



INFLUENCE OF DIETARY FAT ON THE ACTIVITIES OF SUBCELLULAR MEMBRANE-BOUND ENZYMES FROM DIFFERENT REGIONS OF RAT BRAIN

P. SRINIVASARAO,* K. NARAYANAREDDY,† A. VAJRESWARI,
M. RUPALATHA, PADMINI SURYA PRAKASH and PADMINI RAO‡

National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania, P.O.,
Hyderabad-500 007, India

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Abstract—The effect of different dietary fats with varying degrees of unsaturation and essential fatty acid composition, which are commonly consumed in India, on the activity of some important membrane-bound enzymes was assessed in different brain regions of rat. Four groups of male CFY weanling rats were fed nutritionally adequate diets containing groundnut, coconut, safflower or mustard oil as fat source at 20% level for 16 weeks. The synaptosomal, microsomal and myelin membranes were prepared from three brain regions, viz., cerebrum, cerebellum and brain stem from each group. The activities of Na^+ , K^+ -ATPase, Mg^{2+} -ATPase and acetylcholinesterase were assayed and the fatty acid composition was determined in these subcellular membrane fractions. The safflower oil-fed group showed higher Na^+ , K^+ -ATPase activity in most membrane fractions than the coconut or mustard oil-fed groups. The Mg^{2+} -ATPase activity was found to be similar amongst all groups in all the brain regions. The synaptosomal acetylcholinesterase activity was distinctly higher in coconut and groundnut oil-fed groups when compared to safflower or mustard oil consuming groups. Alterations in the activities of these subcellular membrane-bound enzymes are expected to exert a significant impact on the electrophysiological and metabolic functions of brain. Results of the present study show that depending on the nature of dietary fat the fatty acid composition of subcellular membranes is altered, which in turn could regulate the activity of membrane-bound enzymes that are vital for brain function. © 1997 Elsevier Science Ltd. All rights reserved

The influence of dietary fat on compositional changes of body tissues has been well documented in different laboratory animals (Bourre *et al.*, 1990; Sun *et al.*, 1974; Vajreswari and Narayanareddy, 1992a, 1992b). Several studies were carried out with a purpose to assess the impact of dietary deficiency of essential fatty acid (EFA) component on structural and metabolic alterations in tissues (Morgan *et al.*, 1981; Menon and Dhopesarkar, 1982; Galli *et al.*, 1972). The metabolic regulation depends on the function of several membrane-bound enzymes whose activity, to a large extent, is influenced by factors such as degree of unsaturation of constituent fatty acids (Stubbs and Smith, 1984), their quantity and the ratio of cholesterol to phospholipids (Sinensky *et al.*, 1979). There is, however, a need to ascertain in detail, the impact

of dietary fat on the biochemical and metabolic responses of different tissues (central as against peripheral) which may differ widely in their membrane lipid composition.

Studies carried out in this direction at this Institute (Vajreswari and Narayanareddy, 1992a, 1992b) and elsewhere (Awad and Chattopadhyay, 1983; Murphy, 1990) have indicated that dietary fat not only induced changes in membrane fatty acid composition but also affected the activities of enzymes involved in the regulation of cardiac function. In contrast to peripheral organs, brain uniquely possesses high amounts of cholesterol and sphingolipids. In addition, brain membrane phospholipids differ widely in the proportion of n-6 and n-3 fatty acids when compared to those of peripheral organ membranes. We have, therefore, studied the effects of safflower, groundnut, coconut and mustard oil based diets (differing in the fatty acid composition with respect to quality and quantity of unsaturated component) on rat brain membrane lipid composition which revealed significant differences in

*Author to whom all correspondence should be addressed.

†Present address: Dr. Reddy's Foundation, Bolarum Road, Miyapur, Hyderabad-500 138, India.

‡Present address: FDA, Maryland, U.S.A.

the lipid and fatty acid profiles of various subcellular membranes (in press *J. Nutr. Biochem*). In this paper we report the impact of these different oil-based diets on the activities of some crucial membrane-bound enzymes associated with energy linked reactions as well as neural transmission in relation to specific changes in fatty acid composition of myelin and synaptosomal fractions in different regions of the brain.

