ELSEVIER

Acid Phosphatase Localization in Accumulated Membranous Organelles of Optic Nerve Axons Following Acute Elevation of Intraocular Pressure

Bo Ou,* Keitetsu Abe,*
Hai-Bo Chen,* Shinichi Ohno,† and Shigeo Tsukahara*

*Departments of *Ophthalmology and †Anatomy,*
Yamanashi Medical University, Yamanashi, Japan

Abstract: Acid phosphatase localization in accumulated membranous organelles of optic nerve axons of guinea pigs following acute elevation of intraocular pressure (IOP) was determined, employing light and electron microscopic enzymic cytochemistry with β-glycerophosphate as a substrate. Positive reaction products appeared to accumulate in the region of the lamina cribrosa, as revealed with light microscopic enzyme cytochemistry. Electron microscopic enzyme cytochemistry also demonstrated that such reaction products mainly localized on multivesicular or multilamellar bodies and myelin-like structures in the unmyelinated optic nerve axons. Following an acutely elevated IOP, retrograde-moving membranous organelles in the optic nerve axons were found to contain AcPase, suggesting that these organelles could be degraded in the axons through the lysosomal pathway. **Jpn J Ophthalmol 1998;42:373–376** © 1998 Japanese Ophthalmological Society

Key Words: Acid phosphatase, acute elevated intraocular pressure, enzyme cytochemistry, membranous organelles, optic nerve.

Introduction

Acid phosphatase (AcPase), one of more than 60 lysosomal acid hydrolases,[1] is commonly used as a marker enzyme for organelle lysosomes;[2,3] it plays a central role during intracellular digestive processes. Normally a majority of cell organelles containing AcPase in neurons are confined to cell bodies and dendrites, and few such organelles are present in nerve axons.[4,5] Therefore, axonal waste products and endocytotically engulfed materials are assumed to be transported to the neuron cell body for degradation.[6–8] However, under pathologic conditions, such as nerve crush injury, enzyme cytochemical studies have demonstrated that membranous organelles, like multivesicular or multilamellar bodies, are positive for AcPase in the axons.[10–13] In those studies, it is proposed that lysosome-mediated degradation can extend into the nerve axons, in an attempt to maintain the internal milieu of nerve cells.

In the experimental model systems with elevated intraocular pressure (IOP), interruption in anterograde and retrograde axonal transport occurs in the region of the lamina cribrosa of optic nerves.[14–17] Ultrastructural changes have also been observed at these sites, including accumulation of various membranous organelles in axons and disorganization or neurotubules and neurofilaments.[16,17] To establish whether AcPase is present in such membranous organelles after axonal transport blockade, we have investigated AcPase activity in guinea pig optic nerves after 4-hour periods of acute elevation of IOP, employing light and electron microscopic enzymic cytochemistry.

Materials and Methods

The experimental animals were treated in accordance with the ARVO statement on the use of animals in ophthalmic and vision research. Twelve guinea pigs were used in the present study. Four animals without elevated IOP were studied as normal controls and eight were used in the elevated IOP group.

Received: December 17, 1997
Address correspondence and reprint requests to: Shigeo TSUKAHARA, MD, PhD, Department of Ophthalmology, Yamanashi Medical University, 1110 Shimokato, Tamaho, Yamanashi 409-38, Japan

Jpn J Ophthalmol 42, 373–376 (1998)

Published by Elsevier Science Inc.

0021-5155/98/$19.00
PII S0021-5155(98)00032-X

Jpn J Ophthalmol
Vol 42: 373–376, 1998

The IOP was elevated by the following method, as reported previously.[17] After administration of sodium pentobarbital anesthesia, the IOP was monitored by a saline manometer connected to a 25-gauge needle inserted into the anterior chamber of both eyes. The IOP level was controlled by varying the height of the manometer reservoir. In the eight control eyes, the pressure was maintained at 15 mmHg for 4 hours. In the 16 experimental eyes, the IOP of 60 mmHg was kept constant for 4 hours. After 4 hours, all animals were immediately perfused through the heart with 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) for 5–10 minutes. After perfusion, optic nerves were excised and additionally fixed by immersion treatment for 30 minutes. They were rinsed in 0.1 mol/L cacodylate buffer (pH 7.4) containing 7% sucrose, and prepared for various specimens, as described below.

