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Plasmalogenase Activity in Normal and Demyelinating Tissue of the Central Nervous System

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1. The plasmalogenase activity of brain was found to be associated with the white matter but was absent from myelin fractions. 2. Increased enzyme activity was found in demyelinating spinal cords from vitamin B_{12}-deficient monkeys and in white matter from a patient with multiple sclerosis.

Phospholipases that attack the intact choline- and ethanolamine-containing phospholipids are known to be present in mammalian brain tissue. These include a phospholipase A (EC 3.1.1.4), which attacks phosphatidylcholine and phosphatidylethanolamine (Gallai-Hatchard, Magee, Thompson & Webster, 1962), a sphingomyelinase that attacks sphingomyelin (Barnholz, Roitman & Gatt, 1966) and possibly a phospholipase C (EC 3.1.4.3) active towards ethanolamine phospholipids (Ansell & Spanner, 1966). An Mg^{2+}-dependent enzyme (plasmalogenase) specific for the vinyl ether linkage of ethanolamine plasmalogen (2-acyl-1-alkenylglycerylphosphorylethanolamine) has been described by Ansell & Spanner (1965).

In the brain, ethanolamine plasmalogen is found in highest concentration in white matter. In many demyelinating diseases of the central nervous system, in which loss of lipid from white matter occurs, loss of ethanolamine plasmalogen is considerable. For example, the amount in the white matter of spinal cord from lambs with swayback disease was found to be 70% of the normal (Davison & Oxberry, 1966). In multiple sclerosis, the ethanolamine plasmalogen content of plaque tissue is 19–67% of normal values (Davison & Wajda, 1962; Cumings, 1963; Gerstl, Tavaststjerna, Hayman, Eng & Smith, 1965). It seemed relevant therefore to investigate the activity of the plasmalogenase in both grey and white matter and also in demyelinating nervous tissue and to compare these results with those obtained from normal tissue.

MATERIALS AND METHODS

Chemicals were of A.R. quality where possible.

Ethanolamine plasmalogen was prepared essentially as described by Ansell & Spanner (1963). Since this material was occasionally found to be contaminated with another phosphorus-containing component the method was modified as follows. After the saponification step, chloroform-soluble material containing approx. 15μg. atoms of phosphorus/ml. was applied to a column of silicic acid (Bio-Rad Laboratories, Richmond, Calif., U.S.A.) at the rate of approx. 15μg.-atoms of phosphorus/g. of absorbent. The column was then washed through with 10ml. of chloroform/g. of silicic acid, and the ethanolamine plasmalogen was eluted with 20ml. of methanol–chloroform (1:9, v/v). This product had molar proportions phosphorus:acyl ester:vinyl ether 1·00:1·01:0·99. It gave a single spot on thin-layer chromatography on silica gel G with chloroform–methanol–water (65:35:4, by vol.) as solvent. After mild alkaline and acid hydrolysis (Dawson, Hemington & Davenport, 1962) it yielded only one water-soluble compound, glycerylphosphorylethanolamine.

The brain and spinal cord were removed from experimental animals as soon as possible after death. The rats were killed by decapitation after brief anaesthesia with ether, and monkeys by an overdose of Nembutal. Ox brain was obtained at a local slaughterhouse shortly after the death of the animals and cat grey and white matter were removed from a decerebrate animal that was under Fluothane anaesthesia. The human material was removed during post-mortem examination within 12hr. of death. The normal sample of human white tissue was from a male aged 54 years who died from cancer of the oesophagus. The multiple-sclerosis patient was a female aged 38 years who died from bronchopneumonia.

Subcellular fractions of rat brain were prepared as described by Ansell & Spanner (1967). The acetone-dried powders were prepared and extracted with glycerol–sodium hydrogen carbonate, and the extracts were dialysed overnight as described by Ansell & Spanner (1965). Incubations were carried out in glycylglycine buffer–sodium deoxycholate in the presence of $MgCl_2$ (Ansell & Spanner, 1965). Vinyl ether groups in the plasmalogen were determined by the method of Gottfried & Rapport (1962).

Stability of the enzyme preparation. Acetone-dried powders of brain tissue retained their extractable activity for 24hr. after preparation if they were kept at 4° in a vacuum desiccator. In previous experiments (Ansell & Spanner, 1965) the acetone-dried powders of the whole brain were prepared a few minutes after the death of the animal. This was also true for the monkey spinal cord and

Table 1. *Activity of plasmalogenase in grey and white matter and whole spinal cord*

Numbers in parentheses denote the numbers of determinations. Results are given as means ± S.D.

Species	Plasmalogenase activity (μmoles/hr./g. fresh wt.) Cerebral grey matter	Cerebral white matter	Spinal cord
Cat	0·13 ± 0·003 (7)	1·6 ± 0·2 (7)	0·73 (2)
Ox	0·05	1·13	
Monkey			1·18 ± 0·09 (5)
Human		0·90	

Table 2. *Activity of plasmalogenase in the subcellular fractions and myelin of rat brain*

Subcellular fractions of whole rat brain were prepared as described by Ansell & Spanner (1967). Numbers in parentheses denote the numbers of determinations. Results are given as means ± S.D.