Materials

Dietary oils, viz., groundnut, safflower, coconut and mustard oils were obtained locally. Solvents of analar grade were used. All fine chemicals were purchased from Sigma Chemical Co. (St. Louis MO, U.S.A.).

Experimental procedures: Male weanling rats of CFY strain were divided into four groups and were caged individually. Each group of animals received casein based semi synthetic diet adequate with respect to all essential nutrients where the source of dietary fat viz. ground nut, safflower, coconut or mustard oil was included at a level of 20% (w/w) in the diet. The composition of the diet and the fatty acid profiles of oils used in the experiment are given in Tables 1 and 2. The rats were fed the respective diets for a period of 16 weeks and were later killed by decapitation. The major anatomical regions of the brain, i.e. cerebrum, cerebellum and brainstem were quickly dissected out and homogenized in cold 0.32 M sucrose medium. Myelin, synaptosomal and microsomal membrane fractions were isolated by discontinuous sucrose density gradient centrifugation and hypotonic treatment according to the method of Whittaker and Barker (1972). These membrane preparations were used for the assay of ouabain-sensitive Na^+ , K^+ -ATPase, Mg^{2+} -activated ATPase and acetylcholinesterase activities. Synaptosomal and myelin membranes were

Table 2. Fatty acid composition of oils

Fatty acids	Coconut oil	Mustard oil	Groundnut oil	Safflower oil
8:0	4.4	—	—	—
10:0	6.8	—	—	—
12:0	36.7	—	—	—
14:0	26.5	—	—	0.1
16:0	10.5	2.2	12.3	2.9
18:0	3.1	1.3	4.2	2.1
18:1	9.0	10.4	38.6	15.6
18:2	3.0	15.3	38.1	79.4
18:3	—	14.3	2.2	—
20:0	—	—	1.8	—
20:1	—	3.0	—	—
22:0	—	—	2.8	—
22:1	—	53.4	—	—

Expressed as % distribution of fatty acid methyl esters; —, not detectable.

also subjected to fatty acid analysis of their phospholipid fractions.

Na^+ , K^+ -ATPase (EC 3.6.1.3) activity was determined according to Post and Sen (1967) except that the reaction mixture included ethylene glycol-bis (B-aminoethyl ether) N,N,N',N' tetraacetic acid (EGTA). The reaction mixture in a final volume of 0.5 ml contained 50 mM Tris-HCl buffer (pH 7.4), 140 mM NaCl, 14 mM KCl, 3 mM MgCl_2 , 3 mM Tris ATP and 1 mM EGTA. Ouabain, when required, was added at 1.0 mM concentration. Na^+ , K^+ -ATPase was calculated by subtracting the activity obtained with ouabain from that obtained without ouabain. The difference between total ATPase activity and that of Na^+ , K^+ -ATPase was considered as Mg^{2+} -activated ATPase activity.

The activity of acetylcholinesterase (EC 3.1.1.8) was determined according to the method adopted by Ellman *et al.* (1961) except that the reaction was stopped after 15 min of incubation by adding absolute ethanol in equal volume to reaction mixture. The assay medium contained 50 mM Tris-HCl buffer (pH 7.4), 0.2 mM Tris EDTA, 0.1 mM 5,5'-dithio-bis (2-nitro benzoic acid) and 1.0 mM acetylthiocholine iodide.

Total lipids were extracted from membranes by the method of Folch *et al.* (1957). Phospholipids were separated from total lipids by TLC on silica gel H plates, using solvent system comprising chloroform: methanol (100:20) and were visualized by brief exposure to iodine vapours. Phospholipids remained at the origin and were distinctly separated from cerebrosides which had RF value = 0.5. The fatty acids of phospholipids were transmethyated using the method of Kishimoto and Hoshi (1972). Methyl esters of fatty acids were analysed by gas chromatography; (Varian Model 3700), equipped with FID and elec-

Table 1. Diet Composition

Ingredient	Diet composition g 100 g ⁻¹ diet
Casein	20.00
DL-Methionine	0.30
Cellulose	5.00
Oil	20.00
Mineral mixture	4.00*
Vitamin mixture	0.10**
Choline chloride	0.20
Starch	50.40

Diet was prepared as per AIN standards for nutrients *J. Nutr.* **107**, 1340–1348 (1977) and *J. Nutr.* **110**, 1726 (1980).

