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CHRONIC ETHANOL ADMINISTRATION INDUCED AN INCREASE IN PHOSPHATIDYLSERINE IN GUINEA PIG SYNAPTIC PLASMA MEMBRANES

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Chronic ethanol administration to guinea pigs via intragastric intubation elicited a specific increase (50%) in phosphatidylserine in the synaptic plasma membrane. The ethanol-treated group also showed a 53% increase in synaptosomal (Na,K)-ATPase activity. Analysis of the acyl group composition of individual phospholipids in the same membrane fraction revealed only small changes which varied depending on the type of phospholipids. Since the (Na,K)-ATPase is known to be specifically activated by phosphatidylserine, the adaptive increase in enzyme activity during chronic ethanol treatment may be related to the increase in this type of negatively charged phospholipid.

Ethanol is known to exert its anesthetic potency and CNS depressant activity by interacting with the lipid portion of the neuronal membranes (1,2). Furthermore, biological membranes appear to adapt readily to the fluidizing effect of ethanol by altering their membrane lipid composition. Some examples are the changes in cholesterol/phospholipid ratio (3), and alteration in fatty acids of membrane phospholipids (4-6).

Since many membrane-bound enzymes are dependent on the membrane integrity and lipid environment for activity, changes in lipid composition can be an underlying cause for the altered membrane activities after chronic ethanol aministration. An example of this adaptive change is the increase in (Na,K)-ATPase activity in brain synaptosomes (7,8). In spite of previous studies on ethanol-induced changes in membrane parameters, the

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possibility of a change in individual phospholipids has not been eluted. In this study, we present evidence that chronic ethanol administration elicited an increase in phosphatidylserine in SPM of guinea pig cortex. Possible implications of the increase in this negatively charged phospholipid with respect to the adaptive increase in (Na,K)-ATPase activity as well as other membrane functions are discussed.

METHODS AND MATERIALS

A group of twelve male guinea pigs approximately 300 gm were reared in Sinclair Animal Research Facilities under standard conditions with water and lab chow given *ad lib*. At the time of experiment, animals in ethanol group received twice daily a total of 6 gm/kg of ethanol in Lieber-DeCarli liquid diet (711-C, Bioserve, Frenchtown, NJ) via intragastric intubation. The controls were given the same amount of liquid diet except ethanol was substituted with an isocaloric amount of sucrose. The dietary scheme was continued for two weeks during which animals were also given lab chow and water *ad lib*. The ethanol group developed tolerance to the ethanol diet, but body weight of animals was not altered during the administration period.

After ethanol administration, food and ethanol were withdrawn for 14 hr before animals were sacrificed by decapitation. Immediately after decapitation, animal heads were submersed into liquid nitrogen for 35 sec in order to freeze the brain. The cerebral cortex was removed and brain tissue was homogenized in 10 vol of 0.32 M sucrose with 50 mM Tris-HCl (pH 7.4). A portion of the brain homogenate was then taken for subcellular fractionation by differential and sucrose gradient centrifugation. The procedures for isolation of synaptosomes and subsequently the SPM have been described (9).

Synaptosomes were taken for determination of (Na,K)-ATPase activity according to Sun (10). A portion of the SPM was taken for protein determination using Folin reagent with bovine serum albumin as standard (11). Lipids from the plasma membranes were extracted with chloroform/methanol (2:1, v/v) according to the procedure of Folch et al. (12). After phase separation, the organic phase was taken to dryness using a rotary evaporator. Lipids were redissolved in chloroform and spotted on a TLC plate precoated with silica gel G (Analtech, Newark, DE). Lipids were separated by the separation-HCl reaction-separation procedure as described by Horrocks and Sun (13). After solvent development, individual phospholipid spots were scraped into test tubes and the acyl groups were converted to methyl esters by base-methanolysis using 0.5 N NaOH-methanol as the reagent (14). An internal standard was added to each sample. The methyl esters were analyzed by GLC using a Hewlett Packard 5840A research gas chromatograph equipped with dual flame ionization detectors and automatic integrator devices. Conditions for methyl ester analysis were similar to that described earlier (15). This procedure also gives quantitative data for the fatty acids yielded from individual phospholipids.

Table 1. Acyl group composition (%, wt) of individual phospholipids in guinea pig synaptic plasma membranes

Acyl groups	PC		PE		PS		PI	
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
14:0	0.61 (0.16)	0.54 (0.14)	-	-	-	-	-	-
16:0	45.95 (1.32)	48.62* (1.63)	8.39 (1.33)	7.62 (1.04)	3.14 (0.26)	1.78 (0.19)	6.07 (1.85)	6.31 (1.25)
18:0	10.79 (0.50)	9.94 (0.95)	33.65 (0.71)	33.36 (2.22)	40.11 (1.01)	40.60 (0.57)	47.53 (0.83)	47.48 (0.90)
18:1	29.19 (1.24)	28.64 (1.29)	12.18 (1.40)	12.08 (1.53)	19.62 (3.37)	18.13 (1.98)	-	-
18:2	1.68 (0.29)	2.46* (0.35)	0.88 (0.19)	1.43* (0.28)	-	-	-	-
20:1	0.82 (0.08)	0.81 (0.09)	0.70 (0.14)	0.66 (0.11)	0.95 (0.14)	0.87 (0.15)	-	-
20:3	0.22 (0.01)	0.34 (0.10)	-	-	-	-	-	-
20:3	0.19 (0.04)	0.20 (0.06)	0.39 (0.01)	0.42 (0.21)	1.15 (0.29)	0.85 (0.57)	-	-
20:4	5.02 (0.74)	3.89 (0.73)	14.80 (1.20)	14.83 (0.79)	3.77 (0.63)	3.72 (0.61)	43.52 (1.83)	43.60 (2.19)
22:4	1.23 (0.14)	1.02 (0.14)	5.82 (0.59)	5.70 (0.69)	5.90 (0.63)	5.82 (0.20)	2.02 (1.02)	1.92 (0.12)
22:5	1.30 (0.35)	1.06 (0.18)	6.94 (0.99)	8.35 (1.25)	8.40 (1.22)	9.63 (1.58)	-	-
22:6	2.8/ (0.69)	2.40 (0.51)	16.28 (0.51)	17.26 (0.78)	17.22 (1.83)	17.87 (0.78)	1.46 (0.08)	1.30 (0.25)
	(n=4)	(n=4)	(n=6)	(n=5)	(n=3)	(n=4)	(n=4)	(n=4)

