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REDUCED PHOSPHORYLCHOLINE HYDROLYSIS BY HOMOGENATES OF TEMPORAL REGIONS OF ALZHEIMER'S BRAIN

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The hydrolysis of p-nitrophenylphosphorylcholine, p-nitrophenylphosphate, and phosphorylcholine was quantitated in homogenates prepared from the occipital and temporal regions of control and Alzheimer's Disease patient brain. There were no significant differences detected in activities of enzymes cleaving the first 2 compounds. A 78% reduction in the capacity for hydrolyzing phosphorylcholine by temporal homogenates from Alzheimer's patients compared to controls was demonstrated. The hydrolysis of this substrate by occipital homogenates was identical to control values. These results may explain the lack of cognitive improvement of Alzheimer's patients receiving dietary supplements of either choline or lecithin.

Alzheimer's Disease (A.D.), which is characterized by cognitive disabilities and diagnostic neuropathological changes has been reviewed (1). Attempts to treat the disease developed from observed decreases in acetylcholine (AcCh) content and choline acetyltransferase (CAT) activity of patient brain tissue. The neurotransmitter deficiencies of A.D. (2) and the regulation of AcCh formation (3) have been reviewed. This AcCh deficit was the basis for the development of the "cholinergic" hypothesis which attempts to link the cholinergic deficiency with the cognitive disabilities (4). Therapeutic management was undertaken in an attempt to increase the level of AcCh in the effected individuals by dietary supplementation of choline or lecithin. Experimental results in animals have indicated such treatment can elevate plasma choline levels and in some instances brain choline levels (5-7). These changes in brain choline levels, however, did not cause meaningful elevations of AcCh concentration (8-10). Similarly, there has not been consistent improvement of cognitive function of A.D. patients receiving such dietary regimes (11-12).

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The principal biochemical modification of choline administered to intact animals (7) or cell cultures (13,14) is the prompt conversion to phosphorylcholine. The hydrolysis of phosphorylcholine by human and rat brain homogenates has been observed (15). Therefore, it seemed reasonable for us to consider that the hydrolysis of this compound might be altered in A.D. brain tissues.

MATERIALS AND METHODS

Separate homogenates of occipital and superior temporal areas of the same brain tissue from A.D. patients and controls were used as the enzyme source. The average age of the patients with A.D. was 75 $\pm$ 8.9 years and the average post mortem time was 15.5 $\pm$ 6.16 hours. The average age of the controls was 61.64 $\pm$ 14.33 years and the average post mortem time was 14.72 $\pm$ 7.05 hours. There was no statistically significant difference in either the age or post mortem time between the patient and control groups. The liberation of p-nitrophenol from p-nitrophenylphosphorylcholine was used to measure phospholipase C activity (16). The incubation mixtures were composed of 50mM CAPS pH 8.5, 16mM p-nitrophenylphosphorylcholine, 5 mM Na oleate, 200 µg protein in a volume of 200 µl. Release of p-nitrophenol from p-nitrophenylphosphate at 3 different pH's was employed to estimate acid, neutral and alkaline phosphatase activities (17). The incubation mixtures contained 70 ug protein, 10 mM p-nitrophenyl phosphate and either 50 mM acetate pH 4.8, or 50 mM HEPES pH 7.2 or 50 mM CAPS pH 8.5. The release of inorganic phosphate was measured according to a modification of the procedure of Fiske-Subbarow (18) as an estimate of the hydrolysis of phosphorylcholine. The incubation mixtures contained 50 mM CAPS, pH 8.5, 5mM phosphorylcholine and 500 µg protein. All incubations were carried out in duplicate or triplicate at 37° and homogenates placed in boiling water for 10 minutes served as blanks.

RESULTS

A comparison of the hydrolysis of the 3 substrates utilized in these studies by homogenates of occipital and temporal brain regions of controls and A.D. patients is presented in Table 1. The cleavage of p-nitrophenylphosphorylcholine was decreased by 13% in both the temporal and occipital Alzheimer's patient samples, however, this reduction was not statistically significant. This suggests that phospholipase C activity is not severely effected in this disease. There were no differences in the hydrolysis of p-nitrophenylphosphate at the 3 pH's by either temporal or occipital homogenates of A.D. and control brain samples. The greatest activity was found at the acidic pH presumably a reflection of lysosomal acid phosphatase activity. The alkaline phosphatase activity, measured at pH 8.5, was only one tenth as active as the phosphatase measured at the acidic pH.

