Chapter 6

Effect of Picrotoxin-induced Epileptic Condition and Antiepileptic Drug Treatment on Rat Liver Mitochondrial Lipids

Introduction

Liver is the central metabolic organ of the body. Hepatic mitochondrial function is important to maintain the energy status, β-oxidation, TCA cycle and apoptotic activity of the cell (1). Calcium regulated machinery i.e. either downstream process of signal transduction or pro- and anti-apoptotic mechanisms involves functioning of mitochondrion (2, 3). Severe epileptic condition could affect functioning of the aforementioned system. 'Progressive neuronal degeneration of childhood' – a mitochondrial respiratory chain defects have been demonstrated in a number of patients who die of liver failure following severe epilepsy (4). Liver mitochondrial dysfunction of the energy generating system is suggested in pediatric patients with epileptic phenotype (5). Hepatic and pancreatic insufficiency is reported to be associated with Leigh's syndrome characterized by epilepsy and muscle weakness (6).

On the other hand, treatment with AEDs could affect hepatic function by interfering with subcellular systems. Phenobarbital treatment caused oxidative changes in mitochondria and microsomes that are related with alterations in cytochrome P450 levels in rat liver (7). Accumulated evidences suggested that treatment with diazepam, valproic acid, phenobarbitone, phenytoin etc. could lead to severe alterations in functioning of important mitochondrial membrane bound enzymes, oxidative phosphorylation and ultra structure of mitochondrial and RBC membranes as well (8-16). Varying degree of drug induced changes is observed in plasma vitamin levels, protein and fine structure of

hepatocytes in epileptic patients treated with phenobarbitone (17). However, reports on mitochondrial membrane structure and function are lacking for the AEDs under study viz. CBZ, LTG and CLB.

It was shown in Chapter 2 that PTX-induced epileptic condition and treatment with AEDs adversely affected with oxidative phosphorylation in rat liver mitochondria as well as the mitochondrial F_oF_1ATPase kinetic properties (Chapter 4). As mentioned above, the generalized effects of epileptic condition and AEDs treatment on hepatic function are reported, though detailed investigations on mitochondrial lipid/phospholipid compositions and membrane fluidity characteristics have not been reported so far. It was therefore of interest to find out if and what manner the PTX-induced convulsions and treatment with the three AEDs (CBZ, LTG and CLB) affected the mitochondrial lipid/phospholipid profiles. The results of these studies are summarized in this chapter.

Material and methods

Details of chemicals used are as described in Chapter 5.

The treatment with PTX and AEDs were as shown in Chapter 2 and 3. The animals were killed by decapitation after the end of treatment period (i.e. on the day 8^{th} after AEDs treatment and day 21^{st} after PTX or PTX-AEDs treatment). Isolation of mitochondria was essentially the same as described in Chapter2.

Lipid extraction from mitochondrial membrane, separation of phospholipid classes by TLC and determination of membrane fluidity were essentially the same as described in Chapter 5.

Protein estimation was done by the method of Lowry et al. (1951) with BSA as the standard (18).

Results are given as mean ± SEM.

Statistical evaluation of the data was performed using the Students' *t*-test.

Results and Discussion

The results on the effects of PTX treatment on the total phospholipid (TPL) and cholesterol (CHL) content of rat liver mitochondria are summarized in Table 1. As it is evident, CHL content increased by 1.2 fold therefore composite decrease in TPL / CHL molar ratio was observed. Mitochondrial membrane more fluidized after PTX treatment (Table 1).

Further analysis of phospholipid composition of mitochondrial membrane after PTX treatment showed 1.3 fold higher lysophospholipid (Lyso) component while phosphatidylinositol (PI) and diphosphatidylglycerol (DPG) component was decreased by 16-31% (Table 2, 3). From the data presented in Table 3, PTX treatment showed 1.2 to 1.5 fold increase in Lyso and phosohatidylcholine (PC) content with 29% reduction in DPG content. Therefore, PTX treatment resulted in marginal changes in the composition and individual content of phospholipids with fluidization of the mitochondrial membrane (Table 1-3).