	Plasmalogenase activity (μmoles/hr./g. fresh wt.)
Whole brain	2·42 ± 0·4 (9)
Microsomes	0·90 ± 0·2 (16)
Mitochondria	1·53 ± 0·1 (12)
Supernatant	0 (2)
Small myelin	0·07 (2)
Large myelin	0·01 (2)

the samples from cat brain in the present experiments. In one instance where the monkey brain was stored for 24hr. at −40° before the preparation of the acetone-dried powders there was no difference in the activity. Ox brain obtained within 10min. of death was transported in an ice-packed vacuum flask, and the acetone-dried powder was prepared within 2hr. Acetone-dried powders of the human samples were prepared within 12hr. of death; during this time the cadavers had been kept at 4°. Experiment showed that there was a loss of about 5% of the original activity if tissue was kept at 0° for about 12hr. before extraction.

RESULTS

Brain and spinal cord. The activity of plasmalogenase in acetone-dried powders of whole rat brain was 2·42 ± 0·4 μmoles/hr./g. of fresh brain (mean ± S.D. of nine observations). Since it is difficult to obtain adequate amounts of grey and white tissue from rat brain, the distribution of enzyme activity between these two tissues was determined on samples obtained from the cat. In white matter, the activity was 1·6 μmoles/hr./g. of fresh tissue and in grey matter 0·13 μmole/hr./g. of fresh tissue. The greatest activity was therefore found in the part of the brain that contains the highest concentration of substrate. The activity of white matter from ox and human brains was also investigated and found to be of the same order as that of cat (Table 1). The plasmalogenase activity of monkey spinal cord was also similar (Table 1).

Table 3. *Enzyme activity in normal and demyelinating tissue*

The human tissue was obtained at autopsy within 12hr. of death. Spinal cord was taken from normal and vitamin B_{12}-deficient monkeys. Numbers in parentheses denote the numbers of determinations. Results are given as means ± S.D.

	Plasmalogenase activity (μmoles/hr./g. fresh wt.) Normal tissue	Demyelinating tissue
Human, corpus callosum	0·90	1·90
Human, occipital white matter	—	1·81
Monkey, thoracic spinal cord	1·18 ± 0·09 (5)	3·76 ± 0·56 (5)

Subcellular fractions of rat brain. Since the enzyme activity was found almost exclusively in the white matter, subcellular fractions of rat brain were studied. The activity appeared to be confined to the mitochondrial and microsomal fractions (Table 2). The enzyme was not found in the myelin or in the supernatant fraction.

Enzyme activity in demyelinating tissue. Samples of white matter from two examples of demyelinating tissue were examined and compared with normal tissue.

White matter from the frontal lobe and corpus callosum of a patient with multiple sclerosis was compared with that from a patient showing no brain damage. The plaques from the occipital region were extremely large and resembled those seen in cases of Schilder's disease. Even in the old plaques there was considerable active demyelination at their margins. The phospholipid content of the plaque tissue was considerably lower than that of the normal white matter (22·4 μmoles/g. fresh wt. compared with 79·3 μmoles/g. fresh wt.). Of this loss, 30% was due to the loss of ethanolamine plasmalogen. The enzyme activity of the diseased tissue was twice that of the normal white matter on a fresh-weight basis (Table 3).

It has been known for many years that vitamin B_{12} deficiency leads to a degeneration of the spinal cord. This degeneration is most marked in the dorsal columns of the thoracic segments of the cord (Greenfield & Meyer, 1963). For this reason, white matter from the thoracic spinal cord of vitamin B_{12}-deficient monkeys was examined for plasmalogenase activity. The results from these animals were compared with those from normal animals. As can be seen in Table 3, the activity in the vitamin

B_{12}-deficient monkeys was three times that in the normal animals. The difference between the two sets of animals was highly significant ($P < 0.001$, Student's t test). Histologically, the cords from the deficient animals showed characteristic demyelination of the dorsal columns in the thoracic segments. In some of the animals this had extended to the cervical segments. The lumbar region was normal.

DISCUSSION

These results show that the greatest plasmalogenase activity in rat brain was in the mitochondrial and microsomal fractions. Since, in addition, the activity was almost exclusively located in white matter, this finding would indicate that the enzyme is probably located in the glial cells rather than the nerve cell bodies.

Some enzymes that have been demonstrated in glial cells of white matter are lactate dehydrogenase, $NADH_2$–tetrazolium reductase and adenosine triphosphatase. These three enzymes showed increased activity in plaque tissue from early multiple-sclerotic lesions (Ibrahim & Adams, 1963). The neutral proteinase activity of sciatic nerve undergoing demyelination consequent on organophosphorus poisoning showed a significant increase (Porcellati, Millo & Manocchio, 1961). In the present study an increased plasmalogenase activity was found in the plaque tissue of white matter from a patient with multiple sclerosis (Table 3). The plaques studied were well established and the increased enzyme activity was therefore unlikely to be due to increased cell content. A considerable increase in the activity of this enzyme was also observed in demyelinating spinal cord tissue from vitamin B_{12}-deficient monkeys.

Plasmalogenase, like phospholipase A, hydrolyses an intact phospholipid to produce a lysophospholipid. Lysolecithin has been demonstrated to be a myelinolytic agent (Webster, 1957; Thompson, 1964), and it is possible that lysophosphatidylethanolamine may function in a similar manner. It is, however, extremely difficult to prove whether the increased enzyme activity results in demyelination or whether it is merely a response to the demyelination process.

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