TABLE 1

HYDROLYSIS OF PHOSPHATE ESTERS BY TEMPORAL AND OCCIPITAL HOMOGENATES FROM ALZHEIMER'S DISEASE AND CONTROLS

	Alzheimer's		Controls	
Substrate	Temporal	Occipital	Temporal	Occipital
p nitrophenyl-phosphorylcholine(1)	142.5 ± 22.4 (n=15)	135.4 ± 43.5	158.2 ± 83 (n=8)	163.7 ± 34.8
p nitrophenyl phosphate(2)				
acid (pH 4.8)	3.04 ± 0.26	2.85 ± 0.061	2.97 ± 0.27	2.91 ± 0.27
neutral (pH 7.2)	0.84 ± 0.104	0.796 ± 0.0075	0.813 ± 0.095	0.8 ± 0.096
alkaline (pH 8.5)	0.309 ± .05 (n=18)	0.28 ± 0.047	0.299 ± .058 (n=18)	0.3 ± .053
phosphorylcholine	8.08 ± 6.27 (n=24)	21.91 ± 10.9	35.94 ± 12.86 (n=25)	21.87 ± 15.6

1. Activity expressed as nmoles hydrolyzed/mg protein/hr.
2. Activity expressed as u mole hydrolyzed/mg protein/hr.

There was no statistical difference in the hydrolysis of phosphorylcholine by occipital homogenates of A.D. and control brains. However, there was a statistically significant ($p=<.001$) 78% reduction of the hydrolysis of this substrate in the temporal homogenates of A.D. patients as compared to controls. The ratios of activity in temporal to occipital homogenates is 1.64 in the controls but only 0.37 in the A.D. samples. A display of the distribution of activity of all samples is presented in Fig. 1.

DISCUSSION

These results suggest that phospholipase C activity is normal in brain tissue samples from patients with A.D. This in contrast to the 65% reduction this laboratory has observed in phospholipase D activity in A.D. brain homogenates (19). Phospholipase C cleavage of lecithin liberates diglyceride and phosphorylcholine. Choline upon entry to a cell is promptly converted to phosphorylcholine. The data presented in table 1 and figure 1 indicates that there is a reduction of phosphorylcholine hydrolysis in the temporal but not the occipital portions of Alzheimer's brain as compared to controls. It is generally agreed that the temporal regions of the A.D. patient is most

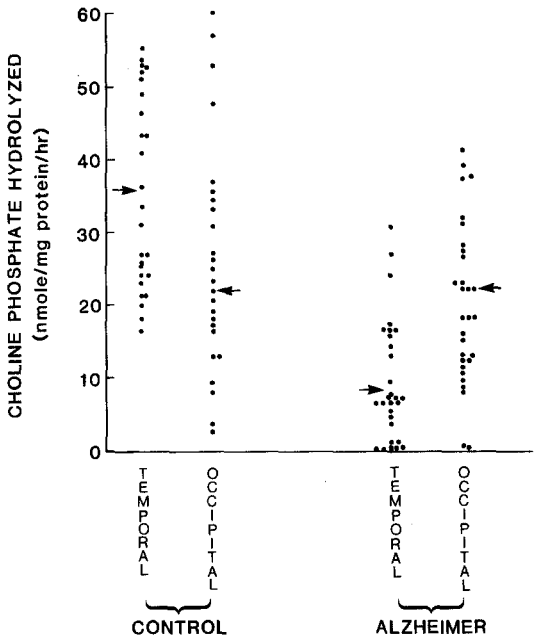

Fig. 1. Phosphorylcholine hydrolysis by Alzheimer's (n=24) and control (n=25) brain tissue homogenates. Each value represents the average of triplicate determinations on each sample. The arrow indicates the average value of the samples.

severely effected (1). This decreased ability to release choline from phosphorylcholine by the affected area of brain could explain the lack of observable clinical improvement of A.D. patients receiving dietary supplements of lecithin or choline. Although plasma choline levels become elevated the principal increase within the brain tissue is in phosphorylcholine. The affected individuals may have reduced capacity to mobilize this potential ACh precursor from the major water soluble choline containing compound of brain tissue (20). This is the second enzyme that has reduced activity in A.D. that potentially could provide free choline from a derivative. This provides a rational biochemical explanation for the lack of efficacy of dietary supplementation in attempts at treating this disease.

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