CBZ treatment to the control animals resulted into 1.6 to 4 folds increase in acidic phospholipids components (PS, PI and DPG) with decreased PC and PE components (21 to 39% decrease, Table 7 and 8). Similar increase in PS and PI by 1.9 to 2.5 fold was also observed with decreased DPG after LTG treatment to the control animals (Table 9 and 10). Opposite trend was seen for PS, PI and DPG after CLB treatment (Table 11 and 12). General increase (1.5 to 4 folds) in Lyso and SPM was noted after AEDs treatment to the control animals (Table 7, 9, 11). The individual phospholipids content followed the similar pattern after AEDs treatment to the control animals (Tale 8, 10, 12) All the three AEDs caused decrease in TPL content by 12 to 26%, thereby lowering of TPL/CHL ratio (Table 4-6).

CBZ treatment to the epileptic animals resulted into 1.4 to 4 folds elevation in Lyso, SPM, PS and PI with decreased PC and DPG components (37 to 50% decrease) (Table 7,

Table 1: Effect of PTX treatment on total phospholipid and cholesterol content, and membrane fluidity of liver mitochondria

	Parameter	Control	PTX
Total phospholipid	TPL (μg/mg protein)	173.66 ± 5.25 (8)	179.60 ± 6.02 (8)
Cholesterol	CHL (μg/mg protein)	31.915 ± 0.60 (8)	38.30 ± 1.68 (8)***
TPL / CHL	Molar ratio	2.71 ± 0.09 (8)	2.34 ± 0.08 (8)*
Membrane fluidity	Fluorescence polarization (P)	0.176 ± 0.0073 (16)	0.155 ± 0.0022 (12)**
	Fluorescence anisotropy (r)	0.125 ± 0.0055 (16)	0.109 ± 0.0016 (12)****
	Limited hindered anisotropy (r∝)	0.066 ± 0.0070 (16)	0.045 ± 0.0022 (12)*
	Order parameter (S)	0.400 ± 0.0200 (16)	0.338 ± 0.0081 (12)**

The results are given as mean ± SEM of the number of observation indicated in the parentheses.

*, $p<0.01$; **, $p<0.02$; ***, $p<0.002$; ****, $p<0.001$

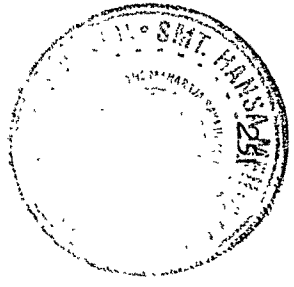

Table 2: Effect of PTX treatment on phospholipid COMPOSITION on liver mitochondria

Phospholipid class	Phospholipid composition (% of total)		Change (%)
	Control	PTX	
Lyso	1.49 ± 0.09	2.00 ± 0.17**	+34
SPM	3.34 ± 0.15	3.20 ± 0.30	-
PC	42.06 ± 0.60	46.86 ± 0.95***	+11
PS	1.97 ± 0.23	2.38 ± 0.20	-
PI	3.58 ± 0.13	3.00 ± 0.23*	-16
PE	34.34 ± 0.63	33.27 ± 0.84	-
DPG	13.26 ± 0.21	9.12 ± 0.25***	-31

The results are given as mean ± SEM of 8 independent observations.
Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
*, $p<0.05$; **, $p<0.02$; ***, $p<0.001$

Table 3: Effect of PTX treatment on phospholipid CONTENT of liver mitochondria

Phospholipid class	Phospholipid content (μg/mg protein)		Change (%)
	Control	PTX	
Lyso	2.73 ± 0.12	4.12 ± 0.51*	+51
SPM	5.83 ± 0.41	6.08 ± 0.73	-
PC	73.02 ± 2.40	83.68 ± 3.25*	+15
PS	3.41 ± 0.39	4.25 ± 0.36	-
PI	6.20 ± 0.24	5.36 ± 0.46	-
PE	59.65 ± 2.19	59.80 ± 2.65	-
DPG	23.00 ± 0.66	16.43 ± 0.92**	-29

The results are given as mean ± SEM of 8 independent observations.
Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG). *, $p<0.02$; **, $p<0.001$

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Table 4: Effect of Carbamazepine (CBZ) treatment on total phospholipid and cholesterol content, membrane fluidity of liver mitochondria in control and epileptic condition.