Light Microscopic Enzyme Cytochemistry

The optic nerves were embedded in Tissue-Tek OCT compound and quickly frozen by direct immersion in liquid nitrogen. A series of cryostat sections, 15 μm in thickness, were cut longitudinally through the optic nerve head. The specimens were routinely incubated for demonstration of AcPase activity using β-glycerophosphate as a substrate.[18] Freshly prepared incubation medium was kept at 37°C for 1 hour and filtered just before use. The incubation was conducted in β-glycerophosphate medium at 37°C for 15 minutes. Some specimens were incubated in substrate-free medium as enzyme cytochemical controls. After washing in the cacodylate buffer, the specimens were exposed to 1% ammonium sulphide for 2 minutes, and then examined by light microscopy.

Electron Microscopic Enzyme Cytochemistry

A series of unfrozen sections, 40 μm in thickness, were cut longitudinally through the optic nerve head on a microslicer. Some specimens were incubated in substrate-free medium as controls. Other were incubated in the β-glycerophosphate medium at 37°C for 30 minutes, and then washed again in the cacodylate buffer, postfixed in 1% osmium tetroxide for 30 minutes, dehydrated in a graded series of ethanol, and embedded in Epon 812. Ultrathin sections were cut, stained with uranyl acetate (1 minute), and observed by a Hitachi H-500 electron microscope.

Results

Light Microscopy

In optic nerves maintained at normal IOP, few AcPase reaction products were seen in nerve bundles in the region of the lamina cribrosa, whereas glial cells around the nerve bundles showed small granular reaction products (Figure 1a). In optic nerves maintained at elevated IOP, some positive reaction products appeared to accumulate in the nerve bundles of the region of the lamina cribrosa in all specimens (Figure 1b).

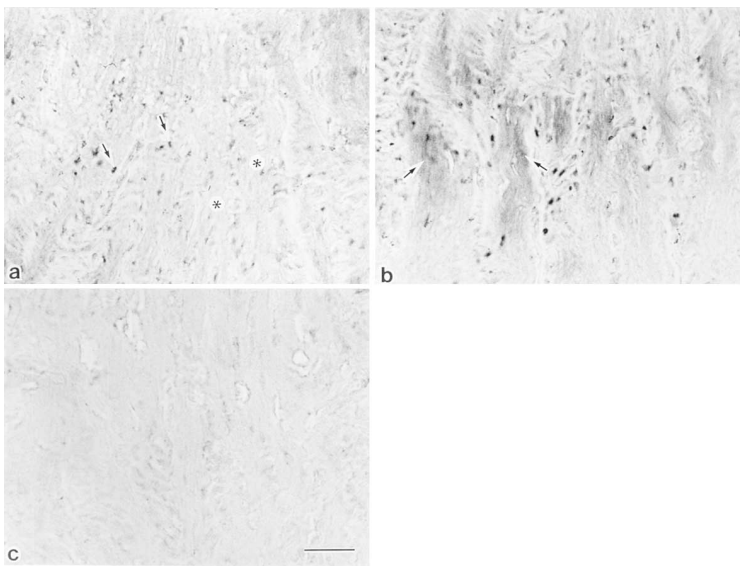

Figure 1. Light micrographs of enzyme cytochemistry for AcPase. (**a**) At normal intraocular pressure (IOP), few AcPase reaction products are seen in region of lamina cribrosa (asterisks), whereas glial cells around nerve bundles show smaller granular reaction products (arrows). (**b**) At elevated IOP, AcPase reaction products appear to accumulate in nerve bundles in region of lamina cribrosa (arrows). (**c**) Substrate-free control section. Upper portions of **a** and **b** show optic nerve head. Bar = 10 μm.

Electron Microscopy

In optic nerves maintained at elevated IOP, enzyme cytochemistry demonstrated AcPase reaction products in some membranous organelles in some parts of unmyelinated axons at the region of the lamina cribrosa (Figure 2a). High magnification electron micrographs showed that multivesicular bodies, multilamellar bodies and myelin-like structures appeared to be positive for the AcPase enzyme activity (Figures 2b, 2c, 2d). However, vesicles and mitochondria were negative for reaction products.

