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Isoforms of the Na,K-ATPase are present in both axons and dendrites of hippocampal neurons in culture

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ABSTRACT **The distributions of isoforms of the Na,K-ATPase α subunit were determined in mature cultured hippocampal neurons and in a polarized epithelial cell line. We find that hippocampal neurons express the α1 and α3 isoforms in the membranes of both axons and dendrites. In contrast the α1 and α3 proteins are exclusively basolateral when expressed endogenously or by stable transfection in renal epithelial cells. These data suggest that epithelial cells and hippocampal neurons localize these proteins by different mechanisms. These observations contrast with those made for the vesicular stomatitis virus and the influenza glycoproteins, which are polarized in both epithelial and neuronal cells.**

Na,K-ATPase is an integral membrane protein complex responsible for establishing the electrochemical gradients of Na^+ and K^+ ions across the plasma membranes of mammalian cells. The enzyme is composed of two subunits, α and β, both of which exist in multiple isoforms (1). The α polypeptide is thought to be the catalytically active subunit, and the β polypeptide seems to be necessary for the assembly and transport of the sodium pump to the plasma membrane (2). Three α-subunit isoforms (α1, α2, and α3), encoded by three genes, have been cloned from the rat (3). The three α isoforms manifest ≈90% identity at the amino acid level and exhibit a tissue-specific and developmentally regulated pattern of expression. Polarized epithelial cells express the α1 isoform and, in most native and cultured epithelial cells, the enzyme is localized exclusively to the basolateral membrane domain (4, 5). In brain, α1 and α2 are expressed both in neuronal and in nonneuronal cells, whereas α3 is the neuron-specific isoform (6).

Although epithelial cells and neurons differ markedly in function, both cell types share the need to polarize their surface membranes. Recent studies that made use of enveloped RNA viruses to examine sorting phenomena in hippocampal neurons in culture (7) have suggested that epithelial cells and neurons may share common sorting mechanisms for membrane proteins. These studies revealed that hippocampal neurons infected with influenza virus target the hemagglutinin glycoprotein to the axons, whereas vesicular stomatitis virus (VSV)-infected neurons sort the VSV glycoprotein to their dendrites. Moreover, Thy-1, a glycosylphosphatidylinositol-linked protein endogenously expressed in hippocampal neurons, is exclusively localized on the axonal surface (8). It is known that glycosylphosphatidylinositol-linked proteins are sorted to the apical surface of polarized epithelial cells (9). These results suggest that the neuronal axon is the counterpart of the epithelial apical domain whereas the dendrite is the counterpart of the basolateral membrane.

In light of the basolateral localization of α1 in epithelia, we were interested to determine whether this protein is restricted in its distribution to the dendrites of polarized hippocampal neurons. Support for this hypothesis can be found in a recent immunocytochemical analysis of rat hippocampal pyramidal neurons *in situ* (10) that suggested a dendritic and axonal localization for α1 and α3, respectively. To test this possibility we assessed the distribution of α1 and α3 proteins by using isoform-specific monoclonal antibodies (mAbs) and polyclonal antibodies.

We find that neither the α1 nor the α3 Na,K-ATPase isoform manifests a qualitatively polarized distribution in mature and fully polarized hippocampal neurons in culture. Furthermore, we have transfected a polarized epithelial cell line (LLC-PK1) with a cDNA encoding the α3 isoform. Immunolocalization analysis of the transfected cell line reveals a basolateral distribution of the exogenous protein and of the endogenous α1 isoform. Thus, both isoforms, which are basolaterally polarized in epithelial cells, are distributed over the entire surface of hippocampal neurons in culture. Immunofluorescence performed on frozen semi-thin sections of rat hippocampus confirm the results obtained in cultured neurons. Both *in situ* and in culture, therefore, the neuronal mechanisms that mediate the subcellular distributions of the α1 and α3 Na,K-ATPase isoforms appear to differ from those that apply to the VSV glycoprotein, despite the fact that all three proteins share a basolateral localization when expressed in epithelial cells.