Phospholipids from guinea pig SPM were separated by two-dimensional TLC as described by Horrocks and Sun (14).
Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol.
* denotes values which are significantly different from controls $p<0.05$.

RESULTS AND DISCUSSION

The acyl group composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) from SPM of control and ethanol treated guinea pigs is shown in Table 1. As reported previously, each phospholipid has its characteristic acyl group profile (9,15). In particular, a distinction in acyl group profile is shown between PI and PS in that acyl groups of PI are enriched in 20:4 whereas the acyl groups of PS are highly enriched in 22:5 and 22:6. Although 18:2 is only present in trace quantity in PC and PE, there is an obvious increase in the proportion of this fatty acid with respect to chronic ethanol administration. Other types

of acyl group changes include an increase in 16:0 and a decrease in 20:4 which are shown only with PC. Although some increase in 22:5 and 22:6 was also observed in PE and PS with respect to the ethanol-treatment, these differences are statistically not significant. In a similar study reported previously (5), we observed a decrease in the proportion of 18:1 in PC with respect to the ethanol-treatment. However, this acyl group change is not found in the present study, although the increase in 22:5 and 22:6 in PE seems to prevail.

When the relative amount of PE present in SPM is expressed as a ratio against PC, there is no difference in the ratios between the controls and the ethanol-treated groups (Table 2). On the other hand, a significant increase ($p<0.001$) in PS/PC (46%) and PS/PE (51%) ratios is shown in the ethanol group as compared to control. The increase seems to be specific for PS because no change in PI/PC or PI/PE ratios in either controls or ethanol-treated groups is observed.

The ethanol-induced increase in PS appears to correlate well with the increase (53%) in (Na,K)-ATPase activity in synaptosomes (Table 2). Studies in the past have correlated (Na,K)-ATPase activity with acyl chain fluidity and membrane phospholipids (16,17). In other studies, PS is known to be the best negatively charged phospholipid for activation of this trans-membrane enzyme (18-20). Floreani et al. (21) also demonstrated an increase in (Na,K)-ATPase activity in brain synaptosomes by interacting with PS vesicles. Since (Na,K)-ATPase is especially sensitive to inhibition by phospholipase A_2 which preferentially removes the polyunsaturated fatty acids from the phospholipids (22), an increase in PS in the SPM may constitute a more fluid microenvironment for active functioning of the enzyme.

Table 2. Phospholipid ratios from guinea pig cortex synaptic plasma membranes and synaptosomal (Na,K)-ATPase activity

Phospholipid ratios	Control	Ethanol	% change	Significance
PE/PC	0.502 ± 0.068 (n=5)	0.508 ± 0.067 (n=4)	—	N.S.
PS/PC	0.556 ± 0.064 (n=5)	0.810 ± 0.068 (n=4)	+ 45.7	$p<0.005$
PS/PE	1.108 ± 0.234 (n=5)	1.675 ± 0.175 (n=4)	+ 51.2	$p<0.01$
PI/PC	0.282 ± 0.033 (n=5)	0.292 ± 0.042 (n=5)	—	N.S.
PI/PE	0.545 ± 0.029 (n=4)	0.546 ± 0.082 (n=5)	—	N.S.
PS/PI	1.817 ± 0.219 (n=3)	2.955 ± 0.370 (n=4)	+ 62.6	$p<0.005$
	Synaptosomal (Na,K)-ATPase activity (umole Pi/min/mg protein)			
	3.14 ± 0.53 (n=6)	4.81 ± 0.81 (n=5)	+ 53.2	$p<0.005$

Phospholipid ratios are based on the amount of fatty acid methyl esters yielded from the individual phospholipids. Methyl esters were analyzed by GLC with an internal standard. Values are evaluated by analysis of variance. N.S., not significant.

The biological significance of PS in neural membranes should not be neglected. Besides its intimate association with (Na,K)-ATPase, this phospholipid is also important in mediating the Ca^{++}-dependent phosphorylation of soluble proteins (23), opiate receptor binding (24,25), stimulation of tyrosine hydroxylase activity (26) and acetylcholine synthesis (27). PS is known to form a complex with Ca^{++} (28), and liposomes made of PS are excellent vehicles for transporting drugs to organ target sites (29). These liposomes are also capable to fuse with cellular membrane and subsequently influence other membrane activities (30,31).

Although chronic ethanol administration is known to elicit a change in membrane cholesterol/phospholipid ratio (3) or modification of membrane phospholipid acyl groups (4), other (32) have concluded that neither of these changes can completely account

for the adaptation to ethanol-induced membrane disordering effect. It is possible that ethanol may exert a more direct effect on membrane lipids by altering specific type of phospholipids such as the cardiolipin in mitochondria (6), and in this case, an increase in PS in SPM. Nevertheless, implication of this lipid change in relation to other membrane functions should be further explored.

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