Biochem. J. (1983) **211**, 507–509
Printed in Great Britain

Effect of dietary lipid on synaptosomal acetylcholinesterase activity

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(*Received 20 December 1982/Accepted 25 February 1983*)

The effect of dietary lipid on the thermotropic properties of acetylcholinesterase activity was examined in rat synaptosomal membrane preparations after feeding diets containing soya-bean oil, sunflower oil or soya-bean phosphatidylcholine as the dietary fats. Arrhenius plots and energies of activation were altered by the duration of feeding as a function of time, as well as by the composition of diet fat fed. Animals fed sunflower oil had the highest maximal velocity for acetylcholinesterase activity. The observations of this study suggest that dietary fat is an important determinant of the physicokinetic properties of lipid-dependent functions in brain synaptosomal membranes.

Studies of the interaction between proteins and lipid constituents of biological membranes indicate that physical characteristics of the microenvironment surrounding a membrane-associated protein affect the catalytic activity of the protein (reviewed previously by Innis & Clandinin, 1981*a*; Foot *et al.*, 1982). In the whole-animal model, demonstration of the significance of the composition of phospholipid milieu surrounding integral membrane proteins has been achieved and establishes that diet fat can dynamically alter membrane phospholipid composition in a variety of membranes (Innis & Clandinin, 1981*a*,*b*; Foot *et al.*, 1982). For mitochondria these diet-induced alterations in membrane composition modulate the lipid-dependent functions of an integral membrane protein (Innis & Clandinin, 1981*c*).

For brain we have recently reported diet-induced changes in fatty acid composition and phosphatidylcholine content for synaptosomal and microsomal membranes (Foot *et al.*, 1982). The present study indicates that these diet fat-induced alterations in synaptosomal structural constituents result in changes in acetylcholinesterase activity.

Materials and methods

Animals and diets

Male Sprague–Dawley rats weighing 49.8 ± 4.2 g were housed and fed diets containing either soya-bean oil as a control diet comprising high levels of both $C_{18:2(9,12)}$ and $C_{18:3(9,12,15)}$ fatty acids ('SBO'), soya-bean phosphatidylcholine ('SBO-PC') or sunflower oil high in $C_{18:2(9,12)}$ and low in $C_{18:3(9,12,15)}$ ('SFO') for 11 days or 25 days as described previously (Foot *et al.*, 1982). Experimental replication ($n = 4$) is indicated in Table 1. After 25 days of feeding, animals weighed 182 ± 4.2, 168 ± 6.2 and 169 ± 10.3 g (means ± S.D.) for rats fed the SBO, SFO and SBO-PC diets respectively.

Membrane isolation and acetylcholinesterase assay

Synaptosomal membrane was isolated from rat brain by preparative ultracentrifugation (Cruz & Gurd, 1978). Acetylcholinesterase activity was assayed by a method providing a clear separation of product from substrate (Lewis & Eldefrawi, 1974). [1-^{3}H]Acetylcholine was purified before use by column chromatography on a Sephadex G-10 column (40 cm × 1 cm). Radioactivity was determined utilizing a Beckman LS7800 liquid-scintillation spectrometer (Beckman Instruments, Palo Alto, CA, U.S.A.). ^{3}H was counted in a toluene-based fluor containing 4.75 g of 2,5-diphenyloxazole, 0.32 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene and 40 ml of NCS solubilizer per litre. All counts were corrected for background and counting efficiency. Protein was measured by a colorimetric method (Lowry *et al.*, 1951).

Statistical analysis

The effect of dietary treatment was examined by analysis-of variance procedures. Comparison between individual diets was made by Neuman–Keuls multiple-range test after an effect of diet treatment was shown by analysis of variance. For Arrhenius plots, data points from four separate experiments were analysed by the method of least squares and straight lines were fitted. Regression coefficients are given on each line illustrated (Fig. 1).

Results and discussion

Interpretation of Arrhenius plots for acetylcholinesterase activity is dependent upon similar affinity of the catalytic site for its substrate at the various temperatures tested. In this regard, the apparent K_m for acetylcholine was found to be 0.04 mM and unaffected by temperature. Thermotropic properties determined from Arrhenius plots of acetylcholinesterase activity show developmental effects of the lipid environment that are related to duration of feeding. Developmental changes in membrane composition have been reported previously (Foot *et al.*, 1982). Maximal reaction velocities were highest at weaning, before dietary treatment (Fig. 1, Table 1). The discontinuity in Arrhenius plots for acetylcholinesterase activity determined by the method of least squares was lowest at weaning and increased after 11 days and 25 days of diet treatment, suggesting that age-dependent changes occur in the membrane microenvironment of this lipid-dependent function (Fig. 1). The apparent K_m and energy of activation above this breakpoint was not altered as a function of age. However, the duration of feeding significantly lowered the energy of activation below the breakpoint (Table 1).

