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Immunocytochemical Demonstration of Na^+,K^+-ATPase in Internodal Axolemma of Myelinated Fibers of Rat Sciatic and Optic Nerves

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Abstract: We used postembedding electron microscopic immunocytochemistry with colloidal gold to determine the ultrastructural distribution of Na^+,K^+-ATPase in the sciatic and optic nerves of the rat. Using a polyclonal antiserum raised against the denatured catalytic subunit of brain Na^+,K^+-ATPase, we found immunoreactivity along the internodal axolemma of myelinated fibers in both nerves. This antiserum did not produce labeling of nodal axolemma. These results suggest that an important site of energy-dependent sodium–potassium exchange is along the internodal axolemma of myelinated fibers in the mammalian CNS and PNS and that there may be differences between the internodal and nodal forms of the enzyme. **Key Words:** Immunocytochemistry—Na^+,K^+-ATPase—Rat—Sciatic nerve—Optic nerve—Internodal axolemma—Myelinated fibers. **Mata M. et al.** Immunocytochemical demonstration of Na^+,K^+-ATPase in internodal axolemma of myelinated fibers of rat sciatic and optic nerves. *J. Neurochem.* **57**, 184–192 (1991).

Na^+,K^+-ATPase is the integral membrane enzyme responsible for active transport of Na^+ and K^+ in eukaryotic cells (Cantley, 1981; Skou, 1988). The activity of Na^+,K^+-ATPase in maintaining Na^+ and K^+ concentration gradients in the nervous system is critical to neuronal electrical function (Albers et al., 1989) and to the transport of other solutes such as Ca^{2+}, H^+, amino acids, and glucose (Albers et al., 1989). Defining the subcellular as well as cellular distribution of the enzyme is important in understanding the integrated functioning of the tissue (Siegel et al., 1984).

The active enzyme contains two polypeptide subunits: the α, or catalytic subunit (apparent M_r of ~100,000), and the β, or glycoprotein subunit (M_r of ~40,000) (Cantley, 1981). The active enzyme complex may exist as an $\alpha\beta$ or $(\alpha\beta)_2$ structure (Cantley, 1981). The catalytic subunit isolated from brain appears as two bands on sodium dodecyl sulfate-containing polyacrylamide gels, originally designated "α" for the lower apparent M_r and "$\alpha(+)$" for the higher apparent M_r (Sweadner, 1979). cDNA clones have been sequenced for three α isoforms, designated $\alpha1$, $\alpha2$, and $\alpha3$, in adult rat brain (Shull et al., 1986; Herrera et al., 1987; Orlowski and Lingrel, 1988). Epitopes specific for each of the $\alpha1$, $\alpha2$, and $\alpha3$ polypeptides have been detected immunologically in brain (Arystarkhova et al., 1989; Rowe et al., 1989; Shyjan and Levenson, 1989; Urayama et al., 1989). $\alpha1$ is identical to the originally defined "α" isoform. It is now known that $\alpha2$ and $\alpha3$ polypeptides are both contained in the brain $\alpha(+)$ band on sodium dodecyl sulfate-containing gels (Schneider et al., 1988). Two mRNA transcripts have been found for β subunit isoforms in brain (Martin-Vasallo et al., 1989). Isoform-specific expression of mRNAs for α (Herrera et al., 1987; Orlowski and Lingrel, 1988; Schneider et al., 1988) and β (Martin-Vasallo et al., 1989) subunits shows tissue-specific developmental regulation.

Prior immunoelectron microscopy studies with polyclonal antisera have localized Na^+,K^+-ATPase to nodal axolemma of central (Wood et al., 1977; Schwartz et al., 1981) and peripheral (Ariyasu et al., 1985; Ariyasu and Ellisman, 1987) nerve. Enzyme cytochemical studies have suggested a more widespread

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distribution of Na^+,K^+-ATPase activity in these tissues (Vorbrodt et al., 1982; Inomata et al., 1983; Nasu, 1983). In this study we have used a polyclonal antiserum to combined α subunits purified from bovine brain Na^+,K^+-ATPase to determine the distribution of the enzyme in the rat sciatic and optic nerves, as revealed by light microscopic immunocytochemistry with biotin-peroxidase and with silver-enhanced colloidal gold and by postembedding electron microscopic immunocytochemistry with colloidal gold. These studies show Na^+,K^+-ATPase immunoreactivity along the internodal axolemma in both the CNS and the PNS.

MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats (weighing 200–250 g) were obtained from Harlan. Trizma, sodium dodecyl sulfate (catalogue no. L5750), and silver stain kits were obtained from Sigma. Protein standards for electrophoresis were from Sigma and Bethesda Research Laboratories. Polyacrylamide, electrophoresis reagents, and nitrocellulose sheets were from Bio-Rad. Horseradish peroxidase-conjugated goat anti-rabbit IgG was from Cappel, goat anti-rabbit IgG bound to 15-nm-diameter colloidal gold was from E-Y Laboratories, and LR White was from Ernest R. Fullam. The silver–gold secondary antibodies and kit were from Janssen Laboratories, and the streptavidin–biotin–peroxidase reagents were from Zymed.

Production of the antibody

Rabbit antiserum (31B), which was prepared against the denatured catalytic subunit purified from bovine brain Na^+,K^+-ATPase and characterized by immunoblot, cDNA selection, and immunolocalization in cerebellum, retina, and kidney, has been described previously in detail (Hieber et al., 1989).

Membrane preparations

Rats were decapitated, and tissues were rapidly dissected, chilled, and used fresh or after storage at −70°C. Microsomal fractions (P2; 30,000-*g* pellet) of rat sciatic nerve and brain were prepared as described (Siegel et al., 1986) except that tissues were dispersed by hand in glass homogenizers in 10 volumes of 0.32 *M* sucrose, 1 m*M* EDTA, and 5 m*M* EGTA and adjusted to pH 7.2 with Tris base. Total membrane fractions of sciatic nerve and brain were prepared by sonicating tissues in 5 m*M* Tris (pH 7.3) and 5 m*M* NaCl, followed by centrifugation at 130,000 *g* for 1 h in a Beckman Airfuge. In the case of nerve homogenate, the material sedimenting at 10,000 rpm for 15 min was removed before the final centrifugation. Protein content was assayed with the method of Bradford (1976).

