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INFLUENCE OF DIETARY *n*-6/*n*-3 POLYUNSATURATED FATTY ACID BALANCE ON THE DEVELOPMENT OF TOLERANCE DURING CHRONIC ETHANOL INTOXICATION IN RATS

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Abstract — The present study addresses the possible interacting effects of dietary *n*-6/*n*-3 polyunsaturated fatty acid (PUFA) balance and chronic ethanol intoxication on the synaptic membrane responses to ethanol and the development of tolerance in rats. Wistar rats were fed either a standard lab chow or various semi-synthetic diets: rich in PUFA (from soya oil: SO), deficient in linolenate (from sunflower oil: SFO) or rich in long-chain (*n*-3) PUFA (cod liver oil: CLO). Male adult rats from the second specially fed generation were submitted to a 3-week alcoholization by daily intubation. Functional tolerance was quantified by the hypothermic response to a challenge dose of ethanol. Synaptic fluidity and sensitivity to ethanol (variations after acute ethanol addition) were assessed by fluorescence polarization (FP) of DPH, TMA-DPH or PROP-DPH. Membrane fatty acid composition was determined by GLC. The fatty acid composition of the synaptic membranes was influenced by the diet, but rearrangements among the lipids occurred, resulting in an apparent stability in brain membrane fluidity parameters. Nevertheless, clear-cut differences were noted in response to ethanol intoxication according to the diet. In the same period of time, rats fed SFO or CLO diets were unable to develop tolerance to ethanol at the membrane level as well as functionally, contrarily to the rats fed SO or standard diets. The structurally specific roles of PUFA are suggested by the negative membrane effects of the α-linolenate deficient diet (SFO) and the positive ones of a diet (SO) rich and well balanced in (*n*-3 + *n*-6) PUFA. Furthermore, the *n*-6/*n*-3 PUFA balance in the synaptic membrane needs to be kept within very narrow limits to allow normal development of the adaptive response to ethanol.

INTRODUCTION

Alterations of membrane physico-chemical properties are important primary effects of ethanol intoxication (Andreas, 1989). Although it is not fully understood how changes in the physical properties of membranes are coupled to the complex behavioural effects of ethanol (Golstein, 1987; Beaugé *et al.*, 1990*a*), distinct alterations of the synaptic membrane 'fluidity' on the one hand and of the sensitivity to acute ethanol-induced fluidization on the other, have been related to the development of behavioural and physical dependence on, or functional tolerance to, ethanol in animal models (Beaugé *et al.*, 1984; Le Bourhis *et al.*, 1986; Leguicher *et al.*, 1987).

Membrane lipids constitute the main part of the solid matter of the brain. Brain phospholipids contain long-chain polyunsaturated fatty acids (PUFA) of the two essential fatty acid classes (Bazan, 1990). Docosahexanoic acid (22: 6 *n*-3) is the predominant fatty acid; it or its *n*-3 fatty acid precursors must be provided in the diet (Bourre *et al.*, 1984). Dietary fat manipulation, such as changes in the *n*-6/*n*-3 polyunsaturated fatty acid ratio, can result in altered membrane composition, thereby dis-

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turbing membrane events (Greenwood *et al.*, 1989).

The present study addresses the possible interaction of *n*-6/*n*-3 PUFA balance with the brain membrane responses to ethanol in rats and especially the development of tolerance.