Parameter	Control	CBZ	PTX treated	PTX-CBZ
Total phospholipid (μg/mg protein)	199.6 ± 4.33 (8)	149.2 ± 7.78 (8)**	185.2 ± 14.42 (8)	108.0 ± 1.81 (8)**, b
Cholesterol (μg/mg protein)	31.51 ± 1.08 (8)	61.16 ± 2.89 (8)**	36.25 ± 1.07 (8)*	40.54 ± 1.73 (8)**
TPL / CHL (Molar ratio)	3.13 ± 0.12 (8)	1.22 ± 0.09 (8)**	2.56 ± 0.11 (8)*	1.33 ± 0.039 (8)**, b
Membrane fluidity:				
Fluorescence polarization (P)	0.156 ± 0.0022 (12)	0.159 ± 0.0056 (12)	0.148 ± 0.0048 (12)	0.171 ± 0.0062 (8)*, a
Fluorescence anisotropy (r)	0.113 ± 0.0017 (12)	0.112 ± 0.0041 (12)	0.104 ± 0.0035 (12)*	0.121 ± 0.0047 (8) a
Limited hindered anisotropy (r∞)	0.057 ± 0.0020 (12)	0.050 ± 0.0055 (12)	0.038 ± 0.0047 (12)*	0.061 ± 0.0063 (8) a
Order parameter (S)	0.340 ± 0.0091 (12)	0.348 ± 0.0200 (12)	0.304 ± 0.0210 (12)	0.387 ± 0.0190 (8)*, a

The results are given as mean ± SEM of the number of observation indicated in the parentheses.
PTX-CBZ treated: PTX-induced epileptic animals treated with CBZ
*, $p<0.01$ and **, $p<0.001$ compared with Control group
a, $p<0.01$ and b, $p<0.001$ compared with PTX treated group.

Table 5: Effect of Lamotrigine (LTG) treatment on total phospholipid and cholesterol content, membrane fluidity of liver mitochondria in control and epileptic condition.

Parameter	Control	LTG	PTX treated	PTX-LTG
Total phospholipid (μg/mg protein)	176.2 ± 6.20 (8)	148.8 ± 5.07 (8)*	179.7 ± 4.67 (8)	162.5 ± 6.91 (8)
Cholesterol (μg/mg protein)	30.17 ± 1.10 (8)	28.72 ± 1.04 (8)	36.98 ± 1.27 (8)**	42.12 ± 2.67 (8)***
TPL / CHL (Molar ratio)	2.89 ± 0.06 (8)	2.61 ± 0.05 (8)**	2.42 ± 0.09 (8)***	1.94 ± 0.05 (8)***, a
Membrane fluidity:				
Fluorescence polarization (P)	0.159 ± 0.0034 (12)	0.118 ± 0.0044 (12)***	0.154 ± 0.0054 (12)	0.172 ± 0.0085 (8)
Fluorescence anisotropy (r)	0.114 ± 0.0018 (12)	0.082 ± 0.0032 (12)***	0.109 ± 0.0040 (12)	0.127 ± 0.0065 (8)
Limited hindered anisotropy (r∞)	0.049 ± 0.0023 (12)	0.0096± 0.0001 (12)***	0.0044± 0.0052 (12)	0.063 ± 0.0087 (8)
Order parameter (S)	0.313 ± 0.0093 (12)	0.158 ± 0.0094 (12)***	0.327 ± 0.00210 (12)	0.387 ± 0.0250 (8)*, a

The results are given as mean ± SEM of the number of observation indicated in the parentheses.
PTX-LTG treated: PTX-induced epileptic animals treated with LTG
*, $p<0.01$; **, $p<0.002$ and ***, $p<0.001$ compared with Control group. a, $p<0.001$ compared with PTX treated group.