Electrophoresis and immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6% polyacrylamide) was performed with the buffer system of Laemmli (1970), and the proteins were transferred to nitrocellulose as described (Towbin et al., 1979) with the addition of 0.1% sodium dodecyl sulfate in the electrode solution. Immunostaining with whole 31B antiserum or an immunoaffinity-purified fraction was carried out as described with dry milk in the blocking solutions, peroxidase-conjugated goat anti-rat IgG as the secondary antibody, and 4-chloro-1-naphthol as the substrate (Siegel et al., 1986). Parallel gels were stained with silver or Coomassie Brilliant Blue.

Light microscopic immunocytochemistry

The animals were perfused with 4% paraformaldehyde for 1 h. The nerves were removed and cryoprotected with 2 *M* sucrose overnight; 1-μm-thick sections were cut at −80°C and placed on gelatin-coated slides.

For silver–gold detection, the sections were coated with 5% ovalbumin, blocked with 1% ovalbumin in 20 m*M* glycine and 20 m*M* Tris saline (pH 7.6) for 15 min, and exposed first to the primary antibody (diluted 1:200) for 2 h at room temperature and then to goat anti-rabbit IgG conjugated to 5-nm-diameter gold (diluted 1:10) for 1 h at room temperature. The gold precipitate was enhanced with silver using the Janssen Intense M Kit.

For avidin–biotin–peroxidase detection, the blocked slides were exposed to the primary antibody (diluted 1:300) overnight and then to goat anti-rabbit IgG conjugated to biotin for 1 h at room temperature. The sections then were incubated with streptavidin-peroxidase (diluted 1:100) for 1 h and developed with diaminobenzidine tetrahydrochloride for 15 min.

Electron microscopic immunocytochemistry

The animals were perfused through the heart with 100 m*M* phosphate buffer followed either by 0.5–1.5% glutaraldehyde and 4% paraformaldehyde or by 4% paraformaldehyde alone in phosphate buffer. The optic nerve, sciatic nerve, and roots were removed, cut into blocks, placed in the same fixative for an additional 2 h, and then embedded in LR White. Ultrathin sections were exposed to the antiserum in dilutions from 1:300 to 1:1,000 for 2 h and then to goat anti-rabbit IgG bound to 15-nm-diameter colloidal gold as described (Mata et al., 1990). The grids were stained for 20 min with uranyl acetate and examined in a JEOL Model 100S electron microscope.

We performed two controls for the immunocytochemical reaction. The first control was deletion of the anti-Na^+,K^+-ATPase antibody. The second control consisted of sections reacted in an identical manner using preimmune serum from the same animal that produced the antibody.

Affinity purification of antiserum 31B

Western transfers of β-galactosidase fusion protein (α4a) containing the 388 C-terminal amino acids of the α subunit derived from *Xenopus laevis* kidney epithelial cells (A6 cell line) (Verrey et al., 1989) were used to affinity-purify antiserum 31B following the general method of Smith and Fisher (1984). An *Escherichia coli* expression vector containing the corresponding 1,500-bp α subunit cDNA insert (α4a) ligated to the *lacZ* gene in a pEX2 plasmid (Verrey et al., 1989) was generously provided by J.-P. Kraehenbuhl (University of Lausanne, Lausanne, Switzerland). The α4a fusion protein was induced, partially purified, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western transfer as described (Verrey et al., 1989). The fusion protein was then excised from Ponceau red-stained blots and used to affinity-purify antiserum 31B as described (Verrey et al., 1989). Antibody was eluted from the nitrocellulose strips with glycine-HCl buffer (pH 2.7) for 10 min with shaking and then neutralized immediately with 100 m*M* Tris base. This affinity-purified antiserum was used in dilutions from 1:3 to 1:100 for immunocytochemistry and undiluted or diluted 1:50 for Western blotting of brain and sciatic nerve protein.

RESULTS

Antibody characterization

In Western blots of membrane fractions from both brain and peripheral nerve, a clear doublet with M_r of ~100,000 was usually recognized, corresponding to $\alpha 1$ and $\alpha(+)$ (Fig. 1a). The proportions of the two bands varied from experiment to experiment, but the predominant form was usually $\alpha(+)$. In unenriched fractions from brain, a fainter band corresponding to the $\alpha 1$ isoform could usually be recognized just below the major band, but its density was quite low. There was no immunostaining in any other region of the gel, nor was there any immunostaining of the supernatant fractions.

The antibody that was affinity-purified on α-subunit fusion protein $\alpha 4a$ identified the same doublet bands, corresponding to $\alpha 1$ and $\alpha(+)$, in the 100,000-*g* particulate fractions and in the microsomal fractions of rat sciatic nerve and in complete homogenate and microsomal fractions of rat brain as did the original antiserum (Fig. 1b). No other immunoreactive bands were detected in these samples. Thus, the antibody purified by its affinity for the polypeptide derived from frog kidney $\alpha 1$ (Verrey et al., 1989) reacts specifically with an epitope in common between the $\alpha 1$ isoform of frog kidney and the $\alpha 1$ and $\alpha(+)$ isoforms in rodent brain and peripheral nerve. It is not known whether this antibody identifies $\alpha 2$ or $\alpha 3$ because both may be present in the $\alpha(+)$ band.

Localization of Na^+,K^+-ATPase in sciatic nerve

In 1-μm-thick cryostat-cut sections of sciatic nerve treated with immune serum and either biotin-labeled secondary antibody (Fig. 2a) or a gold-labeled secondary antibody with silver enhancement (Fig. 3a and b), reaction product representing Na^+,K^+-ATPase immunoreactivity could be seen prominently along the internodal axolemma of myelinated fibers beneath the myelin sheath. At the node of Ranvier, reaction product was not detected along the axolemma (Fig. 3a). Because of the limits of resolution of this technique, it was not possible to determine the precise localization of the immunoreactivity. Control sections showed the absence of reaction products with both techniques (Figs. 2b and 3c).