MATERIAL AND METHODS

Diets

Three different semi-synthetic diets, prepared by INRA (Durand *et al.*, 1986; Beaugé *et al.*, 1988*b*; Zérouga *et al.*, 1989) as well as a standard lab chow (Extra-labo, Ets Pietrement, France) were given to rats from a Wistar strain purchased from Iffa Credo (France). The compositions of the various diets are stated in Table 1. Diets included a vitamin supplement containing vitamin E (alpha-tocopherol) as an antioxidant. The purified diets varied, by the nature of the oil mixture, in the content of the *n*-3 PUFA and therefore in the *n*-6/*n*-3 ratio. Besides the standard diet, the first semi-synthetic diet, soya (S), contained soybean oil and was rich in *n*-3 PUFA (130 mg/100 g); the second diet, sunflower (Sf), contained sunflower oil and was deficient in *n*-3 (3 mg/100 g); whereas the third diet, cod liver oil (CLO), contained in fact ⅔ of oil as sunflower oil and ⅓ as cod liver oil, i.e. a supplementation with a source of long chain *n*-3 PUFA (150 mg/100 g). Diets were freshly prepared and changed every other day. No peroxidation of dietary fatty acids was detectable (Zérouga *et al.*, 1991).

Details of the fatty acid compositions following intakes of various diets are given in Table 2.

Animals

Adult males from the second generation fed with the different controlled semi-synthetic diets were used for chronic alcohol intoxication. The standard diet-fed rats constituted an additional group. Ethanol was administered for 3 weeks by gavage as previously described (Beaugé *et al.*, 1984; Leguicher *et al.*, 1987). The initial dose was 3 g/kg body wt and was increased by 0.5 g/kg every 3 days. Pair-fed control rats received starch in a dose isocaloric with that of ethanol and were given the same amount of diet (semi-synthetic or standard) as their corresponding ethanol-treated rats. Animals were housed individually in plastic cages.

Table 1. Composition of the various experimental diets

Ingredients (g/100 g)	Diets			
	Standard	Soya	Sunflower	Cod liver
Starch	—	45.94	46.24	45.84
Casein	22.00	22.00	22.00	22.00
DL Methionine	0.16	0.16	0.16	0.16
Vitamin mixture*	1.00	1.00	1.00	1.00
Mineral mixture	4.00	4.00	4.00	4.00
Cellulose	4.00	2.00	2.00	2.00
Sucrose	—	23.00	23.00	23.00
Lipidic mixture	5.00	—	—	—
Soybean oil	—	1.9	—	—
Sunflower oil	—	—	1.6	1.3
Cod liver oil	—	—	—	0.7

*Composition of the vitamin mixture (U.S. Biochem. Corp., Cleveland, OH, U.S.A.) triturated in dextrose: in g/kg: α-tocopherol (1000 I.U/g): 5.0; L-ascorbic acid: 45.0; choline chloride: 75.0; D-calcium pantothenate: 3.0; inositol: 5.0; menadione: 2.25; niacin: 4.5; *p*-aminobenzoic acid: 5.0; pyridoxine HCl: 1.0; thiamin HCl: 1.0; vitamin A acetate: 900,000 units; vitamin D_3: 100,000 units; in μg/kg: biotin: 20; folic acid: 90, vitamin B_{12}: 1.35.

Table 2. Indices of lipid alteration in membranes from rats maintained on various diets and chronically ethanol intoxicated: comparison with dietary oils

Diets	Standard	Soya	Sunflower	Cod liver
Dietary oils				
Σsat/Σunsat	0.51	0.19	0.13	0.15
DBI/Σsat	3.30	9.70	13.60	12.90
n-6/*n*-3	14.00	7.30	344.00	6.20
Σ*n*-6+*n*-3	32.70	60.90	70.00	57.20
Controls	(7)	(5)	(5)	(7)
Synaptic FA composition				
Σsat/Σunsat	0.80 ± 0.01	0.84 ± 0.02	0.81 ± 0.02	0.78 ± 0.01
DBI/Σsat	3.78 ± 0.04	3.78 ± 0.08	3.85 ± 0.09	3.97 ± 0.08
n-6/*n*-3	0.97 ± 0.02	1.10 ± 0.04	2.70 ± 0.10	0.70 ± 0.01
Σ*n*-6+*n*-3	29.92 ± 0.42	29.00 ± 0.60	31.30 ± 0.69	28.05 ± 0.58
Cholesterol/phospholipids	0.57 ± 0.03	0.57 ± 0.01	0.60 ± 0.01	0.61 ± 0.04
Alcohols	(7)	(5)	(5)	(7)
Synaptic FA composition				
Σsat/Σunsat	0.85 ± 0.02*	0.83 ± 0.04	0.90 ± 0.04***	0.81 ± 0.03*
DBI/Σsat	3.37 ± 0.09***	3.80 ± 0.10	3.11 ± 0.03***	3.72 ± 0.18*
n-6/*n*-3	1.10 ± 0.03	1.06 ± 0.03	2.50 ± 0.02	0.79 ± 0.03
Σ*n*-6+*n*-3	26.70 ± 0.81**	29.20 ± 0.57	25.70 ± 0.09***	27.50 ± 1.08*
Cholesterol/phospholipids	0.71 ± 0.04***	0.65 ± 0.02***	0.61 ± 0.01	0.64 ± 0.04