*USP XVII.

**AIN Standard.

Table 3. Activity of Na⁺-K⁺-ATPase in subcellular membranes of different brain regions from rats fed different oil diets

	Groundnut oil (Group I)	Coconut oil (Group II)	Safflower oil (Group III)	Mustard oil (Group IV)	Significance at P<0.05 by ANOVA. Comparison between groups			
					I	II	III	IV
					Na ⁺ , K ⁺ -ATPase (umoles Pi mg ⁻¹ protein h ⁻¹)			
CB (Synaptosomes)	8.43 ± 1.16(6)	5.99 ± 0.44(5)	10.44 ± 1.44(6)	6.40 ± 0.81(5)	vs.none	vs.III	vs.II&IV	vs.III
CL (Synaptosomes)	13.15 ± 1.61(6)	11.33 ± 0.74(6)	13.63 ± 1.85(6)	12.39 ± 0.60(5)	vs.none	vs.none	vs.none	vs.none
BS (Synaptosomes)	13.54 ± 1.94(5)	9.03 ± 2.18(4)	10.20 ± 1.28(5)	9.13 ± 0.38(3)	vs.none	vs.none	vs.none	vs.none
CB (microsomes)	6.28 ± 0.45(5)	6.70 ± 1.31(3)	8.53 ± 0.35(6)	5.13 ± 0.82(6)	vs.III	vs.none	vs.I&IV	vs.III
CL (microsomes)	9.58 ± 1.12(5)	8.43 ± 1.09(4)	14.20 ± 0.93(5)	10.88 ± 0.93(5)	vs.III	vs.III	vs.all	vs.III
BS (microsomes)	10.33 ± 0.19(4)	7.30 ± 0.60(5)	13.95 ± 1.48(6)	8.47 ± 0.84(6)	vs.II	vs.I&III	vs.II&IV	vs.III
CB (myelin)	7.63 ± 0.92(6)	3.74 ± 0.30(5)	7.18 ± 0.84(6)	5.63 ± 0.62(6)	vs.II	vs.I&III	vs.II	vs.none
CL (myelin)	14.02 ± 2.86(5)	4.22 ± 0.57(5)	10.00 ± 1.95(5)	9.88 ± 0.81(4)	vs.II	vs.all	vs.II	vs.II
BS (myelin)	5.42 ± 1.16(6)	3.40 ± 0.77(6)	6.63 ± 0.72(4)	2.70 ± 0.21(4)	vs.none	vs.III	vs.II&IV	Nvs.III

Results are Mean ± SEM ≥ 3 observations of pooled samples with a maximum of six. (See number in parentheses). Two cerebra (CB), three cerebella (CL) or three brain stems (BS) were pooled to represent single sample.

tronic integrator (model 4270 varian) using 10% Silar 10C coated on chromosorb W as stationary phase. The carrier gas was nitrogen (20 CC min⁻¹) the column temperature was maintained at 180°C. Fatty acids were identified by comparison with authentic standard mixture (GLC-68B) obtained from Nu-chek preparation (Elysian, MN).

RESULTS

The four dietary fats differed considerably with respect to their fatty acid composition (Table 2). Coconut oil had markedly high proportion (74%) of short chain saturated fatty acids in contrast to safflower oil which had nearly 80% of linoleic acid (18:2) whereas mustard oil possessed high level (53%) of characteristic mono-unsaturated fatty acid, erucic acid (22:1). Groundnut oil, however, had somewhat equal proportions (38% each) of the monoene, oleic acid (18:1) and the diene:linoleic acid (18:2) and saturates constituted to the extent of 21%. The activity of ouabain-sensitive Na⁺, K⁺-ATPase in the different subcellular membranes (depicted in Table 3) varied

