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Phospholipase A Activities in Nervous Tissues

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The phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) activities in snake venoms and in several mammalian tissues have been extensively studied in recent years, but relatively little is yet known about this group of enzymes in nervous tissues.

Evidence for the presence of phospholipase A in brain was first reported by Gallai-Hatchard, Magee, Thompson & Webster (1962), who showed that when aqueous-glycerol extracts of acetone-dried brain were incubated at pH 7.3 with diacylglycerophosphatides and deoxycholate there was substantial formation of lyso derivatives, but the structure of the formed lyso derivatives and the site(s) of action of brain phospholipase on the ester bonds at the 1- and 2-positions of the substrates was not known. Webster & Cooper (1968) incubated rat cerebrum homogenates at pH 7.4 with trace amounts of 2-[¹⁴C]acyl-labelled or [³H]choline-labelled lecithins (phosphatidylcholines). From the pattern of radioactive hydrolysis products it was concluded that both acyl ester bonds of lecithin had been split, forming a mixture of isomeric lysolecithins (1- and 2-acyl-glycerophosphorylcholine), thus indicating that brain contained two positionally specific types of phospholipase, A₁ and A₂ (see van Deenen & de Haas, 1966), operative at physiological pH.

A phospholipase A₁, splitting off only the 1-linked fatty acid of diacylglycerophosphatides, has been isolated from rat and calf brain (Gatt, Barenholz &

Roitman, 1966; Gatt, 1968); this enzyme had optimum activity at pH 4.0 and was inactive at pH 7.4. There is evidence therefore that nervous tissues contain a number of types of phospholipase A activity, differing in pH optima, positional specificity and possibly other properties; recent studies (M. F. Cooper & G. R. Webster, unpublished work) have been concerned with extracting and defining these enzymes more precisely.

Acetone- and butan-1-ol-treated tissue powders were extracted with cold 0.9% NaCl to give clear lipid-free extracts containing about 10mg of protein/ml. Extracts of rat cerebrum were incubated for 1 or 2 h with (1-[³H]stearoyl)lecithin (2.5mm) in the presence of sodium taurocholate (3mg/ml) between pH 3.8 and 8.5. Release of [³H]stearic acid was optimum at pH 4.2 and at this pH only traces of labelled lysolecithin were formed. Production of [³H]lysolecithin, on the other hand, showed a peak at pH 5.5. At higher pH values production of both hydrolysis products fell off progressively and at pH 7.4 were only about one-sixth of the peak amounts. These extracts also exhibited lysophospholipase activity, with a broad pH optimum between 6 and 8, when incubated with 2.5mm-(1-[³H]stearoyl) lysolecithin. However, from control experiments with traces of labelled lysolecithin in the presence of unlabelled lecithin and taurocholate it appeared that secondary breakdown of lysolecithin formed by phospholipase A would be only slight at neutrality and negligible below pH 6.0. The patterns of [³H]stearic acid and [³H]lysolecithin formed from (1-[³H]stearoyl)lecithin by the extracts therefore indicated that they contain phospholipase A₁, with optimum activity at pH 4.2 and thus confirming Gatt's earlier finding, and, in addition, a phospholipase A₂ with a higher optimum at pH 5.5.

Extracts of human cerebral cortex and white matter showed similar patterns of activity, although differing from rat brain with regard to the pH optima of the two phospholipase A activities (see below).

To isolate the phospholipase A₁ activity saline extracts of rat cerebrum were first dialysed against 0.9% NaCl buffered to pH 4.2 with 20mm-sodium acetate. This step precipitated 60% of the protein, and a further 20% was then precipitated by pre-incubating the dialysed supernatant with sodium taurocholate (6mg/ml). The final supernatant contained 90% of the phospholipase A₁ activity (pH 4.2) in the crude extract, but was virtually free of phospholipase A₂ (pH 5.5) and lysophospholipase (pH 7.4) activities; phospholipase A₁ activity at pH 7.4 was also substantially decreased, to 25% of its original low value. This procedure for extracting and isolating the acid phospholipase A₁ of nervous tissue is simple and reproducible and could be applied to small samples of tissue. Extraction of the enzyme from the acetone-dried powder appeared essentially

complete, and doubling the time of extraction with saline did not significantly increase the yield. The average yield from rat cerebrum corresponded to 1.6 (s.d. 0.09) μmol of lecithin hydrolysed/h per g of tissue; the specific activity was 0.24 (s.d. 0.04) $\mu\text{mol/h}$ per mg of protein.