Table 6: Effect of Clobazam (CLB) treatment on total phospholipid and cholesterol content, membrane fluidity of liver mitochondria in control and epileptic condition.

Parameter	Control	CLB	PTX treated	PTX-CLB
Total phospholipid (μg/mg protein)	182.8 ± 7.28 (8)	160.9 ± 4.20 (8)*	174.3 ± 3.64 (8)	155.1 ± 2.07 (8)$^{*, b}$
Cholesterol (μg/mg protein)	30.99 ± 1.04 (8)	40.87 ± 0.81 (8)***	37.71 ± 1.46 (8)***	37.17 ± 1.22 (8)***
TPL / CHL (Molar ratio)	2.95 ± 0.17 (8)	1.96 ± 0.07 (8)***	2.32 ± 0.08 (8)**	2.08 ± 0.05 (8)$^{***, a}$
Membrane fluidity:				
Fluorescence polarization (P)	0.204 ± 0.0050 (16)	0.188 ± 0.0057 (16)*	0.160 ± 0.0050 (12)***	0.181 ± 0.0060 (16)$^{**, a}$
Fluorescence anisotropy (r)	0.146 ± 0.0040 (16)	0.134 ± 0.0044 (16)	0.113 ± 0.0044 (12)***	0.128 ± 0.0046 (16)$^{**, a}$
Limited hindered anisotropy (r∝)	0.076 ± 0.0045 (16)	0.078 ± 0.0058 (16)	0.050 ± 0.0059 (12)*	0.071 ± 0.0061 (16) ^{a}
Order parameter (S)	0.415 ± 0.0150 (16)	0.442 ± 0.0160 (16)	0.349 ± 0.0021 (12)*	0.419 ± 0.0180 (16) ^{a}

The results are given as mean ± SEM of the number of observation indicated in the parentheses.
PTX-CLB treated: PTX-induced epileptic animals treated with CLB
*, $p<0.02$; **, $p<0.01$ and ***, $p<0.001$ compared with Control group
a, $p<0.02$ and b, $p<0.001$ compared with PTX treated group.

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Table 7: Effect of Carbamazepine (CBZ) treatment on liver mitochondrial phospholipid COMPOSITOIN in control and epileptic condition

Parameter	Control	CBZ	PTX treated	PTX-CBZ treated
Lyso	1.93 ± 0.10	9.37 ± 0.24**	2.34 ± 0.08*	7.84 ± 0.63**, b
SPM	3.37 ± 0.16	10.70 ± 0.14**	3.67 ± 0.23	7.82 ± 0.16**, b
PC	43.04 ± 1.39	34.03 ± 0.64**	43.69 ± 1.40	46.69 ± 0.78*
PS	2.29 ± 0.07	5.97 ± 0.18**	2.45 ± 0.11	5.44 ± 0.38**, b
PI	3.35 ± 0.07	6.03 ± 0.19**	3.11 ± 0.19	4.60 ± 0.42*, a
PE	35.03 ± 1.32	21.36 ± 0.63**	33.47 ± 2.50	21.90 ± 0.47**, b
DPG	11.18 ± 0.37	12.55 ± 0.14*	10.37 ± 0.30	5.72 ± 0.14**, b

Phospholipid composition is given as % of the Total phospholipids. The results are given as mean ± SEM of 8 independent observations. Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
PTX-CBZ treated: PTX-induced epileptic animals treated with CBZ
*, $p<0.01$ and **, $p<0.001$ compared with Control group; a, $p<0.01$ and b, $p<0.001$ compared with PTX treated group.