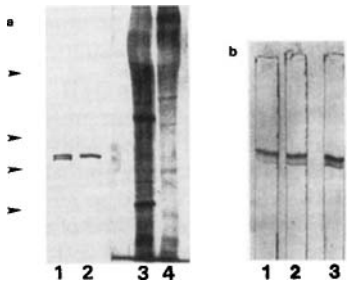

FIG. 1. a: Western blot of 130,000-*g* pellets from dispersions of sciatic nerve and brain with antiserum 31B. Lane 1, nerve pellet (10 μg of protein); lane 2, brain pellet (5 μg of protein). The faint $\alpha 1$ band, which was usually present in low density, is not seen in this blot from brain. The silver stain of an identical gel is shown in lanes 3 (nerve) and 4 (brain). Protein standards are 200, 116, 97, and 66 kDa (arrowheads). **b:** Immunostaining with affinity-purified antiserum. Lane 1, brain homogenate (20 μg of protein); lane 2, 30,000-*g* microsomal fraction of brain (20 μg of protein); and lane 3, 30,000-*g* microsomal fraction of nerve (20 μg of protein). Incubations were performed with antibodies eluted from the $\alpha 4a$ fusion protein (described in Materials and Methods) diluted 1:50 for lanes 1 and 2 and undiluted for lane 3. The lanes are cut at the dye front. No other immunostaining was seen on the original blots except for the $\alpha 1$ and $\alpha(+)$ bands.

With electron microscopy and labeling with colloidal gold, it is clear that the immunoreactivity was present along the junction of the axolemma with the periaxonal membrane of the Schwann cell through the internode (Figs. 4a and b). A similar pattern was seen when the affinity-purified antibody was used (Fig. 4c). The concentration of immunoreactivity along the internodal axolemma within single fibers was not uniform, but we were unable to detect a consistent periodicity (or pattern) of gold deposition. The apparent concentration also varied between fibers, but the degree of immunoreactivity did not seem to correlate with fiber size or degree of myelination, except that unmyelinated fibers consistently showed lower levels of immunoreactivity than myelinated fibers (Fig. 5a).

In cross-section, the gold is clearly seen along the internodal axolemma beneath the myelin sheath (Fig. 6a–c). No immunoreactivity was seen along the axolemma at nodes of Ranvier cut in cross-section (Fig. 6d).

Immunoreactivity was not seen along the Schwann cell membrane, either in the perikaryon or in the paranodal regions (Figs. 2–4), nor was any immunoreactivity seen in fibroblasts or perineurial cells (data not shown).

Localization of Na^+,K^+-ATPase in optic nerve

The distribution of immunoreactivity in optic nerve was similar to that seen in the peripheral sciatic nerve. Immunoreactivity was seen along the axolemma in the internodal regions (Fig. 5b). There was no staining of oligodendroglial cell membranes, perikaryon, or myelin lamellae.

Controls

Control sections incubated either with preimmune serum or with no primary antiserum showed a low concentration of randomly distributed gold particles, which represent inherent background of the methods (data not shown). Tissue fixed with 4% paraformaldehyde, but without glutaraldehyde (Fig. 4a), showed a pattern identical to that seen in tissue fixed with 4% paraformaldehyde and 0.5% glutaraldehyde (Fig. 4b and c).

DISCUSSION

The principal finding of these studies is that an antiserum specific for mammalian brain Na^+,K^+-ATPase

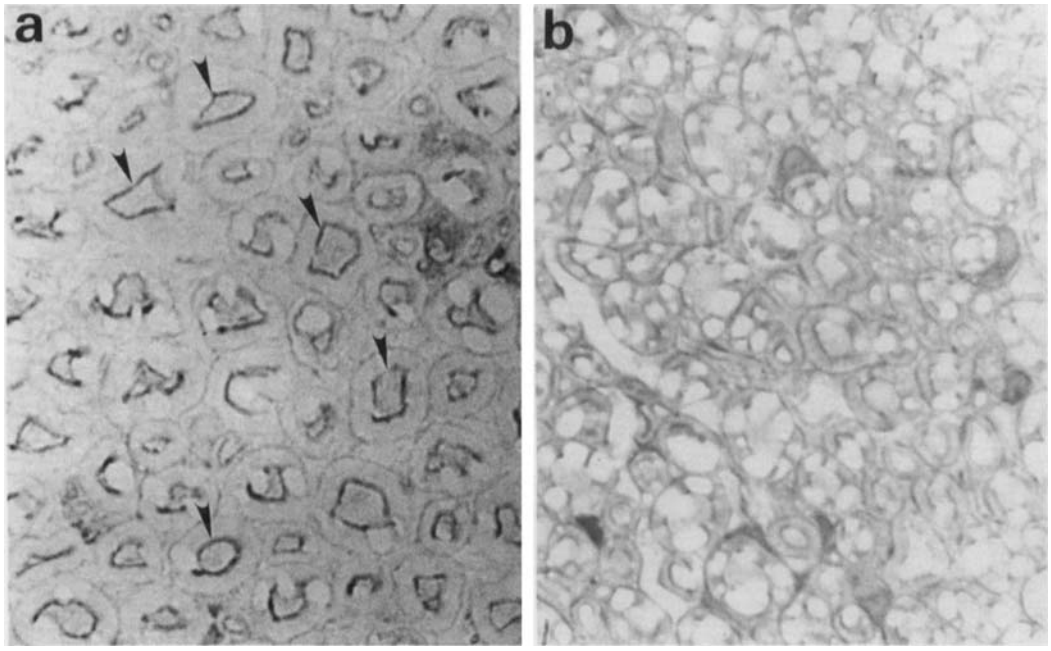

FIG. 2. a: Biotin–streptavidin–peroxidase labeling of a 1-μm-thick cryosection of nerve shows Na^+,K^+-ATPase immunoreactivity along the axolemma (arrowheads). ×5,800. **b:** Control with deletion of primary antibody. ×4,930.

