short communications

Plasmalogenase activities in neuronal perikarya, astroglia, and oligodendroglia isolated from bovine brain

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Abstract Plasmalogenase (EC 3.3.2.-, 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine aldehydohydrolase) activities were assayed with the plasmalogens in dispersed myelin as the substrate. The activities were 6.7 μmoles/mg protein per hr in oligodendroglia from white matter, and 1.1 and 0.6 for astroglia and neuronal perikarya from gray matter. Myelin had no plasmalogenase activity. Thus, both the normal catabolism of myelin plasmalogens and the accelerated hydrolysis of plasmalogens in demyelination is probably carried out by oligodendroglial plasmalogenase.

Supplementary key words ethanolamine plasmalogens · phospholipase · myelin · demyelination

Ethanolamine plasmalogens are the major phospholipid component of central nervous system (CNS) white matter and myelin (1). The CNS plasmalogenase (EC 3.3.2.-), the enzyme that hydrolyzes the vinyl ether linkage of the plasmalogen molecule, is concentrated in white matter, but not in myelin (2), and the activity of plasmalogenase parallels myelination (3). Thus, the oligodendroglia may provide the catabolic activity necessary for the turnover of the plasmalogens associated with myelin. In order to test this hypothesis, we assayed plasmalogenase activities in neuronal perikarya, astroglial, and oligodendroglial fractions derived from adult bovine brain.

EXPERIMENTAL PROCEDURES

Tissues

Whole adult bovine brains were obtained from the Coil Meat Packing Co., Columbus, OH. The brains were excised within 30 min of death and immediately placed on ice for transport (30 min). The tissues were kept at 4°C throughout the isolation procedure. White matter and gray matter were carefully separated.

Cell Preparations

The oligodendroglial fractions were prepared from 25–30 g of white matter by Method B of Fewster, Blackstone, and Ihrig (4) using nylon mesh sieves of 760, 452, 333, and 130 μm pore size. Neuronal perikarya and astroglial fractions were prepared from 10–15 g of bovine gray matter by the method of Blomstrand and Hamberger (5, 6). The cell-enriched fractions were washed with 10 volumes of cold Hank's balanced salt solution and a small portion of each fraction was removed for microscopy. The remaining cells were centrifuged at 1,000 *g* for 10 min, then resuspended and dispersed in 50% glycerol–1% sodium bicarbonate 1:1 (v/v). The dispersions were used for plasmalogenase and protein (7) assays.

Plasmalogenase

The source of the added plasmalogens for the assays was myelin, which was isolated and purified from 10–15 g of bovine white matter according to Toews, Horrocks, and King (8). The purified myelin was sonicated in 0.1 M glycylglycine-HCl buffer, pH 7.4, containing 0.05% Tween 20 to give a suspension containing myelin plasmalogens at a concentration of 4.0 mM. A mixture of 1.0 ml of the myelin suspension and 1.0 ml of the dispersed cell frac-

Abbreviation: CNS, central nervous system.

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TABLE 1. Plasmalogenase activity in neuronal perikarya, astroglia, and oligodendroglia isolated from bovine brain

Cell Type (n)	Activity (mean)
Neuronal perikarya (3)	0.38, 0.22, 1.20 (0.60)
Astroglia (2)	1.42, 0.73 (1.08)
Oligodendroglia (3)	6.60, 6.69, 6.87 (6.72)

Results are expressed as μmoles of plasmalogens hydrolyzed/mg protein per hr. Neuronal perikarya and astroglia were prepared from bovine gray matter and the oligodendroglia were prepared from bovine white matter. Cell dispersions were incubated with purified bovine myelin dispersed with Tween 20 and sonication.

tion was incubated at 37°C. After 0, 20, and 40 min, 0.5 ml portions of the incubation mixture were removed and mixed with 2.0 ml of chloroform–methanol 2:1 (v/v). The plasmalogens remaining in the lower phase at each time were quantitated by the iodine method (9). Activities are expressed as μmoles plasmalogen hydrolyzed/mg cell protein per hr.

RESULTS

Purity of cell fractions

The oligodendroglial and astroglial fractions were substantially free of cell debris, contaminating cell types, and nonviable cells as shown by phase contrast microscopy and staining with 0.1% trypan blue. The neuronal perikarya were less intact than the cells in the other fractions. Cross-contamination of cell fractions was not observed, but cannot be ruled out.