MATERIALS AND METHODS

Antibodies. Two mouse mAbs raised against α1 Na,K-ATPase were used in this study: mAb 6H and mAb C62.4. The production and characterization of mAb C62.4 (kindly provided by D. Biemesderfer and M. J. Kashgarian, Yale University) have been described (11). mAb 6H was prepared according to a similar protocol.

To localize α3 and α2, we used two rabbit polyclonal sera produced against synthetic peptides. Synthetic peptides comprising amino acids 4–17 and 2–14 of the N termini of the rat α2 and α3, respectively, were the kind gift of R. W. Mercer (Washington University). Cysteines were added at their C termini to facilitate coupling to keyhole limpet hemocyanin. Haptenization to keyhole limpet hemocyanin and injection were as described (12). The resultant serum was affinity-purified on columns of peptide immobilized on thiopropyl-Sepharose 6B (Pharmacia). The serum was affinity-purified as described (13) but the specific antibody was eluted with 0.1 M triethylamine (pH 11.5).

The polyclonal anti-microtubule-associated protein 2 (MAP2) antibody (14) was a kind gift of R. B. Vallee

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Abbreviations: VSV, vesicular stomatitis virus; mAb, monoclonal antibody; MAP2, microtubule-associated protein 2; GAP-43, 43-kDa growth-associated protein.

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(Worcester Foundation, Shrewsbury, MA). mAb MAP2, GAP-43, and all secondary antibodies were purchased from Boehringer Mannheim.

Cell Culture and DNA Transfection. Mammalian expression vector pMT21 (Genetics Institute, Cambridge, MA) containing α1, α2, or α3 under control of the simian virus 40 promoter (kind gift of E. J. Benz, Jr., Yale University) were used to transiently transfect COS-1. PMT21-α3 was used to stably transfect LLC-PK1 cells.

COS-1 cells were maintained in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal calf serum. Transient DNA transfection was carried out by the DEAE-dextran/chloroquine shock method (15, 16). After 48 hr, the cells were fixed for 10 min with cold methanol (−20°C) at room temperature and stored at 4°C in phosphate-buffered saline (PBS) containing 0.02% sodium azide.

LLC-PK1 cells (kind gift of K. Amsler, University of Medicine and Dentistry of New Jersey, Piscataway) cells were cultured in minimal essential medium (α modification) containing 10% fetal calf serum. Cotransfection of 36 mg of pMT21-α3 and 6 mg of pCB6 vector (kindly provided by M. Roth, D. Russell, and C. Brewer, Southwestern Medical Center, Dallas) (17), which carries resistance to the antibiotic G418, was performed by coprecipitation with calcium phosphate as described (19). Colonies of cells resistant to G418 were isolated and screened for expression of α3 sodium pump by immunoblot analysis and immunofluorescence. For immunofluorescence, the cells were plated on filters (3095 Falcon, Becton Dickinson). The confluent monolayers were fixed in cold methanol.

Hippocampal neurons were cultured as described by Goslin and Banker (20). For immunofluorescence, the cells were washed with two quick changes of warm PBS and fixed with cold methanol. The cells were then permeabilized (0.3% Triton X-100 in PBS) for 30 min and stored at 4°C in PBS containing 0.02% sodium azide.

Semi-Thin Frozen Sections. Male rats were anesthetized and perfused by cardiac puncture with Ringer's solution followed by 4% (vol/vol) formaldehyde in 0.1 M sodium phosphate (pH 7.4). The whole brain was removed and the hippocampus, coronally bisected, was infiltrated with increasing concentrations of sucrose (12%, 15%, and 18%) in 120 mM sodium phosphate buffer for several hours. Polyvinylpyrrolidone was added as a cryoprotectant (21) and the samples were frozen and stored in liquid nitrogen. Semi-thin cryosections (0.5 μm) were cut (22) using a Reichert Ultracut E ultramicrotome equipped with an FC-4E cryoattachment and mounted on gelatin-coated slides.