The values given for the dietary oils represent the average obtained from at least three separate extractions and analysis of the respective diets and are relative amounts, expressed as a percentage of the total identified fatty acids by weight.
DBI: Double bond index (percent occurrence × number of double bonds).
The values for the membrane lipids are means ± SD. The number of tested animals is shown in parentheses.
Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$ (alcohol group vs control group).

humidity and temperature were kept constant at 65% and 21°C, respectively, with a 12 hr light/12 hr dark cycle.

After 3 weeks of treatment, the animals' tolerance to ethanol was assessed by the hypothermic response to a challenge dose of ethanol (Beaugé *et al.*, 1984; Leguicher *et al.*, 1987).

At the end of the intoxication period, the rats were killed by decapitation. Synaptic membranes were prepared and their purity determined by electron microscopy and enzyme markers as previously described (Le Bourhis *et al.*, 1986; Leguicher *et al.*, 1987).

Biochemical analyses

The synaptic membrane lipids were extracted according to Rose and Oklander (1965). Phospholipid concentrations were quantified by phosphorus determination (Chen *et al.*, 1956) and cholesterol by an enzymatic method (Siedel *et al.*, 1983). After extra washing of the lipid fraction and conversion to the fatty acid methyl esters according to Morrison and Smith (1964), they were analyzed by capillary column gas chromatography (Zérouga *et al.*, 1991).

Membrane fluidity

The membrane fluidity or micro-organization was assessed on aliquots after protein determination (Lowry *et al.*, 1951) by steady-state fluorescence polarization of the fluorophore DPH and its polar derivatives.

TMA-DPH and PROP-DPH (Wood and Schroeder, 1988; Beaugé *et al.*, 1988*b*; Zérouga *et al.*, 1989; Beaugé *et al.*, 1990*b*). The degree of fluorescence polarization P of each probe was determined at 25.00 ± 0.10°C using a T-format 'Fluofluidimetre' SEFAM (Nancy, France). P is related to the order parameter in membrane lipids (Shinitzky and Inbar, 1976). High P values represent high structural order or low membrane fluidity and vice versa. Besides basal fluidity, membrane sensitivity to ethanol was tested after addition *in vitro* of ethanol (0.1–1 M) to the membranes. The slope of the regression line ΔP thus obtained was taken as a measure of membrane tolerance (Leguicher *et al.*, 1987; Beaugé, 1991).

All testing was done with the experimenter blind to the treatment condition of the animal.

Statistical analyses

Student's t-test was used. Data are expressed as means and standard deviations (SD). When SD is not given, it is less than 10%.

RESULTS

Animals

Food consumption and weight gain were similar in the three dietary groups and comparable with those elicited by the standard lab chow. As alcoholization resulted in a decrease in food consumption, the control diet groups have been restricted in the same proportion as the alcohol ones. Nevertheless, all animals either maintained or slightly gained weight, and their general appearance remained normal throughout the alcoholization period.