Table 8: Effect of Carbamazepine (CBZ) treatment on liver mitochondrial phospholipid CONTENT in control and epileptic condition

Parameter	Control	CBZ	PTX treated	PTX-CBZ treated
Lyso	3.83 ± 0.18	13.81 ± 0.88***	4.33 ± 0.17	8.41 ± 0.55***, b
SPM	6.75 ± 0.42	15.72 ± 0.76***	6.57 ± 0.36	8.43 ± 0.69 a
PC	85.91 ± 2.05	50.16 ± 2.99***	81.0 ± 2.46	50.51 ± 1.45***, b
PS	4.54 ± 0.09	8.77 ± 0.48***	4.55 ± 0.25	5.79 ± 0.41**, a
PI	6.68 ± 0.15	8.79 ± 0.32***	5.74 ± 0.35*	4.99 ± 0.49**
PE	69.72 ± 1.57	31.54 ± 2.08***	61.98 ± 1.40**	23.69 ± 0.75***, b
DPG	22.34 ± 1.00	18.38 ± 0.82**	19.15 ± 0.42**	6.17 ± 0.11***, b

Phospholipid content is given as μg of phospholipid per mg protein The results are given as mean ± SEM of 8 independent observations. Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
PTX-CBZ treated: PTX-induced epileptic animals treated with CBZ
*, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$ compared with Control group; a, $p<0.01$ and b, $p<0.001$ compared with PTX group.

Table 9: Effect of Lamotrigine (LTG) treatment on liver mitochondrial phospholipid COMPOSITION in control and epileptic condition

Parameter	Control	LTG	PTX treated	PTX-LTG treated
Lyso	1.44 ± 0.10	4.09 ± 0.13**	2.33 ± 0.12**	2.47 ± 0.18**
SPM	3.65 ± 0.15	8.17 ± 0.16**	3.29 ± 0.19	4.72 ± 0.19**, a
PC	43.09 ± 0.55	40.47 ± 0.89*	44.21 ± 2.48	42.86 ± 0.92
PS	2.04 ± 0.10	3.90 ± 0.12**	2.49 ± 0.14*	2.30 ± 0.08
PI	2.47 ± 0.15	6.10 ± 0.16**	3.06 ± 0.20*	3.19 ± 0.09**
PE	36.09 ± 0.73	29.22 ± 1.00**	33.70 ± 2.53	31.25 ± 0.99**
DPG	11.34 ± 0.18	8.91 ± 0.16**	10.92 ± 0.36	14.06 ± 1.38 a

Phospholipid composition is given as % of the Total phospholipids. The results are given as mean ± SEM of 8 independent observations. Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
PTX-LTG treated: PTX-induced epileptic animals treated with LTG
*, $p<0.05$ and **, $p<0.001$ compared with Control group
a, $p<0.05$ compared with PTX treated group.

Table 10: Effect of Lamotrigine (LTG) treatment on liver mitochondrial phospholipid CONTENT in control and epileptic condition

Parameter	Control	LTG	PTX treated	PTX-LTG treated
Lyso	2.30 ± 0.13	6.07 ± 0.25**	4.19 ± 0.20**	4.00 ± 0.34**
SPM	5.92 ± 0.32	12.10 ± 0.49**	5.91 ± 0.34	7.68 ± 0.48*, a
PC	69.78 ± 2.28	60.54 ± 1.95**	79.45 ± 2.07*	69.50 ± 3.12 a
PS	3.32 ± 0.22	5.78 ± 0.21**	4.48 ± 0.26*	4.36 ± 0.61
PI	4.01 ± 0.31	9.04 ± 0.24**	5.50 ± 0.40*	5.77 ± 0.59*
PE	58.31 ± 1.33	43.78 ± 2.07**	60.57 ± 1.80	50.87 ± 2.86*
DPG	18.31 ± 0.34	13.21 ± 0.35**	19.63 ± 0.79	23.0 ± 2.85

Phospholipid content is given as μg of phospholipid per mg protein The results are given as mean ± SEM of 8 independent observations.
Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
PTX-LTG treated: PTX-induced epileptic animals treated with LTG
*, $p<0.01$ and **, $p<0.001$ compared with Control group
a, $p<0.01$ compared with PTX treated group.