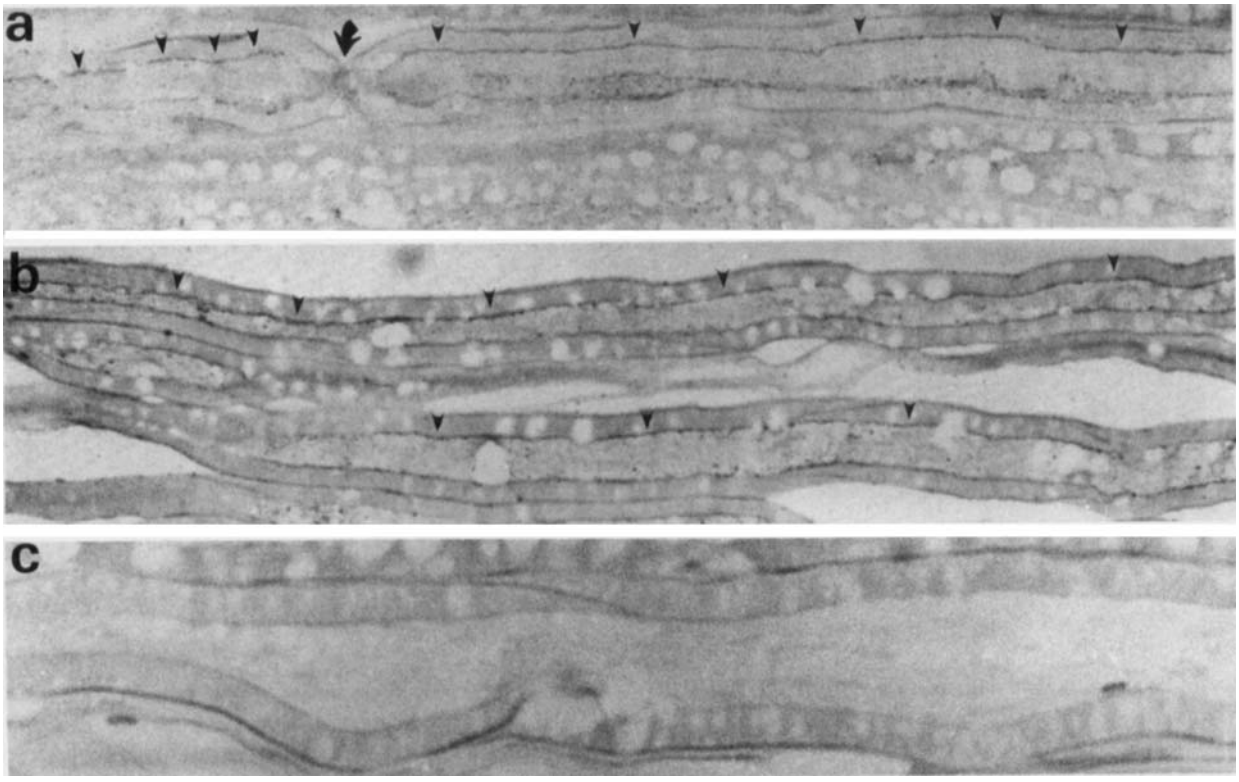

FIG. 3. a and b: Silver-enhanced gold labeling of 1-μm-thick longitudinal cryosections of nerve demonstrates immunoreactivity along the internodal axolemma of myelinated fibers (arrowheads). The node of Ranvier is indicated by an arrow. ×4,930. **c:** Control with deletion of primary antibody. ×8,670.