Discussion

In the region of the lamina cribrosa of eyes maintained at IOP 60 mmHg for 4 hours, the present study has shown that AcPase activity in myelin-like figures, multivesicular bodies, and multilamellar bodies localized in some parts of unmyelinated axons of the optic nerves. Such multivesicular or multilamellar bodies are known to be distinct organelles in the retrograde pathway.[19] The myelin-like figures seem to have originated from multilamellar bodies, because they contain multimembranes. Therefore, membranous organelles containing AcPase were limited to the organelles transported retrogradely.

Whether membranous organelles contain AcPase or not seems to be related to their function. Some in vitro studies have indicated that materials resulting from endocytosis and autophagy might be transported to the neuron cell body for degradation, where lysosomes are usually localized.[6,7] Retrogradely transported organelles are sites of convergence for materials resulting from endocytosis and autophagy. Other in vivo studies have demonstrated that about 50% of materials conveyed by anterograde axonal transport into axons may be unselectively retrieved from the axons by retrograde transport for degradation or recycle.[20] The organelles in axons are still nonlysosomal because they have almost neutral pH, and lack AcPase activity.[8] However, they become acidified during their retrograde transport along neurotubules and may gain acid hydrolase by fusing with lysosomes in proximal axons or cell bodies.[9] The present study suggests that membranous organelles transported retrogradely may acquire some enzymes in the optic nerve axons and not be transported to the neuron cell body for degradation.

Another question immediately arises: How do these membranous organelles gain AcPase activity in the axons? A possible explanation is that the AcPase could be obtained from organelles in the anter-

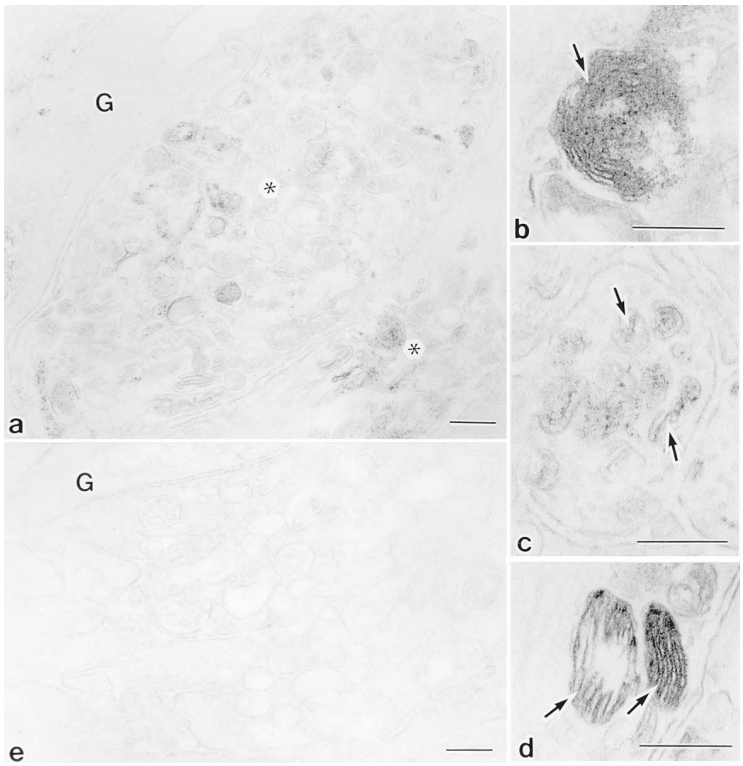

Figure 2. Electron micrographs for AcPase. (**a**) At elevated intraocular pressure (IOP), AcPase-positive bodies and AcPase-negative organelles are observed in unmyelinated axons (asterisks). (**b**)–(**d**) In higher magnification electron micrographs arrows indicate various types of AcPase-positive bodies observed in axoplasm. (**b**) Multilamellar bodies. (**c**) Multivesicular bodies (**d**) Myelin figures. (**e**) Substrate-free control section. Bar = 0.2 μm. G: glial cell.