Relation with fatty acid composition

Distinctive patterns in the percentage of fatty acids in membrane preparations were seen in response to each of the dietary treatments in all rat groups. Table 2 lists some indices of lipid differences in synaptic membranes thought to be of importance in the regulation of membrane fluidity. Individual results concerning the FA composition have been given in detail elsewhere (Zérouga *et al.*, 1991). The various diet oils displayed very different indices as regards total amounts of saturated fatty acids and of PUFA, double-bond index and especially *n*-6/*n*-3 ratios, which varied from 6.2 with CLO to 344.0 with sunflower oil. The same parameters were not statistically different in the synaptic membranes of the studied animals fed the various diets, except the *n*-6/*n*-3 ratio, which varied from 0.7 with CLO to 2.8 with Sf. The cholesterol/phospholipids ratio was also similar in the membranes from the animals fed the various diets.

Alcoholization had clear-cut effects on these indices (Table 2). The amount of saturated fatty acids was increased, the double bond index was decreased and so was the total amount of PUFA in the synaptic membranes of the alcohol-treated animals. The extent of these disturbances depended on the diet. The membranes from animals fed the sunflower diet were the most affected and those from animals fed the soya diet were almost totally unaffected. The alterations in the *n*-6/*n*-3 ratio were more differentiated. The ratio was decreased by 4% with sunflower diet, increased with the standard diet and the CLO diet and remained unaltered with the soya diet. In addition, the cholesterol/phospholipids ratio (as well as the cholesterol content itself) was increased only with the standard and soya diets.

Fluorescence polarization results

The results concerning the fluidity parameter changes after 3 weeks of alcoholization are shown in Table 3. Irrespective of the diet consumed, there was an apparent maintenance of the overall basal or intrinsic fluidity at each probe level and no statistically significant differences from the results obtained with the usual lab chow (standard diet) were observed. Nevertheless, the membranes from the rats fed the sunflower diet were less organized in the pure lipidic region than those from rats fed the soya diet [P (DPH) statistically lower by 3%] as previously found (Beaugé *et al.*, 1988*b*).

In response to ethanol intoxication, some differences appeared. With the animals fed a standard lab chow, changes of the intrinsic degree of polarization in rat synaptic membranes were more marked than previously

Table 3. Fluorescence polarization of DPH probes in synaptic membranes from rats fed the various diets

Probe	Alcohol treatment	Diets			
		Standard	Soya	Sunflower	Cod liver
P(DPH)	−	0.331 ± 0.010	0.336 ± 0.002	0.327 ± 0.010	0.325 ± 0.009
ΔP(DPH)	−	0.0240 ± 0.0051	0.0214 ± 0.0045	0.0233 ± 0.0027	0.0209 ± 0.0037
P(DPH)	+	0.345 ± 0.005***	0.337 ± 0.008	0.339 ± 0.007***	0.340 ± 0.006***
ΔP(DPH)	+	0.0150 ± 0.0035***	0.0152 ± 0.0022**	0.0198 ± 0.0022	0.00217 ± 0.004
P(TMA-DPH)	−	0.359 ± 0.006	0.350 ± 0.004	0.357 ± 0.003	0.352 ± 0.007
ΔP(TMA-DPH)	−	0.0112 ± 0.0034	0.0048 ± 0.0008	0.0062 ± 0.0016	0.0119 ± 0.0048
P(TMA-DPH)	+	0.351 ± 0.002*	0.351 ± 0.005	0.364 ± 0.003**	0.351 ± 0.011
ΔP(TMA-DPH)	+	0.0062 ± 0.0019*	0.0029 ± 0.0011*	0.0051 ± 0.0019	0.0083 ± 0.005
P(PROP-DPH)	−	0.368 ± 0.004	0.372 ± 0.002	0.372 ± 0.005	0.369 ± 0.005
ΔP(PROP-DPH)	−	0.0111 ± 0.0024	0.0087 ± 0.0012	0.0062 ± 0.0013	0.0119 ± 0.0048
P(PROP-DPH)	+	0.374 ± 0.004	0.373 ± 0.003	0.377 ± 0.001	0.376 ± 0.006
ΔP(PROP-DPH)	+	0.0071 ± 0.0024*	0.0065 ± 0.0016	0.0071 ± 0.0014	0.0089 ± 0.0019