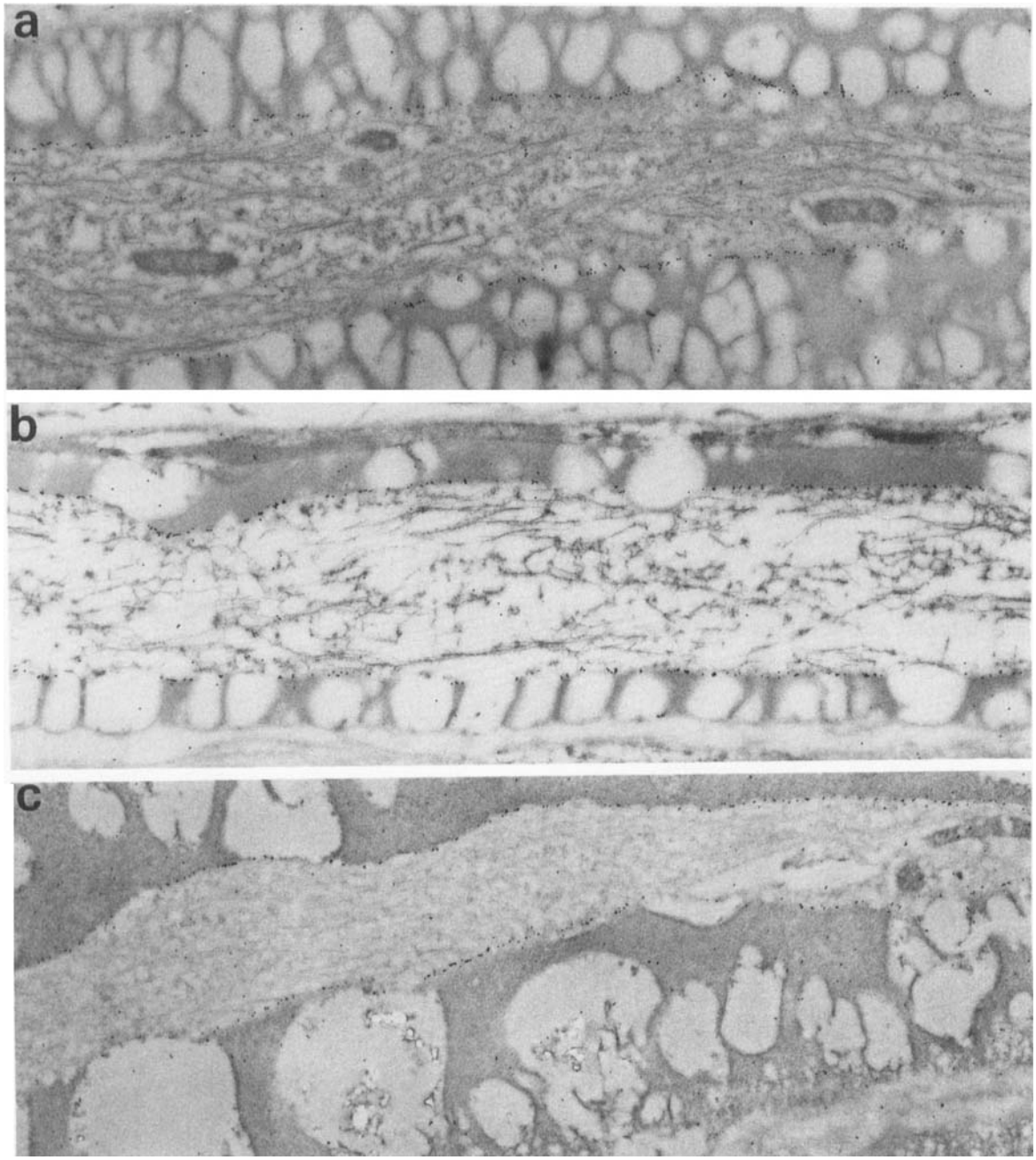

FIG. 4. Colloidal gold-labeled electron microscopic immunocytochemistry of sciatic nerve with antiserum 31B (**a and b**) and with the affinity-purified antibody (**c**). a: 4% paraformaldehyde, ×20,400. b: 0.5% glutaraldehyde, 4% paraformaldehyde, ×20,145. c: 0.5% glutaraldehyde, 4% paraformaldehyde, ×16,830.

catalytic subunit reveals immunoreactivity along the internodal axolemma of myelinated fibers in the CNS and the PNS.

The specificity of the antiserum used in this study is supported by the following criteria. The antiserum, which was raised against Na^+,K^+-ATPase catalytic subunit purified from bovine brain (Hieber et al., 1989), reacts with the catalytic subunit ($\alpha 1$) in purified holoenzyme from lamb kidney (Siegel and Desmond, 1989) and specifically with polypeptides of the same M_r from rat brain and sciatic nerve (Fig. 1) and from mouse and bovine brain (Hieber et al., 1989). It does not react with any other proteins in the crude pelleted membranes or in the soluble fractions of these tissues. Screening a λgt11 cDNA expression library constructed from mouse brain mRNA with the same antiserum

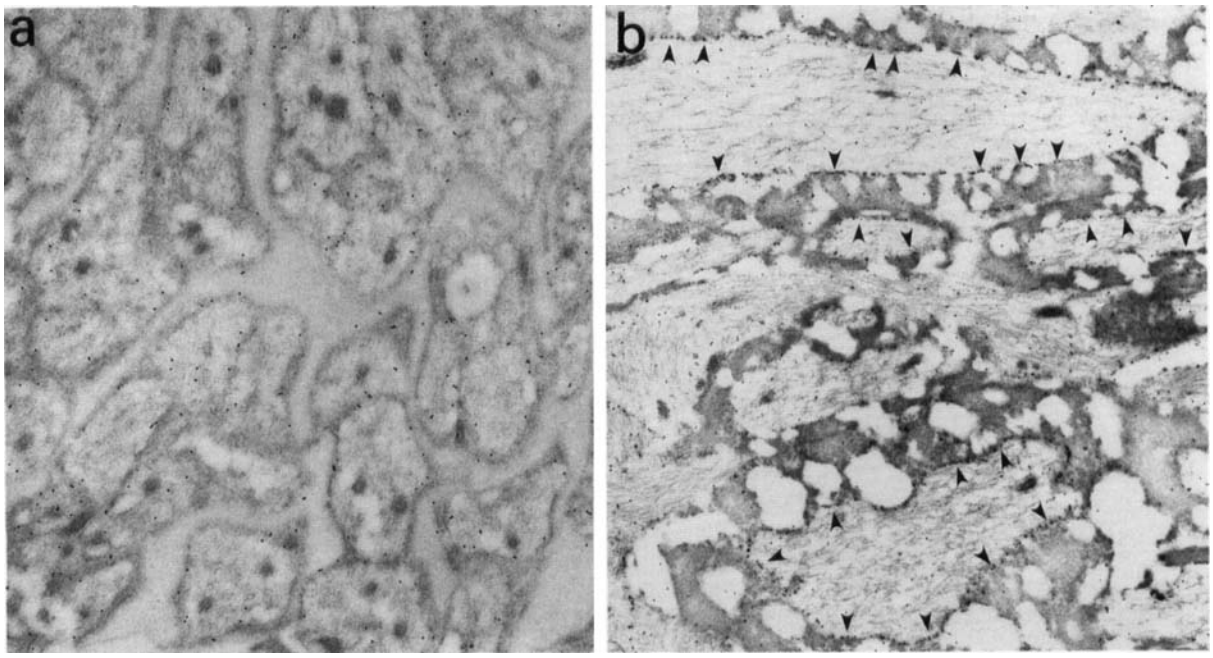

FIG. 5. Colloidal gold immunocytochemistry of the unmyelinated fibers of the cervical sympathetic trunk with antiserum 31B shows relatively sparse labeling of the axolemma (**a**). Under similar conditions of labeling, optic nerve shows strong immunoreactivity along the internodal axolemma (arrowheads; **b**). a: ×24,650. b: ×11,560.

identified a clone with cDNA homologous to that of rat brain α1 by sequence and Northern blot hybridization (Hieber et al., 1989). Light microscopic immunocytochemistry of mouse kidney using the same antibody demonstrated exclusive basolateral membrane staining identical to that described for other antibodies to Na^+,K^+-ATPase in that tissue (Hieber et al., 1989). The antibody also shows strong immunostaining in retinal photoreceptor inner segments (Hieber et al., 1989) and in the gerbil vestibular system (Spicer et al., 1990), correlating with the distribution of the α3 isoform in retina resolved recently with an α3-specific antibody (McGrail and Sweadner, 1989) and with α isoforms using monoclonal antibodies in the gerbil vestibular system (Spicer et al., 1990). Finally, antibody purified from the antiserum by affinity-binding to a fusion protein containing 388 amino acids of known sequence from the carboxy terminus of *Xenopus* kidney (A6 cell) α1 subunit (Verrey et al., 1989) produced a staining pattern identical to that of the whole antiserum 31B (Fig. 4).

