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On the Activity of Brain Phospholipase A_2 towards Specifically Labelled Glycerophospholipids during Subacute Sclerosing Panencephalitis

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Summary. 1,2-Diacyl-sn-glycero-3-phosphorylcholine, -ethanolamine and -serine, specifically labelled with different fatty acids at either the 1- or 2-position, were prepared enzymatically using the acyltransferase system of rat liver microsomes. The substrates were subjected to hydrolysis by phospholipase A_2 obtained from brain tissue of a normal and a case of subacute sclerosing panencephalitis (SSPE). In the pathological tissue and increase of approx. 50% in phospholipase A_2 activity could be observed in comparison to that from the control brain for all investigated substrates. Experiments with phosphatidylethanolamines, specifically labelled with different fatty acids in the 2-position, revealed that the phospholipase A_2 activity of the SSPE brain tissue was enhanced by about 50% when compared to the control brain regardless of the fatty acid constituent at the 2-position of the substrates.

Key words: Subacute Sclerosing Panencephalitis — 1,2-Diacyl-Glycerophosphatides — Fatty Acids — Phospholipase A_2.

Zusammenfassung. Am Hirngewebe eines Kindes mit subakuter sklerosierender Panencephalitis (SSPE) wurden folgende Untersuchungen durchgeführt: 1,2-Diacyl-sn-glycerin-3-phosphorylcholin, -äthanolamin und -serin, spezifisch markiert mit verschiedenen Fettsäuren entweder an der 1- oder 2-Stellung des Glycerins, wurden mit Hilfe der Acyltransferase aus Mikrosomen der Rattenleber enzymatisch dargestellt. Die Substrate wurden der Hydrolyse durch eine Phospholipase A_2 unterworfen, welche aus normalem und pathologischem (SSPE) Gehirngewebe isoliert worden war. Im pathologischen Gehirngewebe wurde ein Anstieg der Enzymaktivität für alle untersuchten Substrate von etwa 50% festgestellt. Versuche mit Phosphatidyläthanolaminen, welche in der 2-Stellung des Glycerins mit verschiedenen Fettsäuren markiert waren, zeigten, daß die Phospholipase A_2-Aktivität im erkrankten im Vergleich zu dem Kontrollgewebe, unabhängig von der Art der Fettsäure in der 2-Stellung des Phosphatidyläthanolamins, um etwa die Hälfte erhöht war.

Schlüsselwörter: Subakute sklerosierende Panencephalitis — 1,2-Diacyl-Glycerinphosphatide — Fettsäuren — Phospholipase A_2.

Abbreviations. SSPE = subacute sclerosing panencephalitis; CSF = cerebrospinal fluid.

Decreases of lipids and changes in the fatty acid pattern of individual lipid classes in whole brain, gray and white matter, and in purified myelin have been observed by several investigators during demyelinating diseases [1, 13, 19, 26, 27, 32].

Chemical studies of subacute sclerosing panencephalitis by Norton *et al.* [17] revealed severe demyelination and an increased water content, very high cholesterol ester, decreased galactolipid, proteolipid protein and ethanolamine plas-

malogen in the white matter of the diseased tissue. The analyses of Cumings [6], Szliwowski and Cumings [20] and Wender [23] of some cases of SSPE showed similar but less severe chemical changes in the white matter than the case presented by Norton *et al.* [17].