Table 11: Effect of Clobazam (CLB) treatment on liver mitochondrial phospholipid COMPOSITION in control andepileptic condition

Parameter	Control	CLB	PTX treated	PTX-CLB treated
Lyso	1.73 ± 0.05	2.54 ± 0.13**	2.32 ± 0.17*	2.32 ± 0.09**
SPM	3.55 ± 0.09	2.63 ± 0.15**	3.00 ± 0.15*	4.45 ± 0.18**, b
PC	41.76 ± 0.59	45.73 ± 0.83*	44.72 ± 1.70	38.41 ± 0.28**, a
PS	3.14 ± 0.28	3.40 ± 0.11	2.52 ± 0.16	2.42 ± 0.14*
PI	3.67 ± 0.10	2.54 ± 0.14**	3.03 ± 0.20*	3.67 ± 0.16 a
PE	36.23 ± 0.60	29.70 ± 0.85**	33.93 ± 2.55	34.28 ± 1.28 a
DPG	10.56 ± 0.24	12.24 ± 0.28**	10.44 ± 0.39	14.46 ± 0.19**, b

Phospholipid composition is given as % of the Total phospholipids. The results are given as mean ± SEM of 8 independent observations.
Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
PTX-CLB treated: PTX-induced epileptic animals treated with CLB
*, $p<0.01$ and **, $p<0.001$ compared with Control group
a, $p<0.01$ and b, $p<0.001$ compared with PTX treated group.

Table 12: Effect of Clobazam (CLB) treatment on liver mitochondrial phospholipid CONTENT in control and epileptic condition

Parameter	Control	CLB	PTX treated	PTX-CLB treated
Lyso	3.15 ± 0.15	4.03 ± 0.20**	4.04 ± 0.22**	3.61 ± 0.16
SPM	6.46 ± 0.24	4.15 ± 0.18***	5.24 ± 0.31**	6.90 ± 0.28 b
PC	76.37 ± 3.26	73.39 ± 1.04	77.99 ± 1.63	59.64 ± 1.14***, b
PS	5.77 ± 0.59	5.45 ± 0.16	4.39 ± 0.27*	3.74 ± 0.22**
PI	6.68 ± 0.22	4.10 ± 0.28***	5.33 ± 0.44**	5.70 ± 0.28*
PE	66.17 ± 2.65	48.06 ± 2.54***	59.08 ± 0.77*	51.28 ± 2.08***, a
DPG	19.34 ± 1.02	19.74 ± 0.83	18.28 ± 1.02	2.41± 0.20**, b

Phospholipid content is given as μg of phospholipid per mg protein The results are given as mean ± SEM of 8 independent observations.
Lysophospholipid (lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).
PTX-CLb treated: PTX-induced epileptic animals treated with CLB
*, $p<0.05$; **, $p<0.01$ and **, $p<0.001$ compared with Control group
a, $p<0.01$ and b, $p<0.002$ compared with PTX treated group.

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Table 13: Effect of PTX treatment on phospholipids parameters of liver mitochondria

Parameter	Control	PTX
PS+PI	5.57 ± 0.424	5.41 ± 0.434
PC/PE	1.23 ± 0.039	1.42 ± 0.006*
SPM/PC	0.08 ± 0.003	0.07 ± 0.006
SPM/PE	0.10 ± 0.006	0.10 ± 0.011
TPL/PS	95.7 ± 9.64	79.5 ± 7.401
TPL/PI	49.2 ± 2.81	68.6 ± 7.731
TPL/PS+PI	31.2 ± 1.66	33.2 ± 1.890
APL/BPL	0.24 ± 0.01	0.17 ± 0.005***
PI/BPL	0.05 ± 0.002	0.04 ± 0.003*
PS/BPL	0.17 ± 0.003	0.11 ± 0.003***
DPG/BPL	2.71 ± 0.091	2.34 ± 0.076**