depending on the brain region. Synaptosomal and microsomal membranes of cerebellum and brain stem showed higher activity compared to cerebrum. A distinctly higher specific activity of this enzyme in myelin fraction of cerebellum in particular, compared to other regions (irrespective of the type of dietary fat) could be explained based on significantly low protein values recorded in myelin fraction of cerebellum. However, myelin membranes from brain stem exhibited a lower activity compared to the other two regions. The pattern of Mg²⁺-ATPase followed the order as synaptosomes > microsomes > myelin (Table 4). The synaptosomal acetylcholinesterase activity was lower in cerebellum compared to cerebrum and brain stem regions (Table 5). The safflower oil-fed group showed significantly higher activity of Na⁺, K⁺-ATPase in most membrane fractions than those animals fed coconut or mustard oil. The activity of this enzyme in groundnut oil-fed group was comparable to that of safflower oil-fed group. Mg²⁺-ATPase exhibited similar activity amongst all the groups suggesting that this enzyme is quite stable and not susceptible to the influence of dietary fat. The brain

Table 4. Activity of Mg²⁺-ATPase in subcellular membranes of brain regions

	Groundnut oil (Group I)	Coconut oil (Group II)	Safflower oil (Group III)	Mustard oil (Group IV)	Significance at P<0.05 by ANOVA. Comparison between groups			
					I	II	III	IV
Mg ²⁺ -ATPase (umoles Pi mg ⁻¹ protein h ⁻¹)								
CB (synaptosomes)	7.02 ± 1.31(6)	5.48 ± 0.78(5)	8.12 ± 0.52(6)	7.81 ± 1.55(5)	vs.none	vs.none	vs.none	vs.none
CL (synaptosomes)	10.67 ± 1.56(6)	6.61 ± 1.02(6)	9.12 ± 4.12(6)	9.49 ± 0.50(5)	vs.none	vs.none	vs.none	vs.none
BS (synaptosomes)	8.25 ± 2.01(5)	12.79 ± 3.02(4)	9.18 ± 4.59(5)	16.71 ± 7.51(3)	vs.none	vs.none	vs.none	vs.none
CB (microsomes)	5.11 ± 1.24(5)	5.87 ± 0.43(3)	6.72 ± 0.41(6)	5.63 ± 0.36(6)	vs.none	vs.none	vs.none	vs.none
CL (microsomes)	3.75 ± 0.86(5)	6.07 ± 1.05(4)	6.84 ± 0.49(5)	6.60 ± 0.49(5)	vs.all	vs.I	vs.I	vs.I
BS (microsomes)	3.98 ± 0.64(4)	4.67 ± 0.11(5)	5.80 ± 0.26(6)	5.75 ± 0.39(6)	vs.III&IV	vs.none	vs.I	vs.I
CB (myelin)	2.43 ± 0.32(6)	2.30 ± 0.18(5)	3.19 ± 0.64(6)	3.10 ± 0.23(6)	vs.none	vs.none	vs.none	vs.none
CL (myelin)	2.79 ± 0.50(5)	1.73 ± 0.53(5)	2.47 ± 0.79(5)	5.32 ± 1.00(4)	vs.IV	vs.IV	vs.IV	vs.all
BS (myelin)	1.23 ± 0.04(6)	2.37 ± 0.68(6)	1.80 ± 0.18(4)	1.95 ± 0.25(4)	vs.none	vs.none	vs.none	vs.none

CB = cerebrum; CL = cerebellum; BS = brain stem

Results are Mean ± SEM ≥ 3 observations of pooled samples with a maximum of six. (See number in parentheses).