Diet altered maximal reaction velocity and energy of activation observed below the transition temperature (Fig. 1, Table 1). Control diet treatments (SBO) resulted in lower maximal velocities when compared with SFO treatments after 11 days and 25 days of feeding or when compared with SBO-PC treatments after 25 days of diet treatment (Table 1). Diet appeared to alter the apparent transition temperatures as follows: SPO-PC > SBO or SFO after 11 days of diet treatment; SBO > SBO-PC > SFO after 25 days of diet treatment (Fig. 1).

Developmental differences in reaction rates and thermotropic properties for acetylcholinesterase activity are consistent with our previous observations on changes in the lipid composition of these membranes (Foot *et al.*, 1982). Differences in maximal reaction velocities observed between SBO and SFO treatments and between SBO and SBO-PC treatments at day 25 indicate that kinetic properties of this membrane-mediated function can be manipulated by diet fat and balance of fatty acids within the diet fed. These changes in function are associated with diet-induced changes in synaptosomal polar lipid composition (Foot *et al.*, 1982). Synaptosomal membranes from these diet treatments differed in

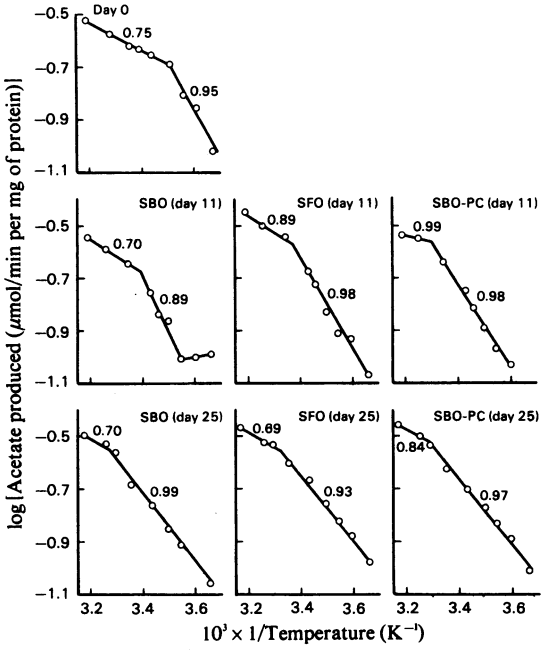

Fig. 1. *Arrhenius plots showing changes in thermotropic behaviour of acetylcholinesterase activity induced by diet fat*

Synaptosomal membranes were prepared from brains of rats fed diets containing soya-bean oil (SBO), sunflower oil (SFO) or soya-bean phosphatidylcholine (SPO-PC). Each point represents the mean of four groups of four rats per diet treatment. Two separate assays were performed on each group. The data were analysed by the method of least squares for determination of break points. Straight lines were fitted by regression analysis. Regression coefficients are given for each line. Energies of activation calculated from these slopes are given in Table 1.

Table 1. *Effect of fat diet on the maximal reaction velocity for acetylcholinesterase isolated from rat brain synaptosomes and the Arrhenius energy of activation observed below the transition temperature*

Values given are means for four separate experiments of four animals fed either soya-bean oil (SBO), sunflower oil (SFO) or soya-bean phosphatidylcholine (SBO-PC). For each variable, values without a common superscript are significantly different: a, b and c, effect of diet fat ($P < 0.05$); *, effect of 'day' ($P < 0.005$). The values in parentheses indicate the pooled standard deviation. The maximal reaction velocity observed for acetylcholinesterase activity in control diet treatments (SBO) was 0.279 μmol of product formed/min per mg of synaptosomal protein.

Dietary treatment	Maximal reaction velocity (% of control values)		Energy of activation below transition temperature (J/mol per °C)	
	Day 11	Day 25	Day 11	Day 25
SBO (control)	100[a]	100[a]	240[a]	150[a*]
SFO	117[b]	115[b]	200[b]	149[a*]
SBO-PC	98.2[a]	113[b]	205[b]	147[a*]
	(1.08)		(34.6)	

phosphatidylcholine/cholesterol ratio and in fatty acyl-tail composition (Foot *et al.*, 1982).

From the present study it may be concluded that for brain synaptosomal membranes, diet-induced changes in phospholipid fatty acid composition and polar lipid content (Foot *et al.*, 1982) significantly alter the functional properties of a lipid-dependent membrane protein within its membrane microenvironment. The interaction between diet, polar head group distribution, cholesterol content and fatty acyl-tail composition of phospholipids is complex and, in our view, likely more specific to the environment of the integral protein than can be assessed by e.s.r. probes. It is noteworthy that gross changes in membrane structure induced by essential fatty acid deficiency have recently been shown not to result in changes in membrane fluidity as monitored by spin-label motion (Elstrom *et al.*, 1981). Most significantly, however, it is apparent that membrane-dependent enzymes of neurotransmitter metabolism, such as acetylcholinesterase, may be sensitive to alterations in the balance of dietary fatty acids consumed, even in a nutritionally complete diet.

These studies were supported by the National Science and Engineering Research Council, The Agricultural Research Council of Alberta and the Medical Research Council of Canada. The technical assistance by P. Sham in the maintenance of animals and L. Birmingham in the laboratory analyses is acknowledged. The assistance of C. P. Chow in the analysis of variance is also acknowledged.

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