Very few information is available on the activity of lipid hydrolyzing enzymes during demyelination of the peripheral and central nervous system. Phospholipase A_1 and A_2 hydrolyze glycerophosphatides at either the 1- or 2-position giving rise to the corresponding 2-acyl- or 1-acyl-lysophospholipids. Webster [24] presented experimental evidence for substantially enhanced phospholipase A_1 and A_2 activity in the peripheral nervous system undergoing Wallerian degeneration, beginning 2 days after transection of the rat sciatic nerve and rising to eight times nor al values by the 2nd week. Investigating the phospholipid metabolism in experimental allergic encephalomyelitis Woelk and Kanig [30] and Woelk *et al.* [31] showed an appreciable increase of brain phospholipase A_1-activity towards various glycerophospholipids and of phospholipase A_2-activity towards specifically labelled 1,2-diacyl-, 1-alk-1'-enyl-2-acyl- and 1-alkyl-2-acyl-sn-glycero-3-phosphorylcholine in the acute stage of the demyelinative disorder. With multiple sclerosis material an increase of approximately 50% in phospholipase A_2-activity could be observed for a number of phospholipid substrates (Woelk and Peiler-Ichikawa [32]). In an effort to elucidate some biochemical aspects of the demyelinating process we felt it necessary to investigate the activity of phospholipase A_2 against various phospholipid substrates during subacute sclerosing panencephalitis.

Experimental Procedures

Brain Tissue. The normal human brain tissue was obtained from the frontal lobes of a male child of 14 years who died by accident. The brain material was subjected to macroscopic and light microscopic examination and showed no pathological changes. The SSPE tissue was taken after autopsy from the frontal lobe of both cerebral hemispheres of a male child of 15 years, with the following case report: The boy was 14 years old on his first admission to our pediatric department. His family was of modest social circumstances. Both parents, two brothers and four sisters were all healthy. He was born after a normal pregnancy, his development had been normal and he was otherwise healthy during childhood. In particular he had never been sick with the measles, at least there is no information on this point. He went to primary school at the age of 7, but after the second grade it was necessary to send him to a special school because of his feeblemindedness. At age 10 he was placed in an orphanage because his parents were unable to take further care of him. In the spring of 1973, at the age of 13, the nurses noticed some behavioral changes (e.g. he would go to school wearing two different shoes, his pyjama or even naked) deterioration of mental capacity and onset of cerebral fits. He was emotionally unstable, laughed or cried without real cause and at times he sat in a corner staring at the wall for hours. During clinical observation in september 1974 he was given anticonvulsive drugs. On october 19, 1974, he was again admitted to hospital because of further deterioration in his condition. Neurologically he showed a weakness of conjugate eye movement, cortical visual disturbances, disturbance of coordination and of speech. His mental condition had gotten worse. During October and November 1974 he became totally bedridden and there was increased clouding of consciousness. He now showed the onset of extrapyramidal symptoms such as tremor, rigidity and ataxia. The further course was characterized by coma, decerebral ridigity, cerebral fever. The patient died on december 2, one year after onset of the initial symptoms. The clinical diagnosis of SSPE was confirmed by 1. a typical EEG pattern with periodic sharp wave complexes on both sides; 2. a pathological globulin fraction of CSF with an increase of γ-globulin to 39.2% and of IgG to 35 mg-%; 3. increase of the antibody titer against measles in serum and CSF; 4. positive reactions against

SSPE virus by fluorescence antibody technique (Dr. M. Y. Käckell, Viruslaboratorium, Universitäts-Kinderklinik, 34 Göttingen, Humboldtallee 38).

Substrates. The glycerophosphatides, listed in the Table 1, specifically labelled at either the 1- or 2-position with different fatty acids were synthesized from the corresponding 1-monoacyl- or 2-monoacyl derivative and ^{14}C-labelled fatty acids using the rat liver microsomal acylating system (Robertson and Lands [18]) as described in detail elsewhere (Woelk and Porcellati [33]). Purification of the substrates was achieved by column chromatography on silicic acid (Debuch [7]) or on Florisil (Etzrodt and Debuch [9]). Hydrolysis of the phosphatides, labelled at the 1-position, by phospholipase A_2 from Crotalus atrox showed that 99% of the radioactivity of the 1,2-diacyl-glycerophosphatides was recovered in the lyso compounds, indicating that specific incorporation of the labelled fatty acid into the 1-position of the lyso derivatives had occurred. Hydrolysis of the 1,2-diacyl-sn-glycero-3-phosphorylethanolamine, labelled at the 2-position with different fatty acids, by the same enzyme, revealed that 98% of the radioactiv ity was recovered in the fatty acids released from the substrates, indicating that specific incorporation of the fatty acids into the 2-position had occurred.