Table 5. Activity of acetylcholinesterase in synaptosomal membranes of brain regions

	Groundnut oil (Group I)	Coconut oil (Group II)	Safflower oil (Group III)	Mustard oil (Group IV)	Significance at P < 0.05 by ANOVA. Comparison between groups				
					I	II	III	IV	
		Acetylcholinesterase (umoles thiocholine mg ⁻¹ protein h ⁻¹)							
CB (synaptosomes)	5.95 ± 0.47(6)	8.80 ± 0.15(6)	4.14 ± 0.32(6)	4.77 ± 0.12(6)	vs.all	vs.all	vs.I&II	vs.I&II	
CL (synaptosomes)	2.42 ± 0.15(6)	2.04 ± 0.16(5)	1.26 ± 0.23(6)	1.66 ± 0.17(6)	vs.III&IV	vs.III	vs.I&II	vs.I	
BS (synaptosomes)	6.65 ± 0.65(5)	7.66 ± 0.80(5)	3.25 ± 0.13(5)	6.67 ± 0.12(4)	vs.III	vs.III	vs.all	vs.III	

CB, cerebrum; CL, cerebellum; BS, brain stem.

Values are Mean \pm SEM; ($n > 4$) with a maximum of six pooled samples. (See number in parentheses).

synaptosomal acetylcholinesterase activity was uniquely higher (Table 5) in coconut and groundnut oil-fed groups compared to safflower or mustard oil consuming animals.

DISCUSSION

The distribution pattern of major fatty acid groups of phospholipid of myelin and synaptosomal membrane fractions for the three regions of brain in response to feeding of different dietary fat are presented in Tables 6 and 7. Feeding of safflower and groundnut oil (rich in linoleic acid) resulted in higher ratio of n-6/n-3 values. A distinctly higher proportion of total n-3 fatty acids could be visualized in both synaptosomal and myelin membranes of rats reared on mustard oil. These higher n-3 values contributed to lowering of n-6/n-3 ratio in this group. However, brain membranes from coconut oil-fed group could maintain adequate proportion of total n-6 and n-3 fatty acids despite this dietary fat being EFA deficient. This is probably the result of adaptation of these animals to compensate for the deficiency of n-6 and n-3 fatty acids that are needed for the brain.

Our study was basically aimed at the assessment of the influence of commonly consumed dietary fats in India (viz. groundnut, safflower, coconut and mustard oils) varying widely with respect to essential fatty acid components on the activities of subcellular membrane-bound enzymes which were considered crucial for brain function. The distinction between brain regions was deemed important because of differences attributable to physiological and functional parameters of specific regions. Since polyunsaturated fatty acid rich phospholipids contribute a great deal to the composition of brain subcellular membranes, dietary fat with varied composition might influence membrane fatty acid profile of phospholipids and thereby alter the structure and activity of membrane-bound enzymes. It is clearly evident from literature that reduction in membrane fluidity has a direct relation to dietary deficiency of essential fatty acids (Alam and Alam, 1986). This in turn could impair the function of membrane ion channels and ultimately influence electrophysiological properties of the membranes. Amongst the factors that influence the functional activity of brain, those related to the transport of neurotransmitters are crucial. Because of intrinsic

Table 6. Distribution pattern of major fatty acid groups of synaptosomal membrane phospholipids