Jpn J Ophthalmol
Vol 42: 373–376, 1998

ograde transport. Some studies about other nerves have suggested that hydrolases were transported into and along the axons, being packaged in the agranular reticulum-like cisterns, which may generate small spherical lysosomes in axons and axon terminals.[12,21] A previous study demonstrated that anterograde axonal transport between ganglion cells and lamina cribrosa was not blocked until the level of IOP approached or exceeded the mean blood pressure.[13] According to this observation, the 60 mmHg IOP used in the present study could not influence the anterograde axonal transport between the ganglion cells and the lamina cribrosa. In cross-cut sections by conventional electron microscopy, we have confirmed that the site for interruption of the axonal transport is the level of the lamina cribrosa along the optic nerve (unpublished). In addition, it is very unlikely that AcPase is incorporated by endocytosis from glial cells into the optic nerves, because such endocytosis of the axons is minimal.[4]

In conclusion, the present study utilizing AcPase enzyme cytochemistry has demonstrated that retrogradely transported organelles contained AcPase after axonal transport blockade, suggesting that their degradation could be accomplished via the lysosomal pathway in axons.

This study was supported by a Grant-in-Aid for General Scientific Research, No. 07407050, given by the Ministry of Education, Science, Sports and Culture to Dr. S. Tsukahara.

References

1. Koenig H. Lysosomes. In Lajtha, A., ed. Handbook of neurochemistry. New York: Plenum Press, 1984:177–204.
2. Holtzman E. Lysosomes: a survey. In Alfert, M. et al., eds. Cell biology monographs. Wren/New York: Springer Verlag, 1976:3.
3. Bainton DF. The discovery of lysosomes. J Cell Biol 1981;91:26–33.
4. Parton RG. Axonal and dendritic endocytic pathways in cultured neurons. J Cell Biol 1992;119:123–37.
5. Augenbraun E. Properties of acidified compartments in hippocampal neurons. Eur J Cell Biol 1993;61:34–43.
6. Koenig ES. Rapid mobility of motile varicosities and inclusions containing a-spectrin, actin, and calmodulin in regenerating axons. J Neurosci 1985;5:715–29.
7. Hollenbeck PJ. Products of endocytosis and autophagy are retrieved from axons by regulated retrograde organelle transport. J Cell Biol 1993;121:305–15.
8. Hollenbeck PJ, Bray D. Rapidly transported organelles containing membrane and cytoskeletal components: their relation to axonal growth. J Cell Biol 1987;105:2827–35.
9. Overly CC. Quantitative measurement of intraorganelle pH in the endosomal-lysosomal pathway in neurons by using radiometric imaging with pyranine. Proc Natl Acad Sci USA 1995;92:3156–60.
10. Broadwell RD, Brightman MW. Cytochemistry of undamaged neurons transporting exogenous protein in vivo. J Comp Neurol 1979;185:31–74.
11. Broadwell RD, Cataldo AM. The neuronal endoplasmic reticulum: its cytochemistry and contribution to the endomembrane system. II, Axons and terminals. J Comp Neurol 1984;230:231–48.
12. Holtzman E, Novikoff AB. Lysosomes in the rat sciatic nerve following crush. J Cell Biol 1965;27:651–69.
13. Krikorian JG. Transport of acid phosphatase in normal and transected rat sciatic nerve. Exp Neurol 1980;70:665–74.
14. Anderson DR, Hendrickson A. Effect of intraocular pressure on rapid axoplasmic transport in primate optic nerve. Invest Ophthalmol 1974;13:771–83.
15. Mincker DS. Orthograde and retrograde axoplasmic transport during acute ocular hypertension in the monkey. Invest Ophthalmol Vis Sci 1977;16:426–41.
16. Quigley HA, Anderson DR. The dynamics and location of axonal transport blockade by acute intraocular pressure elevation in primate optic nerve. Invest Ophthalmol 1976;15:606–616.
17. Ou B, Ohno S, Terada N, Fujii Y, Chen HB, Tsukahara S. Ultrastructural study of axonal cytoskeleton in the optic nerve damaged by acutely elevated intraocular pressure using the quick-freezing and deep-etching technique. Ophthalmic Res 1997;29:48–54.
18. Gomori G. Microscopic histochemistry, principles and practice. Chicago: University of Chicago Press, 1953.
19. Futter CE. Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. J Cell Biol 1996;132:1011–23.
20. Bisby MA. Reversal of axonal transport: similarity of proteins transported in anterograde and retrograde direction. J Neurochem 1981;36:741–5.
21. Novikoff AB. Enzyme localization and ultrastructure of neurons. In Hyden H., ed. The neuron. Amsterdam: Elsevier, 1967:255–318.