The finding of internodal staining for Na^+,K^+-ATPase is surprising because previous electron microscopic immunocytochemical studies showed Na^+,K^+-ATPase in myelinated fibers limited to the node of Ranvier (Wood et al., 1977; Schwartz et al., 1981; Ariyasu et al., 1985; Ariyasu and Ellisman, 1987). These studies have used a polyclonal antibody raised against the Lubrol-solubilized electroplax holoenzyme to localize the enzyme in knifefish brain (Wood et al., 1977), a polyclonal antibody raised against the denatured catalytic subunit purified from goldfish brain Na^+,K^+-ATPase (Schwartz et al., 1980) to localize the enzyme in goldfish optic nerve (Schwartz et al., 1981), and one monoclonal and several different polyclonal antisera raised against kidney holoenzyme to study normal and dystrophic mouse sciatic nerve (Ariyasu et al., 1985; Ariyasu and Ellisman, 1987). In these studies only the axolemma in the dystrophic mouse showed uniform staining (Ariyasu and Ellisman, 1987).

The finding of internodal immunoreactivity is not without precedents. Previous studies using light microscopic immunocytochemistry have suggested the presence of internodal Na^+,K^+-ATPase. Fambrough and Bayne (1983) provided light microscopic fluorescence data suggesting internodal distribution of the enzyme in chicken sciatic nerve with an antibody that recognized isoforms of muscle and nerve but not of Schwann cell or fibroblast (see their Fig. 10). The available light microscopic immunocytochemical figures showing cross-sections of nerve or spinal cord indicate much more axolemmal staining than can be accounted for by nodal sites (Schwartz et al., 1980; McGrail and Sweadner, 1986; Sheedlo and Siegel, 1987). McGrail and Sweadner (1989) recently used light microscopic immunofluorescence to demonstrate that their monoclonal antibody to α3 appears to label internodal fibers in the optic nerve. They suggested that previous failure to demonstrate internodal immunoreactivity might have been due to insufficient antibody sensitivity to

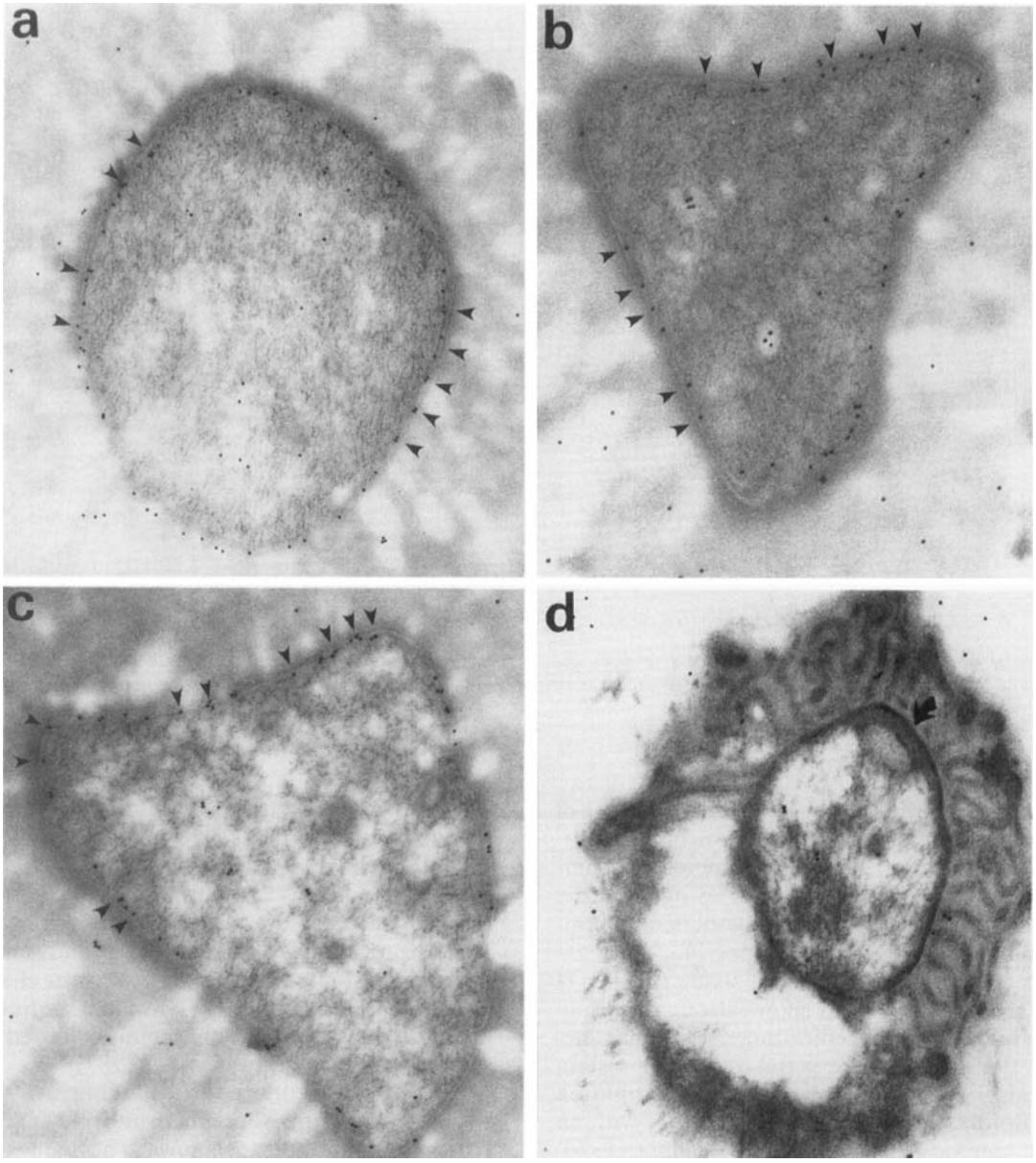

FIG. 6. Colloidal gold-labeled electron microscopic immunocytochemistry with antiserum 31B of sciatic nerve cut in cross-section shows immunolabeling along the internodal axolemma (arrowheads; **a–c**). Note that the gold particles are associated with the axolemma rather than the membrane of the Schwann cell inner mesaxon and that most of the particles appear on the internal side of the axolemma. No labeling of the axolemma is seen at the node of Ranvier (**d**). a: ×30,345. b: ×40,120. c: ×34,680. d: ×40,970. a–d: 2.5% glutaraldehyde, 4% paraformaldehyde.

