The determination of plasmalogenase activity in isolated oligodendroglia from bovine brain white matter

NEIL M. FREEMAN and ERIC M. CAREY
Department of Biochemistry, University of Sheffield, Sheffield S10 2TN, U.K.

Ethanolamine plasmalogen (2-acetyl-1-alk-1'-enyl-sn-glycero-3-phosphoethanolamine) is the major phospholipid component of myelin. Like other phospholipids of the myelin sheath, plasmalogens are continuously being replaced within the myelin membrane. For phospholipids other than ethanolamine phospholipids, this could involve phospholipid-exchange proteins. For ethanolamine phospholipids the mechanism of turnover is unknown, nor the regulation of the biosynthetic and degradative pathways.

The activity of plasmalogenase (2-acetyl-1-alk-1'-enyl-sn-glycero-3-phosphoethanolamine aldehydehydrodrolase, EC 3.3.2.2), which cleaves the vinyl ether bond of plasmalogens, actually increases at the time of myelin membrane deposition in the brain (Ansell, 1971). It has also been reported to be increased in active areas of demyelination within the brain (Ansell & Spanner, 1968). Plasmalogenase activity is probably confined to the myelin-producing oligodendroglial cell, and has low activity in neutrons and astrocytes (Dorman et al., 1977).

The two methods that have been most commonly employed for the determination of plasmalogenase activity require large amounts of protein and lipid substrate and are laborious to perform. They involve extraction of lipid from the assay mixture and determination of the plasmalogen remaining either by reacting the vinyl ether group with an iodine solution (Williams et al., 1962) or by measuring the disappearance of plasmalogens by determination of phospholipid phosphorus of lipid products separated by t.l.c. (D’Amato et al., 1975).

A rapid continuous spectrophotometric assay of plasmalogenase has now been devised that has the sensitivity for use with small amounts of isolated nerve cells and subcellular fractions. The long-chain fatty aldehydes, produced from plasmalogen vinyl ether bond cleavage, are converted into the corresponding alcohols by added liver alcohol dehydrogenase, with the concomitant oxidation of NADH. Liver alcohol dehydrogenase has activity towards longer-chain aldehydes, though less than with acetaldehyde.

Oligodendroglia were isolated from white matter dissected from the cerebral cortex of bovine brain. Cells were disrupted by sonication for 10 s at 80 W in a Branson sonicator. Purified ethanolamine plasmalogen was dispersed in 100 mM-Tris/HCl buffer (pH 7.4)/0.5% Triton X-100, to a final concentration of 2.4 mM, by sonication at 80 W until a clear solution was

We are grateful to the Medical Research Council and the Sir Halley Stewart Trust for financial support. W. J. is a Sir Halley Stewart Research Professor.


The results presented are calculated by dividing the radioactivity incorporated into precipitated protein in the presence of inhibitor by the radioactivity incorporated in the absence of inhibitor.

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<th>[L-Aminocyclopentane-1-carboxylic acid] (mm)</th>
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Enzyme-linked immunoadsorbent assays for myelin basic protein and antibodies to myelin basic protein

N. P. GROOME
Department of Biology, Oxford Polytechnic, Headington, Oxford OX3 0BP, U.K.

The effects of adenosine 3':5'-cyclic monophosphate and selected phosphodiesterase inhibitors on incorporation of L-[^14C]lysine into protein in rat brain and liver slices

SHEILA A. STANDARD, PHILIP W. BEESLEY* and ALAN MACKENZIE
Department of Biochemistry, Royal Holloway College, Egham Hill, Egham, Surrey TW20 0EX, U.K.

Although cyclic AMP is reported to inhibit protein synthesis in several tissues, including liver and muscle (Blochm & Akhtar, 1979), little information is available about the effects of this compound on total protein synthesis in brain. However, Boksa (1976) report that the phosphodiesterase inhibitor pentoxifylline decreases incorporation of precursor amino acid into protein in brain cell suspensions. In view of interest in the therapeutic potential of phosphodiesterase inhibitors (Amer & Kreighbaum, 1975), we have compared the effects of cyclic AMP itself and of selected phosphodiesterase inhibitors on incorporation of L-[^14C]lysine into protein of rat brain and liver slices. We have also measured the tissue contents of cyclic AMP and ATP.

Brain slices (0.3 mm thickness) were prepared from female Wistar rats (200-250 g body wt.) and preincubated for 1 h to decrease endogenous cyclic nucleotide contents. Slices were subsequently incubated for 1 h by the method of Dunlop et al. (1975) in the presence of 0.25 µCi of L-[^14C]lysine (340 nmol/mCi) together with drug additives. After the incubation, slices were washed three times in 170 mm-NaCl at ice temperature (1 litre each; 2 s/wash) and sonicated in 0.6 M-HClO₄ for 90 s at 0°C. Acid-soluble and -insoluble radioactivity were measured essentially by the method of Hill et al. (1975), except that trichloroacetic acid was replaced by HClO₄. The remaining acid-soluble extract was neutralized with K₂CO₃ and freeze-dried. The cyclic AMP and ATP contents of the der-decayed extract (in 50 mm-Tris/HCl, pH 7.5) were determined by the protein-binding assay of Gilman (1970) and the NAD-linked assay of Bücher (1947) respectively. Protein was determined by the method of Bradford (1976).

Liver slices (0.5 mm thickness) were incubated and processed as for brain slices, except that the preincubation step was omitted and the incubation medium was Krebs-Ringer phosphate buffer (134 mm-NaCl, 5.4 mm-KCl, 10.4 mm-Na₂HPO₄, 1.3 mm-KH₂PO₄, 1.34 mm-MgSO₄, 1.34 mm-CaCl₂ and 13 mm-D-glucose, pH 7.4).

The results (Table I) indicate that both exogenous cyclic AMP (2 mm) nor dibutylycyclic AMP (2 mm) has any Plasmalogenase activity has been reported to be influenced by bivalent cations (Ansell & Spanner, 1965). However, the addition of 10 mm-MgCl₂ did not increase the rate of reaction. With plasmalogen dispersed in 0.5% sodium deoxycholate, Mg⁺ caused precipitation of the detergent.

In terms of substrate specificity, the plasmalogenase was only half as active towards lysoplasmalogen (after removal of the acyl group) compared with the acylalkenyl-plasmalogen in isolated glial homogenates. From work with acetone-extracted brain powders, Ansell & Spanner (1965) also suggested that the alkyl group is removed first, followed by the acyl group, by phospholipase A.

Plasmalogenase has been reported to be present in microsomal membranes and mitochondria from whole brain (Ansell & Spanner, 1968) but absent from myelin. The assay method described in the present communication has enabled the subcellular distribution of plasmalogenase in a number of membrane fractions derived from isolated oligodendroglia to be determined, and the use of plasmalogenase as an enzyme 'marker' for oligodendroglia.