Immunofluorescence. All the sections and cells were processed for double immunofluorescence as described (18). With the exception of Fig. 1, all immunolocalizations were carried out with the biotin–avidin amplification system (Vector Laboratories) and an avidin-Texas red-conjugated secondary antibody (Molecular Probes). The samples were mounted in 75% (vol/vol) glycerol in PBS containing 0.1% *p*-phenylenediamine to retard fading.

RESULTS

Antibody Characterization. To examine the subcellular distributions of the α1 and α3 Na,K-ATPase isoforms in rat hippocampal neurons in culture, we have made use of isoform-specific mAbs and polyclonal antibodies. Our studies employed two mAb probes specific for α1 and affinity-purified polyclonal antibodies directed against either α2 or α3. Due to the high degree of homology (≈90%) shared by all three isoforms, we have tested for possible cross-reactivity of these antibodies by Western blot analysis of brain membranes (data not shown) and by immunocytochemistry in COS cells individually transfected with cDNAs encoding each of the three isoforms (Fig. 1).

COS-1 cells transiently transfected with the cDNA clones encoding the α1, α2, and α3 Na,K-ATPase isoforms exhibit high levels of expression for all three proteins. In each case, staining with the relevant isoform-specific antibody reveals a pattern characteristic of the endoplasmic reticulum. Retention of the α isoforms in the endoplasmic reticulum is probably due to the comparatively limited expression of the Na,K-ATPase β subunit in COS-1 cells. This phenomenon has been described in other systems (23).

No cross-reactivity of the α3 antibody was detected with COS-1 cells transfected with α1 (Fig. 1, compare *b* with *a*) and with α2 (data not shown). The same lack of cross-reactivity was detected with α1 antibody in cells transfected with either α2 or α3 (Fig. 1, compare *e* with *d* and *h* with *g*, respectively). The specificity of the other α1 mAb (C62.4) used in this study was also confirmed with this method (data not shown). A low level of endogenous α1 expression is detected by the α1 antibodies but is too faint to be discerned in Fig. 1.

Localization of the α1 and α3 Na,K-ATPase Subunits in Hippocampal Neurons in Culture. To determine the distribution of the three isoforms of the Na,K-ATPase, we double-labeled mature hippocampal neurons with specific dendritic and axonal markers. mAbs and polyclonal antibodies directed against MAP2, a cytoskeletal protein restricted to the somata and dendrites of hippocampal neurons (24), and a mAb directed against the 43-kDa growth-associated protein (GAP-43), which is preferentially distributed to the axonal domain (25), were used as dendritic and axonal markers, respectively. Staining was observed with α1- and α3-specific antibodies in mature hippocampal neurons cultured for 7, 14, and 18 days (Fig. 2), but little or no α2 immunoreactivity was detected (data not shown). Low expression of α2 was expected based on a previous *in situ* hybridization analysis of Na,K-ATPase expression in hippocampal pyramidal cells