The results are given as mean ± SEM of 8 independent observations.
Shingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), Acidic phospholipids (APL), Basic phospholipids (BPL). *, $p<0.05$ **, $p<0.01$ and ***, $p<0.001$

Table 14: Effect of Carbamazepine (CBZ) treatment on phospholipids parameters of liver mitochondria in control and epileptic condition

Parameter	Control	CBZ	PTX treated	PTX-CBZ
PS+PI	5.63 ± 0.173	12.0 ± 0.245**	5.56 ± 0.296	10.12 ± 0.81**, a
PC/PE	1.23 ± 0.017	1.61 ± 0.072**	1.31 ± 0.031	2.41 ± 0.06**, a
SPM/PC	0.08 ± 0.004	0.032 ± 0.008**	0.08 ± 0.006	0.168 ± 0.005**, a
SPM/PE	0.09 ± 0.004	0.505 ± 0.012**	0.11 ± 0.007	0.359 ± 0.014**, a
TPL/PS	88.3 ± 4.35	24.83 ± 1.550**	75.6 ± 2.59*	20.49 ± 1.40**, a
TPL/PI	59.8 ± 2.04	24.84 ± 2.040**	59.6 ± 2.53	24.98 ± 2.43**, a
TPL/PS+PI	35.6 ± 1.34	12.37 ± 0.839**	33.2 ± 1.28	10.68 ± 0.52**, a
APL/BPL	0.21 ± 0.006	0.371 ± 0.007**	0.20 ± 0.012	0.207 ± 0.010
PI/BPL	0.041 ± 0.001	0.091 ± 0.003**	0.04 ± 0.003	0.060 ± 0.006**, a
PS/BPL	0.028 ± 0.001	0.091 ± 0.003**	0.031 ± 0.002	0.071 ± 0.005**, a
DPG/BPL	0.138 ± 0.005	0.190 ± 0.0031**	0.129 ± 0.007	0.075 ± 0.002**, a

The results are given as mean ± SEM of 8 independent observations.
Shingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), Acidic phospholipids (APL), Basic phospholipids (BPL); *, $p<0.05$ and **, $p<0.001$ compared with Control group; a, $p<0.001$ compared with PTX treated group

Table 15: Effect of Lamotrigine (LTG) treatment on phospholipids parameters of liver mitochondria in control and epileptic condition

Parameter	Control	LTG	PTX treated	PTX-LTG
PS+PI	4.54 ± 0.256	9.99 ± 0.211**	5.56 ± 0.328*	5.51 ± 0.165**
PC/PE	1.20 ± 0.039	1.41 ± 0.054*	1.32 ± 0.086	1.39 ± 0.067*
SPM/PC	0.09 ± 0.004	0.200 ± 0.004**	0.08 ± 0.005	0.11 ± 0.006**, a
SPM/PE	0.10 ± 0.004	0.280 ± 0.008**	0.09 ± 0.006	0.15 ± 0.009**, a
TPL/PS	88.4 ± 6.24	38.53 ± 2.01**	72.2 ± 2.89*	71.3 ± 4.20**, a
TPL/PI	73.6 ± 5.09	24.62 ± 1.40**	58.7 ± 2.64*	55.5 ± 6.72**, a
TPL/PS+PI	38.5 ± 1.76	14.99 ± 0.79**	32.3 ± 1.83*	29.5 ± 1.07**
APL/BPL	0.19 ± 0.003	0.243 ± 0.006**	0.17 ± 0.009*	0.25 ± 0.02*, a
PI/BPL	0.030 ± 0.002	0.078 ± 0.002**	0.04 ± 0.002*	0.04 ± 0.004*
PS/BPL	0.025 ± 0.001	0.050 ± 0.002**	0.03 ± 0.002*	0.03 ± 0.001*
DPG/BPL	0.138 ± 0.002	0.114 ± 0.003**	0.135± 0.006*	0.18 ± 0.019**, a

The results are given as mean ± SEM of 8 independent observations.
Shingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), Acidic phospholipids (APL), Basic phospholipids (BPL); *, $p<0.05$ and **, $p<0.001$ compared with Control group; a, $p<0.001$ compared with PTX treated group