Group	Brain region	Saturates	Monoenes	Total n-6	Polyenes* Total n-3	n6/n3	Unsat/sat
Groundnut oil I	CB	53.37 \pm 1.96 ^a	21.7 \pm 0.85 ^{ab}	20.32 \pm 2.08 ^a	4.1 \pm 0.22 ^a	4.93 \pm 0.38 ^a	0.94 \pm 0.04
	CL	59.60 \pm 2.3 ^a	21.9 \pm 2.4 ^a	15.30 \pm 0.30	3.00 \pm 0.06 ^a	5.10 \pm 0.13 ^{ac}	0.68 \pm 0.04 ^{ac}
	BS	47.53 \pm 2.71 ^a	23.83 \pm 1.53 ^a	22.33 \pm 2.03 ^{ab}	6.23 \pm 1.11 ^{ac}	3.9 \pm 0.91 ^a	1.12 \pm 0.12 ^a
Coconut oil II	CB	50.57 \pm 3.18 ^a	23.83 \pm 2.27 ^a	17.70 \pm 2.11 ^{ab}	4.3 \pm 1.5 ^a	5.51 \pm 2.24 ^a	0.95 \pm 0.05
	CL	47.84 \pm 1.46 ^b	24.76 \pm 0.87 ^a	17.36 \pm 1.40	10.0 \pm 0.91 ^b	1.80 \pm 0.26 ^b	1.08 \pm 0.06 ^b
	BS	41.98 \pm 1.39 ^a	29.08 \pm 2.34 ^a	18.07 \pm 1.34 ^a	10.63 \pm 0.55 ^b	1.70 \pm 0.10 ^{bc}	1.39 \pm 0.08 ^b
Safflower oil III	CB	53.80 \pm 2.21 ^a	21.5 \pm 0.76 ^{ab}	21.25 \pm 1.86 ^a	3.45 \pm 0.47 ^a	6.43 \pm 0.79 ^a	0.87 \pm 0.09
	CL	56.18 \pm 2.52 ^a	22.8 \pm 0.57 ^a	17.08 \pm 2.34	3.95 \pm 0.16 ^a	4.26 \pm 0.49 ^a	0.79 \pm 0.08 ^a
	BS	50.23 \pm 1.68 ^{ab}	28.2 \pm 1.03 ^a	15.05 \pm 1.24 ^b	7.25 \pm 0.93 ^a	2.19 \pm 0.32 ^b	0.99 \pm 0.06 ^a
Mustard oil IV	CB	54.07 \pm 1.59 ^a	21.0 \pm 0.48 ^b	14.22 \pm 0.73 ^b	14.34 \pm 0.87 ^b	1.04 \pm 0.09 ^b	0.91 \pm 0.04
	CL	47.8 \pm 3.03 ^b	21.1 \pm 0.85 ^a	16.03 \pm 4.10	14.77 \pm 1.96 ^c	1.12 \pm 0.36 ^b	1.11 \pm 0.13 ^b
	BS	47.03 \pm 1.36 ^c	29.67 \pm 1.67 ^a	10.23 \pm 0.72 ^c	13.03 \pm 1.01 ^b	0.80 \pm 0.12 ^c	1.14 \pm 0.05 ^{ab}

All are Mean \pm SEM. Number of observations > 3 pooled samples.

*Includes fatty acids 20:4 n-6; 22:4 n-6; 22:5 n-3 and 22:6 n-3 species.

Comparisons are made among the various regions of four groups and values in the vertical rows not showing common superscript are significantly different from each other at 5% level ($P < 0.05$) by analysis of variance.

Table 7. Distribution pattern of major fatty acid groups of myelin membrane phospholipids

Group	Brain region	Saturates	Monoenes	Total n-6	Total n-3	n6/n3
Groundnut oil I	CB	49.98 ± 2.84	41.12 ± 1.15 ^a	4.64 ± 0.79 ^a	4.18 ± 0.79	1.14 ± 0.14 ^a
	CL	47.47 ± 4.87	41.95 ± 5.95 ^a	6.95 ± 1.36 ^a	3.56 ± 0.67 ^a	2.26 ± 0.85 ^a
	BS	46.85 ± 3.05	45.55 ± 1.85 ^a	3.35 ± 0.95 ^a	4.55 ± 0.72	0.85 ± 0.36 ^a
Coconut oil II	CB	52.67 ± 7.56	37.77 ± 2.95 ^a	3.02 ± 0.05 ^b	2.05 ± 0.45	1.42 ± 0.45 ^b
	CL	49.37 ± 2.79	37.87 ± 1.52 ^a	3.77 ± 0.61 ^b	3.91 ± 0.75	0.95 ± 0.22 ^b
	BS	42.27 ± 3.66	42.31 ± 1.06 ^a	3.44 ± 0.25 ^b	5.99 ± 0.93	0.64 ± 0.09 ^{ab}
Safflower oil III	CB	50.97 ± 2.38	33.31 ± 1.1 ^b	9.5 ± 0.56 ^{ab}	1.85 ± 0.25	5.3 ± 0.74 ^b
	CL	49.88 ± 3.05	39.88 ± 1.43 ^a	7.23 ± 0.99 ^b	4.61 ± 0.75	1.46 ± 0.08 ^b
	BS	43.94 ± 5.55	44.18 ± 0.13 ^a	6.6 ± 1.72 ^a	6.35 ± 0.68	1.13 ± 0.21 ^b
Mustard oil IV	CB	53.16 ± 4.5	40.83 ± 4.26 ^a	1.49 ± 0.27 ^c	3.20 ± 0.83	0.72 ± 0.12 ^c
	CL	48.6 ± 1.73	41.8 ± 0.71 ^{ab}	2.97 ± 0.44 ^b	7.31 ± 0.39	0.51 ± 0.09 ^b
	BS	43.06 ± 4.92	48.13 ± 4.48 ^a	4.39 ± 1.06 ^a	6.02 ± 1.39	0.87 ± 0.13 ^b