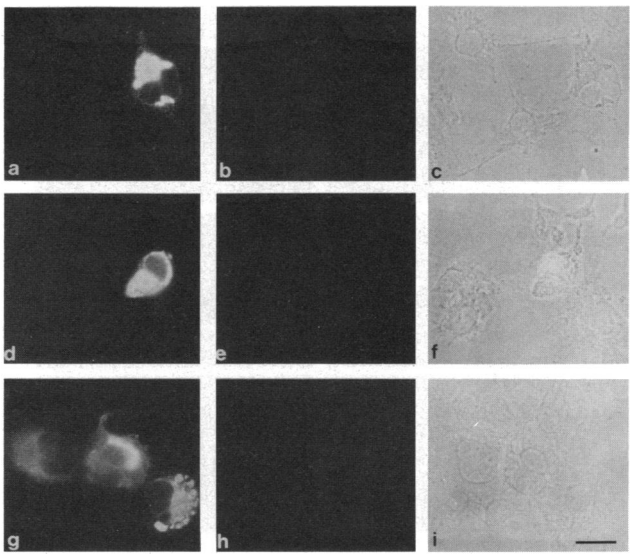

FIG. 1. Characterization of antibodies by immunocytochemistry on COS-1 cells individually transfected with cDNAs encoding the three α Na,K-ATPase isoforms. Double-label immunofluorescence analysis was performed on cells transfected with cDNA encoding α1 (*a–c*), α2 (*d–f*), or α3 (*g–i*). After methanol fixation, cells were stained with anti-α1 (*a*, *e*, and *h*), -α2 (*d*), or -α3 (*b* and *g*) antibodies, followed by fluorescently labeled secondary antibodies (rhodamine-conjugated goat anti-rabbit and fluorescein-labeled anti-mouse) against the respective species isotypes. (*c*, *f*, and *i*) Corresponding phase-contrast micrographs. No interisoform cross-reactivity was detected among these antibodies. (Bar = 18 μm.)

(26). The distribution of α1 subunit in hippocampal neurons, as revealed by staining with mAb 6H, is illustrated in Fig. 2 *a–d*. Virtually every neuronal process visible by phase-contrast microscopy was stained. In double-label experiments, α1 immunostaining is associated with both MAP2-positive and MAP2-negative processes. The labeling of MAP2-negative processes clearly demonstrates the presence of α1 immunoreactivity in axons. Some of the α1 staining associated with dendrites in older cultures undoubtedly can also be attributed to axons, which run along the dendritic surface. In younger cultures, whose dendrites are free of axonal interactions, unequivocal examples of dendritic staining were readily detectable (Fig. 2 *a* and *b*). Although dendrites are stained with both anti-MAP2 and anti-α1 antibodies, the pattern of staining with the two antibodies is quite distinct. Whereas MAP2 immunostaining appears to fill the cytoplasm, as expected for a cytoskeletal antigen, α1 staining is concentrated at the edges of the dendrites, consistent with a surface localization. We did not observe changes in the distribution of the α1 isoform at different stages of development, but its level of expression changed significantly. The comparatively weak staining observed at 7 and 14 days increased markedly during the third week in culture and remained approximately constant between 18 and 28 days (data not shown). Identical results have been obtained using a second α1-specific mAb, C62.4 (data not shown).

