Ultracytochemical Demonstration of Alkaline Phosphatase Activity in Astrocytes and Subependymal Cells in the Rat Brain

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Summary. Ultrastructural localization of alkaline phosphatase activity was studied in astrocytes of the cerebral cortex and corpus callosum, and in the ependymal and subependymal cells of the lateral ventricle of normal rat brain by means of electron microscopic histochemistry employing the lead citrate method. Astrocytes exhibited alkaline phosphatase activity on the plasma membrane of the endfeet facing the vascular basement membrane and at the gap junctions, but not on other parts of the plasma membrane or in the cytoplasm. Immature astrocytes containing small bundles of filaments, a few glycogen granules and other cell organelles, exhibited alkaline phosphatase activity on the inner and outer nuclear membranes, the outer mitochondrial membrane and on portions of the plasma membrane. Thus, it was concluded that the distribution of alkaline phosphatase in astrocytes shifts from the cytoplasm and plasma membrane to the endfeet and gap junctions with cell maturation. Alkaline phosphatase activity was detected on the plasma membrane of the subependymal cells and surrounding processes, but not on the ependymal cells.

Recent electron microscopic studies have shown that mature oligodendrocytes have alkaline phosphatase (ALP) activity in their plasma membrane, but that immature oligodendrocytes have ALP activity in their cytoplasmic cell organelles (Mori et al., 1984; Mori and Nagano, 1985). Whether astrocytes show such changes in ALP activity with maturation is unknown. Similarly, it is not clarified yet whether subependymal cells which are supposed to be immature, glial cell precursor exhibit the activity in their cytoplasm.

Few investigations are available on the localization of ALP activity in astrocytes at the electron microscopic level. The activity of ALP has been localized to astrocytic vascular endfeet (Mayahara et al., 1967; Ovtscharoff, 1973; Gotow, 1984).

Here we report on the ALP activity on the astrocytic vascular endfeet, astrocytic cell bodies, processes in the neuropil and subependymal cells.

MATERIALS AND METHODS

Thirty-four male Wistar rats each weighing 80–120 g were used. The rats were kept in an animal cage with solid food and water ad libitum. These animals were anes-
S. MORI and M. NAGANO:

The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital in the morning and killed by intracardiac perfusion of a fixative consisting of 2% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.4) and 8% sucrose for 10 min at room temperature.

Sections (50 μm in thickness) were obtained from small pieces of tissue from the corpus callosum, cerebral cortex and the superior lateral corner of the lateral ventricle including the ependyma and subependyma, at the level of the optic chiasma, using a microslicer (DTK-1000, Dosaka). The sections were immersed in a buffer solution containing 0.05 M Tris-maleate buffer, pH 7.4, and 8% sucrose for 3 hrs.

The sections were incubated in lead citrate medium (MAYAHARA et al., 1967) containing 14 ml of 0.2 M Tris-HCl buffer (pH 8.5), 20 ml of 0.1 M sodium β-glycerophosphate, 26 ml of 0.015 M MgSO4, 40 ml of 0.5% lead citrate (K & K Laboratories, Plainview, New York) and 8 g of sucrose for 30 min at 37°C. The pH of the solution was adjusted to 9.4 with 0.1 N NaOH.

Control experiments were performed as follows: 1) sodium β-glycerophosphate substrate was omitted from the incubating medium, 2) tetramizol (10 mM) was added as an inhibitor or, 3) sections were preheated (60°C, 30 min) prior to incubation. The sections were washed briefly in distilled water after incubation and then immersed in 1% osmium tetroxide in veronal acetate buffer (pH 7.4) for 1 hr at 4°C, dehydrated with graded acetone (30%, 50%, 70%, 90%, 99% and 3 times of 100%) and embedded in Epon. Semithin Epon sections (0.5 μm) were cut from the superficial layer of the specimen on a Porter-Blum ultramicrotome and then stained with 0.2% toluidine blue in saturated sodium borate in order to identify whether the cutting surface included the cerebral cortex (VI layer), corpus callosum or the ventricular wall containing the ependyma and subependyma. Ultrathin sections were then cut and stained with saturated uranyl acetate in 50% ethyl alcohol for 15 min. They were examined with a Hitachi HU-12B electron microscope, as were unstained ultrathin sections (Fig. 4).

RESULTS

Many cells in the cerebral cortex, corpus callosum and wall of the lateral ventricle had ALP activity. They were morphologically classified using the criteria outlined in previous investigations (MORI and LEBLOND, 1969b, 1970; PRIVAT and LEBLOND, 1972; SKOFF et al., 1976; PETERS et al., 1976) into astrocytes, oligodendrocytes, nerve cells, subependymal cells and vascular wall cells. Interstitial microglia (MORI and LEBLOND, 1969a) and ependymal cells (MORI, 1966; PETER et al., 1976) did not exhibit ALP activity.