α3. The light microscopic immunocytochemistry presented in this article (Figs. 2 and 3) is in agreement with the general localization reported by McGrail and Sweadner (1989). In addition, the resolution obtained with electron microscopic immunocytochemistry (Figs. 4–6) shows that the localization of internodal Na^+,K^+-ATPase is along the axolemma. Three different studies using the ouabain-sensitive, K^+-dependent *p*-nitrophenyl phosphatase cytochemical reaction have previously shown Na^+,K^+-ATPase *activity* in the internodal (as well as nodal) axolemma of myelinated fibers of mammalian CNS and PNS (Vorbrodt et al., 1982; Inomata et al., 1983; Nasu, 1983).

The current study used postembedding immunocytochemistry in which the antibody is applied to the ultrathin sections on grids, so that there are no tissue barriers to antibody penetration and the epitopes at the transversely cut surfaces have maximal exposure to the antiserum. This methodologic feature might account for the resolution of internodal immunoreactiv-

ity in this electron microscopic study, which was not found in the previous studies that used preembedding methods. However, this feature cannot explain the absence of nodal immunoreactivity with antibody 31B. Further, using our antiserum on cryosections for light microscopy produced identical internodal immunostaining (Figs. 2 and 3), a finding suggesting that a critical difference lies in the epitope(s) recognized rather than only with the method used.

There are two possible explanations for the absence of nodal immunoreactivity with the antibody used in the current study. It is possible that a different isoform is present at the node compared with the internode and that antibody 31B does not recognize the isoform that is present at the node or that the antibody recognizes but has a low affinity for the isoform present at the node. Alternately, the same isoform may be present both at the node and internodally, but specific post-translational modifications to the Na^+,K^+-ATPase α subunit at the node may obscure the epitope recognized by the antibody. We are in the process of obtaining other characterized antibodies to the α subunits and to the β subunits to address these issues.

Recent studies have made it clear that the axolemma of mammalian myelinated fibers displays regional heterogeneity. For example, in mammalian nerve, Na^+ channels are located almost exclusively at the node of Ranvier (Ritchie, 1986), whereas K^+ channels are located internodally (Kocsis, 1986; Ritchie, 1986). Thus, Na^+ influx accompanying action potentials occurs mainly at the node, whereas K^+ efflux occurs mainly internodally. Nodal enzyme might be regulated primarily by intracellular Na^+ entering during the action potential. Internodal enzyme might be regulated primarily by extracellular K^+ to pump K^+ into the axon in the internode, thus maintaining ionic homeostasis in the periaxonal space between axolemma and inner mesaxon. Additional studies will be required to test for expression of unique α-subunit epitopes at different sites and to determine the relationship between isoform distribution and site-specific function.

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REFERENCES

Albers R. W., Siegel G. J., and Stahl W. L. (1989) Membrane transport, in *Basic Neurochemistry*, 4th ed. (Siegel G. J., Agranoff B. W., Albers R. W., and Molinoff P. B., eds), pp. 49–70. Raven Press, New York.

Ariyasu R. G. and Ellisman M. H. (1987) The distribution of (Na^+-K^+)ATPase is continuous along the axolemma of unensheathed axons from spinal roots of 'dystrophic' mice. *J. Neurocytol.* **16,** 239–248.

Ariyasu R. G., Nichol J. A., and Ellisman M. H. (1985) Localization of sodium/potassium adenosine triphosphatase in multiple cell types of the murine nervous system with antibodies raised against the enzyme from kidney. *J. Neurosci.* **5,** 2581–2596.

Arystarkhova E. A., Lakhtina O. E., and Modyanov N. N. (1989) Immunodetection of Na,K-ATPase alpha3-isoform in renal and nerve tissues. *FEBS Lett.* **250,** 545–548.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Cantley L. C. (1981) Structure and mechanism of the Na,K-ATPase. *Curr. Top. Bioenerg.* **11,** 201–237.

Fambrough D. M. and Bayne E. K. (1983) Multiple forms of (Na^+-K^+)-ATPase in the chicken: selective detection of the major nerve, skeletal muscle, and kidney form by a monoclonal antibody. *J. Biol. Chem.* **258,** 3926–3935.

Herrera V. L., Emanuel J. R., Ruiz Opazo N., Levenson R., and Nadal Ginard B. (1987) Three differentially expressed Na,K-ATPase alpha subunit isoforms: structural and functional implications. *J. Cell. Biol.* **105,** 1855–1865.

Hieber V., Siegel G. J., Desmond T., Lee-Hwa Liu J., and Ernst S. A. (1989) Na,K-ATPase: comparison of the cellular localization of alpha-subunit mRNA and polypeptide in mouse cerebellum, retina, and kidney. *J. Neurosci. Res.* **23,** 9–20.

Inomata K., Mayahara H., Fujimoto K., and Ogawa K. (1983) Ultrastructural localization of ouabain-sensitive potassium-dependent *p*-nitrophenylphosphatase (Na^+,K^+-ATPase) activity in the central nervous system of the rat. *Acta Histochem. Cytochem.* **16,** 277–285.

Kocsis J. D. (1986) Functional characteristics of potassium channels of normal and pathological mammalian axons, in *Ion Channels in Neural Membranes* (Ritchie J. M., Keynes R. D., and Bolis L., eds), pp. 123–144. Alan R. Liss, New York.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Martin-Vasallo P., Dachowski W., Emanuel J. R., and Levenson R. (1989) Identification of a putative isoform of the Na,K-ATPase beta subunit. *J. Biol. Chem.* **264,** 4613–4618.

Mata M., Fink D. J., Ernst S. A., and Siegel G. J. (1989) Immunocytochemical localization of (Na^+,K^+)-ATPase to internodal axolemma in myelinated fibers of the rat. (Abstr.) *J. Neurochem.* **52** (Suppl.), S186.