The results are expressed as in Table 1.
Twelve animals were fed the standard diet, of which six were alcohol treated.
Ten animals were fed the soya or the sunflower diet, of which five were alcohol treated.
Fourteen animals were fed the cod liver diet, of which seven were alcohol treated.

observed. P (DPH) was significantly increased by 4% whereas P (TMA-DPH) was decreased (−2%) and P (PRO-DPH) unchanged. The polar part of the membranes is more organized than the apolar one, as seen by the higher P values and the membrane region probed by PROP-DPH is definitively different (more rigid) from the one probed by TMA-DPH. We have already reported differentiated disturbances of the polar part of the membranes after alcoholization (Beaugé *et al.*, 1990*c*; Stibler *et al.*, 1991). Taking all groups into consideration, P (DPH) was increased by about 4%, except in animals fed the soya diet. If a slight decrease in P (TMA-DPH) was found with the standard diet, the sunflower diet gave a significant increase in this parameter. No changes at all were seen at the PROP-DPH level.

In alcohol-naïve membranes, no actual differences were found in the sensitivity of the membrane to the acute fluidizing effect of ethanol. The development of a synaptic (and erythrocyte) membrane resistance to the acute fluidizing effect of ethanol has been the most consistent finding after chronic alcohol intoxication (Chin and Goldstein, 1977). This tolerance effect was seen here in the standard group, at the DPH level (38%) as well as at the TMA-DPH and the PROP-DPH levels. Nevertheless, only the soya diet allowed the development of a significant resistance (more than 30% difference with the alcohol-naïve membranes) at the DPH and TMA-DPH levels, as found with the standard diet. The membranes from rats fed the sunflower, with or without CLO, were still very sensitive to ethanol after 3 weeks of alcoholization (Table 3). The protocol used here does not permit us to know whether it is only a delay in, or an actual impediment to, the development of membrane tolerance.

Functional tolerance to ethanol

The importance of the development of tolerance to the hypothermic effect induced by intraperitoneal (i.p.) administration of a test dose of ethanol after ethanol intoxication with the diverse diets is shown in Fig. 1. Rectal temperatures under baseline conditions were found not to differ significantly among the dietary groups. The maximal drop in tempera-

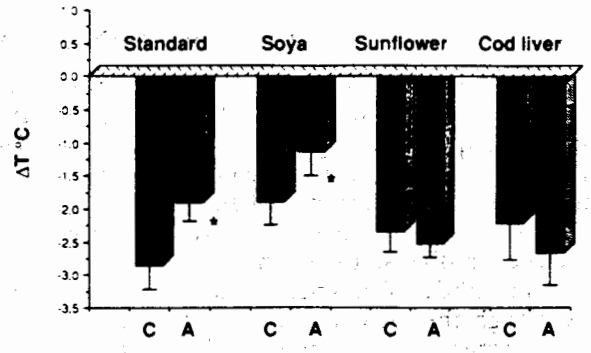

Fig. 1. Maximal drop in body temperature following acute i.p. administration of a challenge dose (3 g/kg) of ethanol to rats fed the various diets and after 3 weeks of chronic alcohol intoxication by daily gavage. ΔT is the maximal drop in body temperature (°C). Results are expressed as means and the bars represent SD. The number of animals are the same as in Table 3.

ture (ΔT_{max}) occurred during the first hour after ethanol injection. In control rats, as previously found, the soya-fed animals were slightly less sensitive to acute ethanol than the other dietary groups (ΔT_{max}: −1.9°C versus −2.9, 2.4 and 2.3 in the standard, sunflower and CLO groups, respectively). In ethanol-treated rats, ΔT_{max} was decreased by 30 to 40% in standard and soya-fed rats, but slightly increased in the sunflower groups. As well as membrane tolerance [represented as ΔP (DPH) in Fig. 2], tolerance to the hypothermic effect of acute ethanol injection had not developed over the 3 weeks in the sunflower-fed animals.