Values are Mean ± SEM. Number of observations ≥ 3 pooled samples.

Comparisons are made among the various regions of four groups and values in the vertical rows not showing common superscript are significantly different from each other at 5% level ($P < 0.05$) by analysis of variance.

existence of Na^+ , K^+ -ATPase in synaptic and myelin membranes and its regulation in the cation fluxes, we have chosen to determine activities of these membrane-bound enzymes in particular. Earlier reports showed that membranes with high fluidity or elevated unsaturated to saturated fatty acid ratio display higher Na^+ , K^+ -ATPase activity (Vajreswari *et al.*, 1990; Kimelberg and Papahadjopoulos, 1972). Our experiments showed significantly higher activity of Na^+ , K^+ -ATPase in the groups of animals fed safflower or groundnut oil which had relatively higher proportions of linoleic acid. Interestingly, coconut oil-fed group also showed similar synaptosomal Na^+ , K^+ -ATPase activity despite being EFA deficient. This may be explained by the observation that phospholipids of these membranes exhibited an adequate proportion of n-6 and n-3 fatty acids which favoured activity of the enzyme comparable to those fed dietary fats rich in essential fatty acids.

A relative decrease in the ratio of unsaturated fatty acids as seen in the case of dietary fat derived from coconut and groundnut oil (Table 2) resulted in higher activity of synaptosomal acetylcholinesterase as compared to those fed safflower or mustard oil diets rich in unsaturated fatty acids (Table 2). This observation corroborates the literature reports which showed an inverse relationship between acetylcholinesterase activity and membrane fluidity (Bloj *et al.*, 1973; Massa *et al.*, 1975).

Cholesterol, by virtue of its ability to inhibit molecular motion/conformational freedom within phospholipid bilayer, could also influence the activities of these enzymes. However, this possibility may not assume importance in this study because of the lack of differences in cholesterol to phospholipid molar

ratio among different membranes in response to feeding these dietary fats (data not shown).

Apart from influencing the catalytic activity of these enzymes in their respective membrane milieu, the possible direct or indirect effect of the dietary fatty acids on the induction/expression of the genes for these enzyme proteins cannot be ignored. For instance, the markedly elevated Na^+ , K^+ -ATPase activity of the synaptosomal and microsomal membrane fractions in the safflower oil-fed group could reflect an augmented gene expression for the enzyme protein in this group (Clarke and Jump, 1996; Takeuchi *et al.*, 1995).

Results of the present study also throw light on the prospects of dietary management of clinical conditions with known underlying enzymatic derangements. For example, Alzheimer's disease is characterised by depressed cholinergic transmission in brain, and safflower oil-based diet could be a better therapeutic choice by virtue of its ability to diminish brain acetylcholinesterase activity thereby enhancing cholinergic transmission. Furthermore, safflower oil-diet could result in augmented neural activity in brain by increasing synaptosomal Na^+ , K^+ -ATPase activity which leads to enhanced reuptake of the inhibitory neurotransmitter, gamma-aminobutyric acid (Iversen and Kelly, 1975). On the contrary, coconut oil-based diet could be contra-indicative in the management of Alzheimer's disease because it manifested augmented acetylcholinesterase activity and attenuated Na^+ , K^+ -ATPase activity in brain synaptosomes.

Whatever may be the implications of altered activities of membrane-bound enzymes on functional attributes of brain, data presented here show that depending on its fatty acid composition, the dietary fat

can regulate the activity of membrane-bound enzymes which are considered crucial for brain function.

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