Enzyme. Acetone-dried tissue powder was obtained from human cerebral cortex as reported previously [28] and the partially purified phospholipase A_2 prepared according to Cooper and Webster [5].

Incubation. The incubation procedure, chromatographic separation and isolation of the reaction products and radioactivity measurements were as previously described by Woelk and Porcellati [33].

Analytical. Phospholipid P was determined by a modified (Debuch *et al.* [8]) procedure of Bartlett [3], plasmologen (as dimethylacetal) according to Feulgen *et al.* [10] as modified by Klenk and Debuch [14]. Protein was determined according to Lowry *et al.* [15] with crystalline bovine serum albumin as a standard.

Results and Discussion

Various 1,2-diacyl-glycerophosphatides, specifically labelled with different fatty acids at either the 1- or 2-position were prepared enzymatically using the acyltransferase system of rat liver microsomes (Table 1) and the compounds subjected to hydrolysis by phospholipase A_2 obtained from normal and SSPE-brain tissue (Table 2 and 3).

In the diseased tissue an increase of approx. 50% in phospholipase A_2 activity could be observed in comparison to that from the control brain for all investigated substrates (Table 2 and 3). Phospholipase A_2 obtained from normal brain and from that afflicted with the demyelinating disease had the highest affinity for phosphatidylcholine and the lowest one for phosphatidylserine, phosphatidylethanolamine being cleaved at an intermediate rate (Table 2).

Table 1. Specifically labelled glycerophosphatide preparations

Glycerophosphatide	Specific activity (nCi/μmol)
1-[^{14}C] Stearoyl-2-acyl-sn-glycero-3-phosphorylcholine	45.6
1-[^{14}C] Stearoyl-2-acyl-sn-glycero-3-phosphorylethanolamine	58.1
1-[^{14}C] Stearoyl-2-acyl-sn-glycero-3-phosphorylserine	35.9
2-[^{14}C] Stearoyl-1-acyl-sn-glycero-3-phosphorylethanolamine	32.8
2-[^{14}C] Oleoyl-1-acyl-sn-glycero-3-phosphorylethanolamine	50.4
2-[^{14}C] Linoleoyl-1-acyl-sn-glycero-3-phosphorylethanolamine	28.6
2-[^{14}C] Linolenoyl-1-acyl-sn-glycero-3-phosphorylethanolamine	25.3

Table 2. Phospholipase A_2-activity of human cerebral cortex against different 1,2-diacyl-glycerophosphatides in normal and demyelinating conditions

Substrate	Normal	SSPE	Difference percentage
1-[^{14}C] Stearoyl-2-acyl-sn-glycero-3-phosphoryl-choline	148.4	223.2	+ 50.6
1-[^{14}C] Stearoyl-2-acyl-sn-glycero-3-phosphoryl-ethanolamine	102.3	157.5	+ 54.2
1-[^{14}C] Stearoyl-2-acyl-sn-glycero-3-phosphoryl-serin	95.6	145.1	+ 52.1

Results are expressed as nmol substrate hydrolyzed $\times mg^{-1}$ protein $\times h^{-1}$. For details see Experimental section.

Table 3. The effect of unsaturation of phosphatidylethanolamines on the phospholipase A_2-activity of human cerebral cortex in normal and demyelinating conditions

Substrate	Normal	SSPE	Difference percentage
2-[^{14}C] Stearoyl-1-acyl sn glycero-3-phosphoryl-ethanolamine	50.3	77.1	+ 53.4
2-[^{14}]C Oleoyl-1-acyl-sn-glycero-3-phosphoryl-ethanolamine	98.4	147.7	+ 50.1
2-[^{14}C] Linoleoyl-1-acyl-sn-glycero-3-phosphoryl-ethanolamine	87.5	129.0	+ 47.5
2-[^{14}C] Linolenoyl-1-acyl-sn-glycero-3-phosphoryl-ethanolamine	71.2	107.0	+ 50.3

Results are expressed as nmol substrate hydrolyzed $\times mg^{-1}$ protein $\times h^{-1}$. For details see Experimental section.