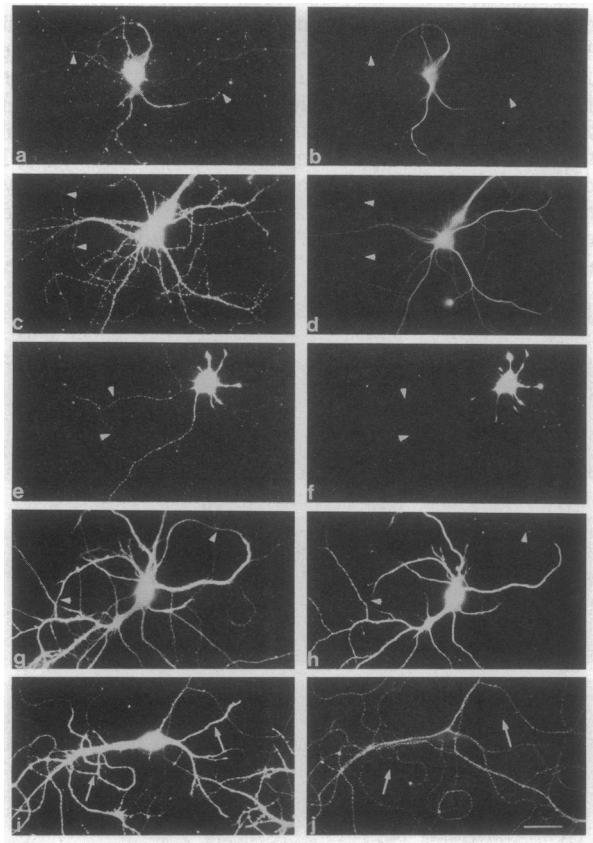

FIG. 2. Immunolocalization of α1 and α3 Na,K-ATPase isoforms in hippocampal neurons in culture. Hippocampal neurons cultured for 7 (*a*, *b*, *e*, and *f*), 14 (*g–j*), and 18 (*c* and *d*) days were double-labeled with the α1 (*a* and *c*) or α3 (*e*, *g*, and *i*) specific antibodies and with anti-MAP2 polyclonal antibodies (*b* and *d*) or mAbs (*f* and *h*). In *j* the cultured neurons were double-stained with anti-GAP-43. Arrowheads indicate MAP2-negative processes (axons) that are positively stained with α1- and α3-specific antibodies. Arrows show processes negative for GAP-43 (dendrites) but positively stained for α3. The staining observed with both α1 and α3 indicates that both isoforms are present in dendrites and in axons. (Bar = 16 μm.)

Immunostaining with a polyclonal antibody selective for the α3 isoform demonstrates that it also has an unrestricted distribution in hippocampal neurons. α3 immunoreactivity was detected in both MAP2-positive and MAP2-negative processes, as shown in Fig. 2 *e–h*. The latter result demonstrates that the α3 isoform is present in axons. To rule out the possibility that this isoform is exclusively associated with axons, 14-day-old cultures were double-stained with antibodies against α3 and GAP-43 (Fig. 2 *i* and *j*, respectively). Although at 14 days in culture the density of the axonal network is such that axons frequently run along dendrites, there are always processes recognizable as dendrites by their GAP-43 negativity (arrows) and their distinctive morphology. These results clearly demonstrate that the α3 isoform, like α1, is present in both dendrites and axons. Double-labeling experiments with α1- and α3-specific antibodies showed precise colocalization of the two isoforms (data not shown). The time course of expression of the α3 polypeptide differed somewhat from that of α1. α3 was expressed at high levels at 14 days and increased little thereafter.

The pattern of staining with the two antibodies was different in the occasional glial cells present in these cultures (data not shown). Glia were stained with α1- but not with α3-specific antibody, as expected from previous results (10).

Localization of α3 in a Transfected Epithelial Cell Line. The observations presented above demonstrate that neurons express α3 Na,K-ATPase in dendrites and axons. The sorting behavior of this isoform in polarized epithelia, however, has not previously been examined. It was necessary to determine, therefore, whether the unpolarized expression of α3 reflects its behavior in epithelia. Toward this end, we have stably transfected LLC-PK1 cells with the cDNA encoding α3. LLC-PK1 is a cell line derived from pig kidney proximal tubule that, like the MDCK line, retains the morphological and biochemical characteristics of polarized epithelia (5). Immunofluorescence analysis of two transfected lines reveal a basolateral staining pattern for α3. The exclusively basolateral localization has been confirmed by confocal imaging of cells transfected with α3 and double-labeled with α1 antibody (Fig. 3). Endogenous α1 and exogenous α3 colocalize on the basolateral membrane of transfected LLC-PK1 cells.

Localization of α1 and α3 Isoforms in Sections of Hippocampus. To determine whether our results with cultured neurons apply as well to hippocampal pyramidal cells *in situ*, we immunostained frozen semi-thin sections (0.5 μm) of adult rat hippocampus. Double-labeling experiments with the α1 mAbs (C62.4) and the α3 polyclonal antibodies reveal different levels of expression of the two proteins in different cell types (Fig. 4).