Plasmalogenase activities

The oligodendroglia isolated from adult bovine white matter hydrolyzed the plasmalogens of myelin at the rate of 6.72 μmoles/mg protein per hr. This catabolic activity was 10-fold greater than in the neuronal perikarya and 6-fold greater than in the astroglia (**Table 1**). For all cell fractions the assays were linear for 40 min. The plasmalogenase activities with a purified ethanolamine substrate are about 50% higher (10), but the activities with the dispersed myelin substrate are more reproducible. The myelin suspensions had no plasmalogenase activity because blank incubations without the cell dispersions had no loss of plasmalogens. The plasmalogens present in the incubation mixtures were almost entirely from the added myelin and were predominantly ethanolamine plasmalogens.

DISCUSSION

The oligodendroglial cell fraction from white matter was capable of hydrolyzing ethanolamine plasmalogens to a much greater extent than the neuronal perikarya and astroglial fractions from gray matter. Ansell and Spanner (2) reported that CNS white matter contained 10 times more plasmalogenase activity than gray matter. The oligodendroglia isolated from white matter had 10 times the plasmalogenase activity of neuronal perikarya and 6 times the activity of astroglia. Plasmalogenase activities in neuronal and astroglial fractions are probably not due to oligodendroglial contamination because the former cells have a substantial turnover of plasmalogens (11–14).

Increased metabolic activities, required for the development and maintenance of myelin, have been found in the oligodendroglia when compared to neurons and astroglia. Deshmukh, Glynn and Pieringer (15) found that galactosyl diglyceride synthesis was about 20-fold greater in oligodendroglia from rat white matter when compared to neuronal perikarya. Also, the incorporation of fatty acids into lipids occurred at a much faster rate in the oligodendroglia, with the greatest incorporation corresponding to the period of myelination (16), and cerebroside sulfotransferase was 8-fold more active in isolated oligodendroglia than in neurons (17).

The oligodendroglia appear to contain the plasmalogenase enzyme necessary for the normal turnover of the ethanolamine plasmalogens associated with myelin. The rapid turnover of ethanolamine plasmalogens in the CNS (1, 18) cannot be accounted for by phospholipase A_2 activity, since phospholipase A_2 was least active with plasmalogens as the substrate and plasmalogenase has less activity with the deacylated plasmalogen than with the intact plasmalogen (20). The counterpart of phospholipase A_1 for plasmalogens is plasmalogenase (10), which is probably responsible for most of the turnover of plasmalogens in the CNS.

The concentration of plasmalogenase activity within the oligodendroglia is also consistent with an oligodendroglia location for the increased plasmalogenase activities associated with demyelinating conditions. Demyelination of the CNS, as reported by Ansell and Spanner (2) and McMartin, Horrocks, and Koestner (21), was accompanied by activation of plasmalogenase in the areas of demyelination. The evidence presented here suggests that the oligodendroglia are the CNS cells most capable of supplying the increased plasmalogenase activities seen during demyelination of the CNS. It is most probable that the activation of the plasmalogenase activity within the oligodendroglia is a necessary step in the pathogenesis of white matter injury that results in the destruction of myelin and lysis of the oligo-

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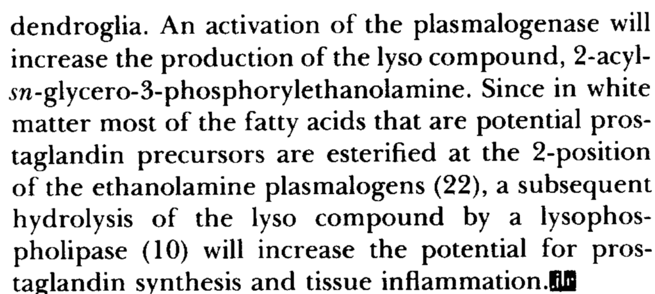
dendroglia. An activation of the plasmalogenase will increase the production of the lyso compound, 2-acyl-*sn*-glycero-3-phosphorylethanolamine. Since in white matter most of the fatty acids that are potential prostaglandin precursors are esterified at the 2-position of the ethanolamine plasmalogens (22), a subsequent hydrolysis of the lyso compound by a lysophospholipase (10) will increase the potential for prostaglandin synthesis and tissue inflammation.

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