kidney, basolateral targeting of Na^+K^+-ATPase and of a 120-kD membrane protein was not perturbed by colchicine, whereas both apical and basolateral vacuolar H^+-ATPase of renal intercalated cells were perturbed (46). Thus, the disruptive effects of colchicine are not surface specific, but rather are protein specific. Another possible interpretation might be that any proteins, apical or basolateral, which bind via ankyrin to the membrane cytoskeleton are resistant to delocalization by colchicine.

A second mechanism for partial reversal of polarity in the choroid plexus might be the terminal web, which in exocrine glands plays a role in the control of apical exocytosis (3, 42). If the choroid plexus epithelium lacked an apical terminal web, one could speculate that the loss of this exocytotic barrier would facilitate apical incorporation of basolateral proteins. But since actin and the principal actin cross-linking proteins of the terminal web (α-actinin, myosin, and spectrin) are enriched underneath the apical cell surface of the plexus epithelium, the terminal web appears to be well developed in these cells. Therefore, an altered terminal web structure cannot serve to explain the partial reversal of polarity in the choroid plexus.

A third cytoskeletal mechanism that is believed to be involved in placement of an ever-growing number of integral membrane proteins (i.e., Na^+K^+-ATPase and voltage-gated Na^+ channel) to specialized sites of the cell surface is linkage of these proteins via ankyrin to the spectrin/actin-based membrane cytoskeleton (see Introduction). This mechanism may also operate in the choroid plexus epithelium, where Na^+K^+-ATPase and immunoreactive ankyrin are colocalized at the apical cell surface and are absent from the basolateral membrane domain. If Na^+K^+-ATPase is delivered in a nonpolarized fashion to both the apical and basolateral surfaces (4, 8, 39), only the apical fraction of Na^+K^+-ATPase will be stabi-

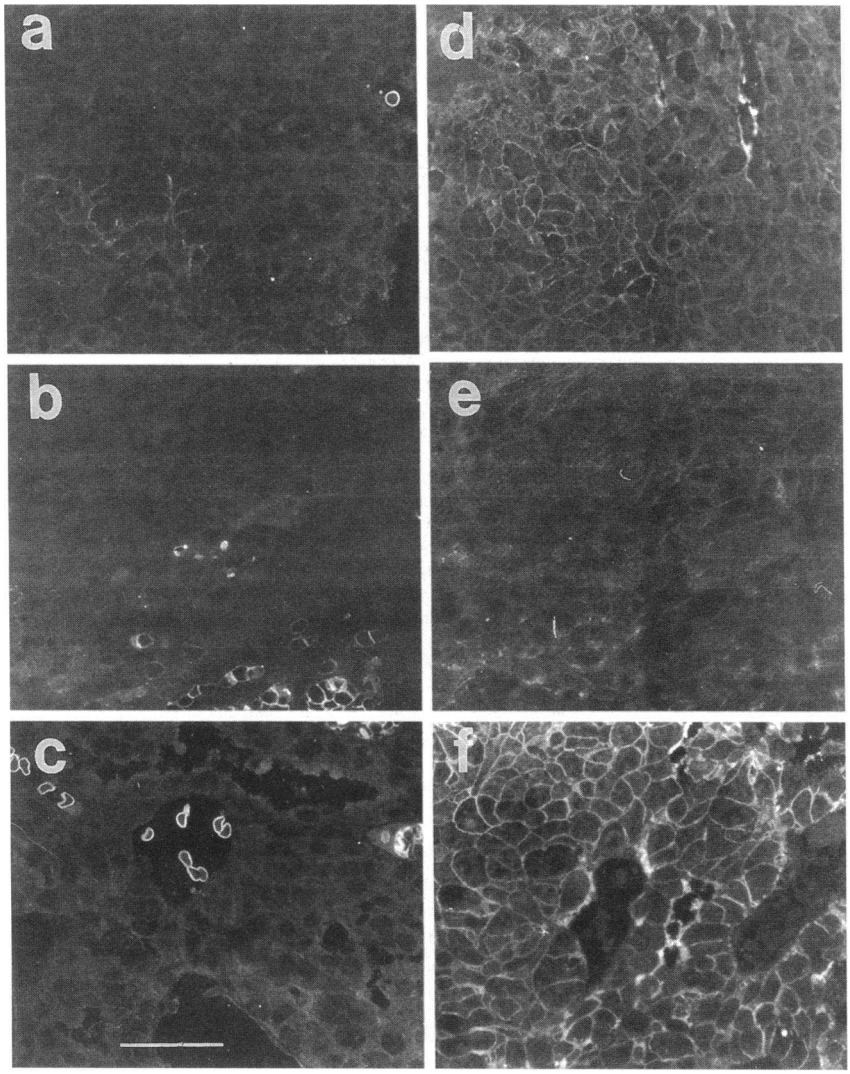

Figure 10. Selected fields of semithin sections from choroid plexus papilloma removed from an unperfused mouse transgenic for SV40 large T antigen driven by the SV40 early promoter-enhancer immunostained with antibodies to AE2 (*a–c*) and to Na^+K^+-ATPase α1 subunit (*d–f*). Bar = 10 μm.

lized via linkage to apical ankyrin to the underlying membrane cytoskeleton. In contrast, molecules inserted basolaterally may be rapidly endocytosed and either degraded or delivered to the apical cell surface by transcytosis. Such a transcytotic pathway has been well established for the poly-Ig receptor (47) and various F_c receptors (48) in polarized monolayers, as well as for hepatocyte proteins of the biliary canalicular membrane in vivo (49). Direct apical targeting is also possible.

However, none of these explanations for the apical localization of Na^+K^+-ATPase easily explains the apical enrichment of ankyrin in the choroid plexus. E-cadherin has been shown to be directly or indirectly associated with ankyrin and may play a role in stabilizing and assembling the membrane cytoskeleton and the Na^+K^+-ATPase at the basolateral cell surface of MDCK cells (50). Recent studies indicate that chick choroid plexus epithelial cells express B-cadherin (51) which, when transfected into fibroblasts, fails to condense endogenous fodrin and Na^+K^+-ATPase at the lateral membrane. This behavior contrasts with that of E-cadherin which in fibroblasts (52) and in retinal pigment epithelial cells (53) condenses fodrin and Na^+K^+-ATPase at the lateral membrane. Thus, the absence of cadherins capable of assembling ATPase–cytoskeleton complexes at the basolateral cell surface of the choroid plexus could contribute to accumulation of ankyrin at the apical surface. However, the possibility remains of apical membrane binding sites for choroid plexus ankyrin in addition to the Na^+K^+-ATPase itself. These could include one of the ankyrin-binding glycoproteins (54). To further test this hypothesis, as well as to assess protein-sorting mechanisms in the choroid plexus, we will need an in vitro system in which to study development of polarity under controlled conditions.

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