As a subsequent step the hydrolysis rate of 1,2-diacyl-sn-glycero-3-phosphorylethanolamines, specifically labelled with different fatty acids in the 2-position, by the phospholipase A_2 from normal and pathological human cerebral cortex was measured. As can be seen from the Table 3 oleic acid was removed most and stearic acid less actively from the substrates by the enzyme obtained from both the normal and the diseased tissue. Phospholipase A_2 activity of the SSPE brain tissue was enhanced by about 50% when compared to the control brain regardless of the fatty acid constituent at the 2-position of the ethanolamine phosphoglycerides (Table 3).

The occurrence, properties and subcellular distribution of phospholipases A_1 and A_2 of brain tissue have been the subject of several investigations [4, 11, 12, 25, 28, 33]. Recently an extensive work has appeared on the distribution of these enzymic activities in rat brain subcellular particles, which indicates that phospholipase A_1 is almost exclusively located in microsomes, whereas phospholipase

A_2 activity predominates in mitochondria (Woelk and Porcellati [33]). Pronounced differences in the enzyme activities of phospholipase A_1 and A_2 were found in neurons and glial cells, phospholipase A_1 activity being 8-fold and A_2 activity 5-fold higher in neurons than in glial cells (Woelk *et al.* [29]). Neuronal and glial phospholipase A_1 had optimal activities at pH 7.2 and 5.4 respectively, whereas phospholipase A_2 activities in neurons and glial cells were optimal at pH 5.4 and 8.0, respectively [29]. A phospholipase A_2 with a pH optimum of 5.0 was extracted by Cooper and Webster [5] from acetone-dried tissue powder obtained from human cerebral cortex, and the enzyme partially purified by heat treatment and gel filtration on Sephadex. It may be supposed from our previous studies on phospholipase A activities in neurons and glial cells that the enzyme described by Cooper and Webster [5] is of neuronal origin. Investigating the substrate specificity of the acidic phospholipase A_2 Cooper and Webster [5] found that 2-[^{14}C]-linoleoyl lecithin and 2-[^{14}C]oleoyl lecithin were hydrolyzed most actively and at similar rates, while 2-[^{14}C]palmitoyl lecithin was split about half as fast. Similar results were obtained in the present work for the phospholipase A_2 catalyzed hydrolysis of phosphatidylethanolamines labelled with different fatty acids at the 2-position (Table 3). Appreciable differences were observed between the mitochondrial phospholipase A_2 and the corresponding enzyme purified from cerebral cortex. Waite and Sisson [22] presented experimental evidence for a higher affinity of the mitochondrial phospholipase A_2 for phosphatidylethanolamine and -serine than for phosphatidylcholine, whereas phosphatidylcholine was hydrolyzed most and phosphatidylserine less extensively by the enzyme purified from cerebral cortex (Table 2). Furthermore phospholipase A_2 obtained from cerebral cortex showed similar hydrolysis rates for oleic and linoleic acid, esterified at the 2-position of phosphatidylethanolamines (Table 3) whereas the enzyme from rat liver mitochondria released linoleic acid more actively from the substrate than oleic acid.

Only little is known on enzymes involved in the demyelinating and remyelinating process. Ansell and Spanner [2] described raised activities of plasmalogenase, cleaving the vinyl-ether bond of plasmalogens in demyelinating spinal cord from B_{12}-deficient monkeys and in plaque tissue from a case of multiple sclerosis. Vasan and Bachhawat [21] and Maggio *et al.* [16] observed an increase in the activity of arylsulfatase A, regulating the concentration of sulfatides, during demyelination of the central nervous system. Most recently Woelk and Kanig [30] and Woelk *et al.* [31] got evidence for an elevated phospholipase A_1 and A_2-activity during the acute stage of experimental allergic encephalomyelitis, suggesting that this demyelinating disorder is principally characterized by an enhanced phospholipid turnover accompanied by active remyelination preventing the accumulation of greater quantities of lyso-phospholipids. It is probable that the depletion of phospholipid and the appearance of lyso-phosphatides during multiple sclerosis [26,27] and SSPE [6,17,20,23] is caused by an increase in the activities of phospholipase A. Experiments are in progress to inhibit the action of phospholipase A_1 and A_2 by glycerophospholipid analogs.