The α3 isoform is expressed at high levels in pyramidal cells. In CA3 and CA4, there was bright staining in stratum radiatum and stratum oriens, where the apical and basilar dendrites of pyramidal cells ramify, and much weaker staining in stratum lucidum, which contains mossy fiber axons (that arise from granule cells of the dentate gyrus) (Fig. 4*a*). Double-label immunofluorescence with anti-MAP2 shows "lines" of α3 staining along the edges of MAP2-labeled dendrites, just as in cultured neurons (Fig. 4 *c* and *e*). Since α3 is not expressed in glial cells (10), we feel confident in assigning this staining to the surface membranes of the pyramidal cell dendrites. In addition there was intense staining in the alveus and fimbria (data not shown), where the axons of pyramidal cells accumulate as they leave the hippocampus. Thus the pattern of staining observed with α3-specific antibody is just that expected for a protein that is expressed in both the axons and the dendrites of pyramidal cells.

In contrast to α3, the pattern of α1 immunoreactivity is dominated by intense staining within the stratum lucidum. This staining is almost certainly associated with mossy fiber axons, since it does not extend into the CA1 region and since equally bright staining is also present in the hilus of the

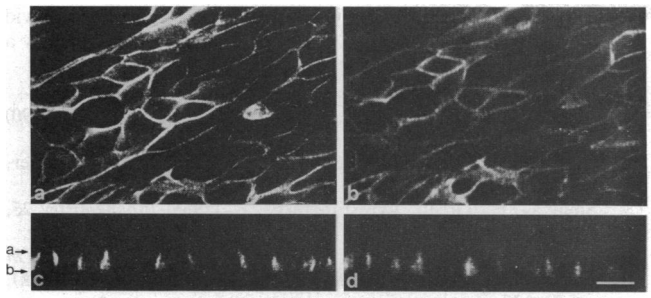

FIG. 3. Basolateral localization of $\alpha 3$ in transfected LLC-PK1 cells. Immunofluorescence performed on a confluent monolayer of transfected LLC-PK1 cells double-stained with the anti-$\alpha 1$ (*b* and *d*) and anti-$\alpha 3$ (*a* and *c*) antibodies. En-face micrographs (*a* and *b*). Confocal microscopy was used to generate the *x*–*z* cross-sections in *c* and *d*. Arrow a indicates the apical surface; arrow b indicates the basolateral surface. (Bar = 15 μm.)

dendate gyrus, where mossy fibers emerge from granule cells. The intense fluorescence of mossy fibers masks any staining that might be present in the proximal dendrites of pyramidal cells. This, together with the low level of expression in pyramidal cells, precludes any conclusions concerning the distribution of the $\alpha 1$ isoforms in this cell type. Interestingly, staining of both the dendrites and the axons of the granule cells of the dentate gyrus was observed with the $\alpha 1$-specific antibody (data not shown). This result suggests that the distribution of the $\alpha 1$ Na,K-ATPase isoform is not restricted to the dendrites of the granule cells of the dentate gyrus. This finding is entirely consistent with our observations derived from fully polarized hippocampal neurons in culture. It should be noted that the isolation conditions employed result in a culture that is composed predominantly of pyramidal cells and that contains few, if any, neurons derived from the granule cell layer (27, 28).