DISCUSSION

The primary aim of this study was to ascertain the influence of dietary PUFA on neuronal membrane organization and function, as assessed by fluidity measurements, which can affect the membrane responses to alcohol and alcoholization. Chronic ethanol intoxication exerts effects on the fluidity of these membranes, the extent of which has been correlated with the behaviours of tolerance to and dependence on the drug (Beaugé *et al.*, 1984; Le Bourhis *et al.*, 1986, Leguicher *et al.*, 1987, Beaugé *et al.*, 1990*a*).

Different dietary levels of *n*-3 PUFA have

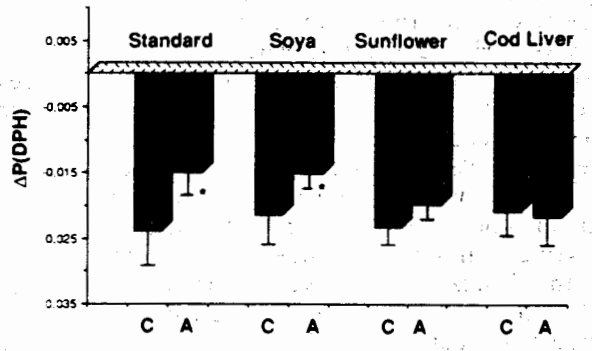

Fig. 2. Synaptic membrane sensitivity to acute ethanol addition [ΔP (DPH)] after 3 weeks of chronic alcohol intoxication by daily gavage in adult male rats fed with the various diets. ΔP (DPH) is the slope of the linear variation of the degree of polarization of DPH (P) after acute *in vitro* addition of ethanol (0.1 to 1 M). Results are from the same animals and are expressed as in Fig. 1.

been given to male Wistar rats through two generations. The total dietary fat was nutritionally adequate in all groups, avoiding overt symptoms of essential fatty acid (EFA) malnutrition even after chronic ethanol treatment, which is known to enhance markers of EFA deficiency in diverse tissue phospholipids (Sun and Sun, 1985; Wainwright *et al.*, 1989).

Although the fatty acid composition of the cell membrane is susceptible to dietary manipulations, the induced changes occur within very narrow limits (Gibson *et al.*, 1984). The very different contents in saturated and polyunsaturated fatty acids, DBI, and *n*-6/*n*-3 ratios found in the three semi-synthetic diets used here are not reflected in the synaptic fatty acid composition of the animals fed these diets. The only significant differences are the changes of the *n*-6/*n*-3 ratios and to a lesser extent in the total long chain PUFA percentages. An influence of metabolic changes due to the different intakes, rather than a direct dietary effect could be suggested.