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References

1. Alling, C., Vanier, M. T., Svennerholm, L.: Lipid alterations in apparently normal white matter in multiple sclerosis. Brain Res. **35**, 325—336 (1971)
2. Ansell, G. B., Spanner, S.: Plasmalogenase activity in normal and demyelinating tissue of the central nervous system. Biolchem. J. **108**, 207—209 (1968)
3. Bartlett, G. R.: Phosphorus assay in column chromatography. J. biol. Chem. **234**, 466—468 (1959)
4. Bazán, N. G., Jr.: Phospholipase A_1 and A_2 in brain subcellular fractions. Acta physiol. lat.-amer. **21**, 101—106 (1971)
5. Cooper, M. F., Webster, G. R.: On the phospholipase A_2 activity of human cerebral cortex. J. Neurochem. **19**, 333—340 (1972)
6. Cumings, J. N.: Lipid Chemistry in subacute leucoencephalitis. In: L. Van Bogaert, J. Radermecker, J. Hozay and A. Lowenthal. Eds.: Encephalitides, p. 638. Amsterdam: Elsevier Publishers, 1961
7. Debuch, H.: Über die Bildung der Plasmalogene zur Zeit der Myelinisierung bei der Ratte. Hoppe-Seylers Z. physiol. Chem. **338**, 1—9 (1964)
8. Debuch, H., Mertens, W., Winterfeld, M.: Placenta-Lipoide des Menschen während der Schwangerschaft. I. Quantitative Bestimmung der Phosphatide mit Hilfe einer zweidimensionalen dünnschichtchromatographischen Methode. Hoppe-Seylers Z. physiol. Chem. **349**, 896—902 (1968)
9. Etzrod, A., Debuch, H.: Über den Einbau von 1-[^{14}C] Acetat in die Fettsäuren und Aldehyde der äthanolaminhaltigen Phospholipoide des Gehirns junger Ratten. Hoppe-Seylers Z. physiol. Chem. **351**, 603—612 (1970)
10. Feulgen, R., Boguth, W., Andresen, G.: Quantitative Bestimmung der Acetalphosphatide (Plasmalogen) im Serum unter Berücksichtigung des „Waelsch-Effektes". Hoppe-Seylers Z. physiol. Chem. **287**, 90—108 (1951)
11. Gallai-Hatchard, J., Magee, W. L., Thompson, R. H. S., Webster, G. R.: The formation of lysophosphatides from Di-acyl phosphatides by brain preparations. J. Neurochem. **9**, 545—554 (1962)
12. Gatt, S.: Purification and properties of phospholipase A_1 from rat and calf brain. Biochim. biophys. Acta (Amst.) **159**, 304—316 (1968)
13. Gerstl, B., Kahnke, M. J., Smith, J. K., Tavaststjerna, M. G., Hayman, R. B.: Brain lipids in multiple sclerosis and other diseases. Brain **84**, 310—319 (1961)
14. Klenk, E., Debuch, H.: Progress in the chemistry of fats and other lipids, R. T. Holman, W. O. Lundberg and I. Malkin, eds., Vol. 6, pp. 3—29. London: Pergamon Press 1963
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the folin phenol reagent. J. biol. Chem. **193**, 265—275 (1951)
16. Maggio, B., Maccioni, H. J., Cumar, F. A.: Arylsulfatase A (EC 3.1.6.1) activity in rat central nervous system during experimental allergic encephalomyelitis. J. Neurochem. **20**, 503—510 (1973)
17. Norton, W. T., Poduslo, S. E., Suzuki, K.: Subacute sclerosing leucoencephalitis. II. Chemical studies including abnormal myelin and an abnormal ganglioside pattern. J. neuropath. exp. Neurol. **25**, 582—597 (1966)
18. Robertson, A. F., Lands, W. E. M.: Positional specificities in phospholipid hydrolyses. Biochemistry **1**, 804—810 (1962)
19. Ställberg-Stenhagen, S., Svennerholm, L.: Fatty acid comparison of human brain sphingomyelins: normal variation with age and changes during myelin disorder. J. Lipid Res. **6**, 146—155 (1965)
20. Szliwowski, H. B., Cumings, J. N.: Les lipides cérébraux dans l'encéphalite subaiguë. Acta neurol. belg. **61**, 153—161 (1961)
21. Vasan, N. S., Bachhawat, B. K.: Enzymic studies on sulphatide metabolism in different stages of experimental allergic encephalomyelitis. J. Neurochem. **18**, 1853—1859 (1971)
22. Waite, M., Sisson, P.: Partial purification and characterization of the phospholipase A_2 from rat liver mitochondria. Biochemistry **10**, 2377—2383 (1971)