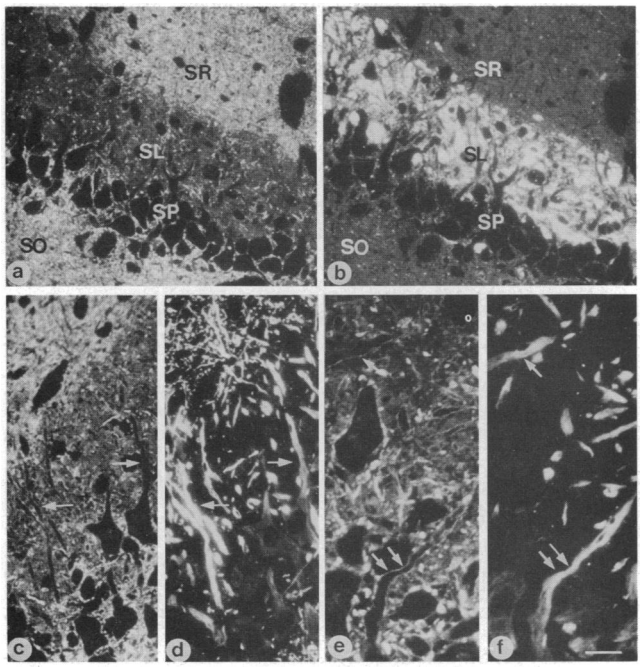

FIG. 4. Localization of $\alpha 1$ and $\alpha 3$ Na,K-ATPase isoforms in frozen semi-thin sections of hippocampus. Semi-thin cryosections ($\approx$0.5 μm thick) were double-labeled with the anti-$\alpha 3$ (*a*) and anti-$\alpha 1$ (*b*) antibodies or with the anti-$\alpha 3$ (*c* and *e*) and anti-MAP2 (*d* and *f*) antibodies. SO, stratum oriens; SP, stratum pyramidum; SL, stratum lucidum; SR, stratum radiatum. Arrows indicate MAP2-positive processes (dendrites) in *d* and *f* and the dendritic surfaces stained with anti-$\alpha 3$ antibodies in *c* and *e*. (Bars: *a*–*d*, 20 μm; *e* and *f*, 9.2 μm).

DISCUSSION

Distribution of Na,K-ATPase Isoforms in Hippocampal Neurons in Culture. The results presented in this paper demonstrate that the $\alpha 1$ and $\alpha 3$ Na,K-ATPase isoforms are present in both axons and dendrites of cultured hippocampal neurons during all of the stages examined. Further, within the limits of resolution of the immunofluorescence technique employed, the two sodium pump isoforms appear to colocalize precisely. LLC-PK1 cells transfected with the cDNA encoding $\alpha 3$ localized both this exogenous protein and the endogenous $\alpha 1$ polypeptide exclusively to their basolateral surfaces. We conclude, therefore, that these two transmembrane proteins, which are restricted to the basolateral surfaces of polarized epithelial cells, do not exhibit a qualitatively polarized distribution in hippocampal neurons in culture.

In addition to examining cultured neurons, we have used our antibodies to localize sodium pump isoforms in semi-thin sections prepared from rat hippocampus. While $\alpha 1$ expression in pyramidal cells is too low to permit definitive localization, the staining revealed by the $\alpha 3$ antibody suggests a localization of the protein in both the axons and dendrites of pyramidal neurons. A previous examination of the sodium pump isoform distribution in the hippocampus suggested a concentration of the $\alpha 1$ protein in the stratum radiatum, the region rich in pyramidal cell dendrites (10). Our studies suggest that this staining is in fact contributed by the axons of the mossy fibers and resides in the stratum lucidum, which immediately underlies the stratum radiatum in CA3 and CA4. Thus, our observations on the distribution of the sodium pump isoforms *in situ* are consistent with our findings in the culture model. Neither isoform appears to be exclusively concentrated in one or the other plasmalemmal domain.

It is interesting to consider how hippocampal neurons may be served by the concomitant expression of $\alpha 1$ and $\alpha 3$. This issue is all the more difficult to resolve because the reason for the existence of multiple isoforms of the Na,K-ATPase α subunit is essentially unknown. Differences in substrate affinities among sodium pump isoforms have been demonstrated (29). The $\alpha 3$ isoform displays 2–3 times lower affinity for sodium than the other two isoforms, suggesting that the activity of the $\alpha 3$ isoform can be modulated by relatively high levels of intracellular sodium, such as those that might follow repeated action potentials. This different threshold for sodium could explain the necessity for the expression of two isoforms in neuronal cells and for their localization in all of the electrically active neuronal processes.