Astrocytes, which had pale nuclei, extended vascular endfeet and a light cytoplasm containing bundles of filaments and other cell organelles, had ALP activity on the plasma membrane of the endfeet facing the vascular basement membrane (Fig. 1), as well as at the junctions between the vascular endfeet (Fig. 2) or processes which contained astrocytic filaments. These junctions were identified as gap junctions between adjacent astrocytic plasma membranes because similar pentalaminar structures to those reported in previous studies (BRIGHTMAN and REESE, 1967, 1969) could be observed in the junction. There were many reactive gap junctions in the neuropil. ALP activity was associated with both intimately apposed membranes of the gap junctions (Fig. 2), whereas astrocytes did not have ALP activity on most of their plasma membrane or inside the cell.
Small cells that had characteristic nuclear features of astrocytes exhibited ALP activity on the inner and outer nuclear membranes, the outer mitochondrial membrane, and part of the plasma membrane (Fig. 3). The cells were identified as immature astrocytes because of small bundles of filaments, a few glycogen granules, many free ribosomes and poorly developed rough endoplasmic reticulum.

In the subependymal plate of the lateral ventricle, subependymal cells exhibited ALP activity on the plasma membrane (Fig. 4). ALP reaction deposits were also
associated with the plasma membrane of various processes appearing between the subependymal cells (Fig. 4). The origin of these processes is not known. No activity was detected in the subependymal cell cytoplasm. While ependymal cells lacked both cytoplasmic and plasma membrane ALP activity. The enzymatic activity was also absent in ependymal cell microvilli and cilia.

In the various control experiments performed, neither the plasma membrane nor the cytoplasm exhibited dark membranes resulting from ALP activity.

DISCUSSION

Although numerous light microscopic studies have been published on ALP activity in the brain, none have examined the activity in the astrocytes. Electron microscope observation showed that astrocytes had ALP on their endfeet plasmalemma facing the vascular basement membrane (MAYAHARA et al., 1967; OVTSCHAROFF, 1973; GOTOW, 1984). These findings are supported in the present study (Fig. 1), although astrocytic plasmalemma facing the subpial basal lamina did not have ALP activity (GOTOW, 1984). The ALP activity on the plasma membrane of astrocytic vascular endfeet may play a role in the transmembrane transport of nutritious substances as has been shown for other tissues (MÖLBRET et al., 1960; CLARK, 1961; REALE, 1962; MIJUTANI and BARNETT, 1965; HUGON and BORGERS, 1968). Thus, astrocytes with vascular endfeet may transport various trophic agents from the vascular lumen through the vascular basement mem-

![Electron micrograph of the ALP reaction in a small cell from the corpus callosum. ALP activity is present in both the inner and outer nuclear membranes (n) and in the outer membrane of some mitochondria (M). Partial plasmalemma (m) also shows the presence of ALP. The cell has characteristic nuclear features of astrocytes (N), and small bundles of filaments (thick arrows) and few glycogen granules (thin arrow). There are small, scattered rough endoplasmic reticulum (ER), mitochondria (M) and many free ribosomes (R). The cell may be identified as an immature astrocyte. ×18,000](image)
brane and ALP-positive endfeet plasmalemma. Similarly ALP in the plasma membrane of immature astrocytes may help in absorbing trophic agents mainly from the surrounding intercellular spaces.

There have been many investigations of gap junctions between astrocytes (Brightman and Reese, 1967, 1969; Tani et al., 1973; Landis and Reese, 1974; Sipe and Moore, 1976; Massa and Mugnaini, 1982). This study demonstrates firstly the existence of ALP at gap junctions between astrocytes although the presence of other enzymes has been demonstrated (Fujimoto et al., 1981; Fujimoto and Ogawa, 1982a, b).
It is not certain yet whether ALP is present at every gap junction between astrocytes and oligodendrocytes or between astrocytes and the outer tongue process of myelin sheaths (Massa and MUGNAINI, 1982), though oligodendrocytes showed ALP activity on their plasmalemma (Mori et al., 1984; Mori and NAGANO, 1985). Similarly, it is not clarified yet whether ALP localized at gap junctions may function to facilitate the passage of ions or trophic molecules between glial cells.

Immature oligodendrocytes possessed ALP in both the cytoplasm (in the nuclear membrane and mitochondrial outer membrane) and the plasma membrane, while in the mature cells, only the plasma membrane did (Mori et al., 1984; Mori and NAGANO, 1985). Interestingly, the change in ALP distribution and cellular maturation in astrocytes was similar to that in oligodendrocytes. Most mature astrocytes demonstrated ALP activity in the endfeet or gap junctions, while immature astrocytes exhibited ALP mainly in the cytoplasm (in the nuclear membrane and mitochondrial outer membrane) and in the plasma membrane as was the case with immature oligodendrocytes.

In the subependyma, ALP was present throughout the entire plasmalemma of the subependymal cells and in various processes between cells. Most of these processes are believed to have originated from neighboring subependymal cells. Since this site is near the lateral ventricle from which the cerebrospinal fluid may percolate through intercellular spaces (BRIGHTMAN and REES, 1969; BRIGHTMAN et al., 1970), ALP may play a role in the transmembrane absorption of nutritious substance from the intercellular space. It is worthwhile noting that subependymal cells, which have generally been believed to be immature cells, did not show cytoplasmic ALP activity, although immature astrocytes and oligodendrocytes usually did (Mori et al., 1984; Mori and NAGANO, 1985). Further investigation is required to resolve this discrepancy.

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