23. Wender, M., Contribution to the study of subacute sclerosing leucoencephalitis with particular regard to the problem of lipid chemistry in the brain. Psychiat. et Neurol. (Basel) **141**, 381 (1961)
24. Webster, G. R.: Phospholipase A activities in normal and sectioned rat sciatic nerve. J. Neurochem. **21**, 873—876 (1971)
25. Webster, G. R., Cooper, M.: On the site of action of phosphatide acyl-hydrolase activity of rat brain homogenates on lecithin. J. Neurochem. **15**, 795—802 (1968)
26. Woelk, H., Borri, P.: Glycerinphosphatide und Sphingolipide der normalen weißen Substanz bei der Multiplen Sklerose. Z. Neurol. **205**, 243—256 (1973)
27. Woelk, H., Borri, P.: Lipid and fatty acid composition of myelin purified from normal and MS brains. Europ. Neurol. **10**, 250—260 (1973)
28. Woelk, H., Fürniss, H., Debuch, H.: Enzymkinetische Untersuchungen einer Phospholipase A_1 dargestellt aus Menschengehirn. Hoppe-Seylers Z. physiol. Chem. **353**, 1111—1119 (1972)
29. Woelk, H., Goracci, G., Gaiti, A., Porcellati, G.: Phospholipase A_1 and A_2 activites of neuronal and glial cells of the rabbit brain. Hoppe-Seylers Z. physiol. Chem. **354**, 729—736 (1973)
30. Woelk, H., Kanig, K.: Phospholipid metabolism in experimental allergic encephalomyelitis: activity of brain phospholipase A_1 towards specifically labelled glycerophospholipids. J. Neurochem. **23**, 739—743 (1974)
31. Woelk, H., Kanig. K., Peiler-Ichikawa, K.: Phospholipid metabolism in experimental allergic encephalomyelitis: activity of mitochondrial phospholipase A_2 of rat brain towards specifically labelled 1,2-diacyl-, 1-alk-1′-enyl-2-acyl- and 1-alkyl-2-acyl-sn-glycero-3-phosphorylcholine. J. Neurochem. **23**, 745—750 (1974)
32. Woelk, H., Peiler-Ichikawa, K.: Zur Aktivität der Phospholipase A_2 gegenüber verschiedenen 1-Alk-1′-enyl-2-acyl- und 1-Alkyl-2-acyl-Verbindungen während der multiplen Sklerose. J. Neurol. **207**, 319—326 (1974)
33. Woelk, H., Porcellati, G.: Subcellular distribution and kinetic properties of rat brain phospholipases A_1 and A_2. Hoppe-Seylers Z. physiol. Chem. **354**, 90—100 (1973)

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