Implications for Neuronal and Epithelial Sorting Mechanisms. The fact that the sodium pump does not appear to comply as well as the VSV glycoprotein with the equation between basolateral membrane in epithelial cells and dendrites in hippocampal neurons suggests that there are important differences in the mechanisms employed by neurons and epithelia to localize this protein. Several hypotheses can be generated to explain this interesting phenomenon. It could be argued, for example, that the sorting information embodied in the neuronal sodium pump differs in some characteristic from that manifest by its epithelial counterpart. In seeking the possible source of such heterogeneity, we can eliminate the primary structures of the $\alpha 1$ and $\alpha 3$ subunits themselves. Genetic analysis reveals that only three genes encode the three sodium pump α isoforms (3), and there is no evidence for heterogenous splicing. Similarly, the possibility that differences in posttranslational modifications of α subunits in these two systems contributes to sorting behavior also appears unlikely, since no biochemical studies of the α subunits have provided consistent evidence for the existence of classical posttranslational modifications. Finally, it could be suggested that variations in β-subunit expression account for the observed distributions. Molecular cloning studies provide

evidence for at least three β isoforms that manifest cell-type-specific distributions (30–32). Epithelial cells appear to express exclusively the β1 isoform (33). In contrast, glia express β2 in association with α2 (31). It is conceivable that neurons express multiple β isoforms and that sorting information contained in the β isoforms may contribute to the α-subunit localizations observed. We feel this is unlikely for two reasons. (*i*) Although we lack probes for the β2 and β3 isoforms, we find that the endogenously expressed β1 shares the axonal and dendritic localization characteristic of α1 and α3 (data not shown). Furthermore, data from our laboratory demonstrate that the sodium pump β subunit in epithelial cells does not appear to carry information important for sorting (C. Gottardi and M.J.C., unpublished data).

If there are no distinctions to be found in the sorting information associated with neuronal and epithelial sodium pumps, then explanations for their different sorting behaviors must be sought in the cellular machinery that mediates the targeting and retention of domain-specific proteins. The possibility that the Na,K-ATPase might not be actively sorted but instead pursues a default pathway has received support from studies in polarized epithelial cells. Hammerton *et al.* (34) found that newly synthesized Na,K-ATPase is delivered in roughly equal proportions to the apical and basolateral surfaces of MDCK cells. They suggest that a difference in the turnover rates at the two surfaces produces the observed steady-state basolaterally polarized distribution of Na,K-ATPase. They postulate that the greatly enhanced stability of the basolateral sodium pump is due to its well-established interaction with the cytoskeletal meshwork of ankyrin and fodrin that underlies the basolateral cell surface (35, 36).

In view of these results, the contrasting distribution of the Na,K-ATPase in neurons and epithelia might reflect differences in the distribution or composition of the membrane-associated cytoskeleton rather than in the sorting of the sodium pump itself. Indeed, several isoforms of ankyrin and fodrin have been described in various neurons (37–39). Evidence gathered for ankyrin suggests that distinct isoforms can occupy unique subcellular localizations (37, 38). The distribution of ankyrin and fodrin in cultured hippocampal neurons is consistent with an active role for cytoskeletal proteins in the localization of the sodium pump isoforms. A polyclonal antibody directed against erythrocyte ankyrin (kindly provided by K. Angelides, Baylor College of Medicine, Houston), which does not distinguish among the different ankyrin isoforms, labels both axons and dendrites of hippocampal neurons in culture (data not shown). Fodrin has a similar distribution in these cells (P. De Camilli, personal communication). It may also be worth noting that in choroid plexus and retinal pigment epithelium, which are derivatives of the neuroepithelium, the Na,K-ATPase and the ankyrin–fodrin cytoskeleton are restricted to the apical, rather than the basolateral, surface (40). Thus, although neurons and epithelial cells may share common mechanisms for the sorting of integral membrane proteins, these similarities may not extend to proteins whose distribution is influenced by their association with the membrane cytoskeleton.

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