Along with this fine tuning of the membrane fatty acids profile, there is an apparent maintenance of the overall fluidity reflecting mainly the structural order at each level probed into the membrane. Despite the limited diet-related alterations, clear-cut differences were noted in response to ethanol intoxication of the rats receiving the various diets. The effect of alcoholization is clearly different between the lipid core, which became less fluid and less sensitive, and the polar region probed by TMA-DPH and PROP-DPH, also less sensitive, but with an unchanged or slightly increased intrinsic fluidity (Hitzemann *et al.*, 1986; Beaugé *et al.*, 1988*a*; Beaugé and Zérouga, 1989). This complex rearrangement is probably not only the result of an adaptive phenomenon and the rigidification when observed cannot be considered as the inverse of the fluidization achieved by acute ethanol (Beaugé *et al.*, 1990*a*). The alcoholization impact is greater in the lipid core, probed by DPH. It has recently been suggested that *n*-6 and *n*-3 PUFA have structurally specific roles depending on their particular double bond distribution, which gives them special conformation and thus their relative percentages could change to a small extent the membrane fluidity (Belcher *et al.*, 1986; Ehringer *et al.*, 1990; Léger *et al.*, 1990). Nevertheless, the decreases in the unsaturation state of the membrane, in the double-bond index and in the total amount of *n*-6 + *n*-3 PUFA could partly explain the increase in *P* (DPH), possible index of dependence (Le Bourhis *et al.*, 1986), observed with all tested diets except soya (Zérouga *et al.*, 1991). The metabolic effects evoked earlier are probably amplified by the alcohol treatment.

The protocol used here was not suitable for assessing the state of dependence of the animals. Nevertheless, we have found, in separate experiments, that a high percentage (70% and more) of the animals fed the sunflower diet, whether or not supplemented with cod liver oil, developed behavioural dependence, versus 50% of the animals fed soya diet (Le Bourhis *et al.*, 1986; Aufrère *et al.*, 1990).

In spite of the apparent fine regulation of the membrane fluidity, differences are noted when a gross membrane property is monitored. Thus, the sensitivity of synaptosomal membranes to the disruptive effects of acute alcohol addition on membrane order was very differentially affected after ethanol intoxication, depending on the concomitant diet. Over the 3 weeks of chronic ethanol treatment, a resistance to fluidization occurred in the animals fed the standard or the soya diets, but surprisingly, no resistance developed with the two diets containing sunflower oil.

An increase in membrane cholesterol content may be considered as one of the adaptive responses to the continued presence of ethanol. The acute fluidizing effect of ethanol is closely linked to the partitioning of ethanol in the membrane (Leguicher *et al.*, 1987; Lalitha *et al.*, 1990; Chiou *et al.*, 1991) and can be considered to reflect the permeability of the membrane to ethanol. As cholesterol is known to decrease the partition of lipid-soluble substances into membranes, the increase in cholesterol in alcohol-treated animals may be related to a decrease in the partition of alcohol into these membranes. Furthermore, as also indicated in recent work (Ehringer *et al.*, 1991) the position of acyl chain unsaturation has a great importance in regulating membrane permeability.

As in our previous work (Beaugé *et al.*, 1984, 1990*a*; Leguicher *et al.*, 1987; Beaugé, 1991), we did find a clear parallel between the two tolerance phenomena, synaptic membrane tolerance (as seen by ΔP (DPH), synaptic membrane sensitivity to acute ethanol addition) and the whole animal's functional tolerance (as assessed by the hypothermic effect of acute ethanol injection). After chronic alcoholization, the animals fed the sunflower or the CLO diet did not develop tolerance to ethanol functionally as well as at the synaptic membrane level, contrarily to those fed the standard or soya diets.

The relationship between membrane fluidity and sensitivity on the one hand, and membrane fatty acid composition on the other, is far from simple. Nevertheless, PUFA appear to have some relatively non-specific beneficial effects possibly through membrane stabilization, and an *n*-6/*n*-3 PUFA ratio well-balanced and maintained within very narrow limits seems to be necessary to allow an adaptive response of tolerance to ethanol.

In conclusion, several recent studies, as well as the present one, suggest a major role of nutritional status in alcohol-related effects in experimental animals and also in man (Glen *et al.*, 1987; Laksman *et al.*, 1988; Wainwright *et al.*, 1989; Lalitha *et al.*, 1990). Regardless of the exact mechanism, these results emphasize the importance of dietary supply on synaptic membrane sensitivity in connection with animal response to ethanol.

A better understanding of the mechanisms might lead to the development of effective therapies to influence alcohol tolerance and perhaps, to moderate excessive alcohol consumption.

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