Mata M., Alessi D., and Fink D. J. (1990) S100 is preferentially distributed in myelin-forming Schwann cells. *J. Neurocytol.* **19,** 432–442.

McGrail K. M. and Sweadner K. J. (1986) Immunofluorescent localization of two different Na,K-ATPases in the rat retina and in identified dissociated retinal cells. *J. Neurosci.* **6,** 1272–1283.

McGrail K. M. and Sweadner K. J. (1989) Complex expression patterns for Na^+,K^+ ATPase isoforms in retina and optic nerve. *Eur. J. Neurosci.* **2,** 170–176.

Nasu F. (1983) Ultracytochemical demonstration of ouabain-sensitive, K^+-dependent, *p*-nitrophenylphosphatase (Na^+,K^+-ATPase) activity in the rat hippocampal formation. *Acta Histochem. Cytochem.* **16,** 368–373.

Orlowski J. and Lingrel J. B. (1988) Tissue-specific and developmental regulation of rat Na,K-ATPase catalytic alpha isoform and beta subunit mRNAs. *J. Biol. Chem.* **263,** 10436–10442.

Ritchie J. M. (1986) The distribution of sodium and potassium channels in mammalian myelinated nerve, in *Ion Channels in Neural Membranes* (Ritchie J. M., Keynes R. D., and Bolis L., eds), pp. 105–122. Alan R. Liss, New York.

Rowe P. M., Link W. T., and Albers R. W. (1989) Demonstration of three isoforms of Na,K-ATPase alpha-subunit in rat brain. (Abstr) *Trans. Am. Soc. Neurochem.* **20,** 156.

Schneider J. W., Mercer R. W., Gilmore-Hebert M., Utset M. F., Lai C., Greene A., and Benz E. J. (1988) Tissue specificity, localization in brain, and cell-free translation of mRNA encoding

the A3 isoform of Na^+,K^+-ATPase. *Proc. Natl. Acad. Sci. USA* **85,** 284–288.

Schwartz M., Siegel G. J., Chen N., and Agranoff B. W. (1980) Goldfish brain (Na^+,K^+)-ATPase: purification of the catalytic polypeptide and production of specific antibodies. *J. Neurochem.* **34,** 1745–1752.

Schwartz M., Ernst S. A., Siegel G. J., and Agranoff B. W. (1981) Immunocytochemical localization of (Na^+,K^+)-ATPase in the goldfish optic nerve. *J. Neurochem.* **36,** 107–115.

Sheedlo H. J. and Siegel G. J. (1987) Comparison of the distribution of Na^+,K^+-ATPase and myelin-associated glycoprotein (MAG) in the optic nerve, spinal cord and trigeminal ganglion of shiverer (*shi/shi*) and control (+/+) mice. *Brain Res.* **415,** 105–114.

Shull G. E., Greeb J., and Lingrel J. B. (1986) Molecular cloning of three distinct forms of the Na^+,K^+-ATPase alpha-subunit from rat brain. *Biochemistry* **25,** 8125–8132.

Shyjan A. W. and Levenson R. (1989) Antisera specific for the alpha1, alpha2, alpha3, and beta subunits of the Na,K-ATPase: differential expression of alpha and beta subunits in rat tissue membranes. *Biochemistry* **28,** 4531–4535.

Siegel G. J. and Desmond T. J. (1989) Effects of tetradecyl sulfate on electrophoretic resolution of kidney Na,K-ATPase catalytic subunit isoforms. *J. Biol. Chem.* **264,** 4751–4754.

Siegel G. J., Holm C., Schreiber J. H., Desmond T., and Ernst S. A. (1984) Purification of mouse brain (Na^+,K^+)-ATPase catalytic unit, characterization of antiserum, and immunocytochemical localization in cerebellum, choroid plexus, and kidney. *J. Histochem. Cytochem.* **32,** 1309–1318.

Siegel G. J., Desmond T., and Ernst S. A. (1986) Immunoreactivity and ouabain-dependent phosphorylation of ($Na^+ + K^+$)-adenosine triphosphatase catalytic subunit doublets. *J. Biol. Chem.* **261,** 13768–13776.

Skou J. C. (1988) Overview: the Na,K pump, in *Methods in Enzymology, Vol. 156* (Fleischer S. and Fleischer B., eds), pp. 1–125. Academic Press, New York.

Smith D. E. and Fisher P. A. (1984) Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in *Drosophila* embryos: application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. *J. Cell Biol.* **99,** 20–28.

Spicer S. F., Schulte B. A., and Adams J. C. (1990) Immunolocalization of Na^+,K^+-ATPase and carbonic anhydrase in the gerbil vestibular system. *Hear. Res.* **43,** 205–217.

Sweadner K. J. (1979) Two molecular forms of ($Na^+ + K^+$)-stimulated ATPase in brain. Separation and difference in affinity for strophanthidin. *J. Biol. Chem.* **254,** 6060–6067.

Towbin H., Staehelin T., and Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.

Urayama O., Shutt H., and Sweadner K. J. (1989) Identification of three isozyme proteins of the catalytic subunit of the Na,K-ATPase in rat brain. *J. Biol. Chem.* **264,** 8271–8280.

Verrey F., Kairouz P., Schaerer E., Fuentes P., Geering K., Rossier B. C., and Kraehenbuhl J.-P. (1989) Primary sequence of *Xenopus laevis* Na^+-K^+-ATPase and its localization in A6 kidney cells. *Am. J. Physiol.* **265,** F1034–F1043.

Vorbrodt A. W., Lossinsky A. S., and Wisniewski H. M. (1982) Cytochemical localization of ouabain-sensitive, K^+-dependent *p*-nitrophenylphosphatase (transport ATPase) in the mouse central and peripheral nervous systems. *Brain Res.* **243,** 225–234.

Wood J. G., Jean D. H., Whitaker J. N., McLaughlin B. J., and Albers R. W. (1977) Immunocytochemical localization of the sodium, potassium activated ATPase in knifefish brain. *J. Neurocytol.* **6,** 571–581.