The phospholipase A_1 activities in dialysed and taurocholate-treated preparations of human cerebral cortex and white matter gave broader pH peaks than rat brain, with maximal activities at pH 4.6, averaging 0.85 and 0.62 $\mu\text{mol/h}$ per g of tissue respectively. The substrate specificity of purified A_1 prepared in this way from rat cerebrum or from human cerebral cortex was in general agreement with that reported for rat brain by Gatt (1968). In the presence of taurocholate, (1-[^3H]stearoyl) lecithin and (1-[^3H]palmitoyl) lecithin were hydrolysed most rapidly, at similar rates, and (1-[^3H]stearoyl) phosphatidylserine about 20–30% as fast. Only slight activity against (1-[^3H]stearoyl) phosphatidylethanolamine was detected in this system but, as Gatt had found, there was appreciable hydrolysis in a mixture of taurocholate and a non-ionic detergent. Only relatively slight breakdown of (1-[^3H]stearoyl) lysolecithin, glycerol tri[^{14}C]oleate or cholesteryl [^{14}C]oleate was found.

Preparations showing phospholipase A_2 activity free of phospholipase A_1 have not as yet been isolated. Some preliminary information about this enzyme was obtained, however, with unpurified saline extracts. The pH optimum for phospholipase A_2 activity in human grey and white matter was 5.0, somewhat less than in rat brain, and no activity was detectable above pH 6.5.

Comparative studies on the regional distribution of phospholipase A in the human nervous system were carried out with (1-[^3H]stearoyl)lecithin as substrate for both types of activity. Phospholipase A_1 activity was calculated from the amounts of [^3H]stearic acid released by dialysed and taurocholate-treated extracts at pH 4.6, and phospholipase A_2 from the [^3H]lysolecithin formed by incubation with crude saline extracts at pH 5.0. In the central nervous system phospholipase A_1 activity showed a wide range of values between various regions, in contrast with phospholipase A_2 , which was more uniformly distributed. Phospholipase A_1 /phospholipase A_2 activity ratios therefore differed considerably (cerebellar cortex, 5.0:1; cerebral cortex, 3.1:1; centrum semiovale, 2.0:1; cervical cord, 1.4:1); a small phospholipase A_1 activity but no phospholipase A_2 was found in sciatic nerve.

The finding that certain lysophosphatides can be actively reacylated by brain (Webster, 1965), in the manner described originally in liver by Lands (1960), suggested that in normal nervous tissue phospholipase A plays a part in fatty acid exchange reactions. Whether also some abnormality of intrinsic phospho-

lipase A action is involved in loss of lipid in degenerative neurological disease is still unexplored.

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The Catabolism of Ethanolamine Phospholipids in Brain Tissue

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Some 9% of the dry weight of whole brain consists of ethanolamine phospholipids and 85% of this is in membranes. Undoubtedly a considerable proportion, particularly the ethanolamine plasmalogen, serves a structural function and is metabolically stable in adult life. In pathological conditions where demyelination occurs and loss of ethanolamine lipid is evident, this loss can be extremely serious. The measurement of the rates of breakdown of the ethanolamine lipids of the brain *in vivo* has never been achieved, though it is apparent from the measurements of the uptake of radioactive phosphate and ethanolamine in adult brain that the phosphate base moiety of both the major ethanolamine phospholipids undergoes some turnover (Ansell & Spanner, 1967).

Although early studies indicated the presence of enzymes hydrolysing ethanolamine phospholipids in brain (see Ansell & Hawthorne, 1964), the first demonstration of a specific enzyme, phospholipase A, was by Gallai-Hatchard, Magee, Thompson & Webster (1962). Some corroborative evidence of their breakdown *in vivo* was demonstrated by Knauff, Marx & Mayer (1961), who showed a rise in the free ethanolamine concentration in brain in insulin hypoglycaemia, which indicated a breakdown of ethanolamine lipid.

When a brain homogenate was incubated in oxygenated Krebs-Ringer bicarbonate medium for short periods, there was a breakdown of the endogenous phospholipids including those containing ethanolamine. The low rate of loss of ethanolamine lipids of about 1.5 $\mu\text{mol/h}$ per g fresh wt. was accompanied by a rise in the concentration of water-soluble ethanolamine compounds, particularly ethanolamine