Table 16: Effect of Clobazam (CLB) treatment on phospholipids parameters of liver mitochondria in control and epileptic condition

Parameter	Control	CLB	PTX treated	PTX-CLB
PS+PI	6.85 ± 0.399	5.94 ± 0.191*	5.56 ± 0.359*	6.11 ± 0.31
PC/PE	1.16 ± 0.018	1.55 ± 0.069**	1.32 ± 0.023**	1.12 ± 0.02[a]
SPM/PC	0.09 ± 0.002	0.06 ± 0.003**	0.07 ± 0.003**	0.12 ± 0.005**, [a]
SPM/PE	0.10 ± 0.002	0.09 ± 0.007	0.09 ± 0.005	0.13 ± 0.006**, [a]
TPL/PS	58.2 ± 6.44	47.9 ± 2.50	71.2 ± 4.97	66.2 ± 4.71 [a]
TPL/PI	49.8 ± 1.68	64.5 ± 3.20**	59.2 ± 3.88*	42.9 ± 2.04*, [a]
TPL/PS+PI	26.7 ± 1.31	27.3 ± 1.14	31.4 ± 1.34*	25.4 ± 0.64
APL/BPL	0.21 ± 0.008	0.23 ± 0.003*	0.20 ± 0.007	0.27 ± 0.00**, [a]
PI/BPL	0.045± 0.001	0.03 ± 0.002**	0.04 ± 0.003*	0.05 ± 0.002 [a]
PS/BPL	0.040 ± 0.002	0.04 ± 0.002	0.03 ± 0.002*	0.03 ± 0.002[a]
DPG/BPL	0.130 ± 0.003	0.157± 0.004**	0.13 ± 0.005	0.19 ± 0.003**, [a]

The results are given as mean ± SEM of 8 independent observations.
Shingomyelin (SPM), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), Acidic phospholipids (APL), Basic phospholipids (BPL); *, $p<0.05$ and **, $p<0.001$ compared with Control group; a, $p<0.001$ compared with PTX treated group

8). TPL content was further decreased by 50% with increased CHL content and thereby decreased TPL/CHL ratio (Table 4). LTG treatment caused increased Lyso, SPM and PI by 1.3 to 1.7 folds as compared to controls with marginal effects on PS, PC, DPG and PE components (Table 9, 10). Lyso, SPM and DPG were elevated by 1.3 folds in CLB treated epileptic animals (Table 11, 12). TPL content decreased with the lowering of TPL/CHL ratio after CLB treatment to the epileptic animals (Table 6). There was only a marginal change in the membrane fluidity of mitochondrial membrane after AEDs treatment to the epileptic animals. In general Lyso, SPM, PS and PI component remained elevated above the control values in epileptic animals after AEDs treatment (Table 7, 9, 11). PE component decreased in all the AED treated epileptic animals. The molar ratio of TPL/CHL in the epileptic animals decreased with out any appreciable change in the membrane fluidity after AEDs treatment (Table 4-6).

Taken together, PTX treatment showed only marginal changes in phospholipid composition of liver mitochondria, however, membrane fluidization was evident. Elevation of Lyso was the common feature in AEDs treatment to either control or epileptic animals. Increased lyosophospholipids indicate activation of phospholipases, which are known to be activated by Ca^{2+} (19). Similarly, alterations in acidic phospholipid after AEDs treatment could lead to distorted charge distribution in the membrane microenvironment. Oxidative energy metabolism is regulated by proper functioning of F_oF_1ATPAse. However, ATPases activity is known to be dependent on acidic phospholipids, in particular PS and PI (20). Hence, it may be suggested that the

changes in the kinetic properties of mitochondrial ATPase (Chapter 4) could be attributed to altered PS and PI components and altered membrane charge distribution after AEDs treatment to control and epileptic animals. A decreased efficacy of ATPase means that mitochondrion is less able to sequester energy required by no. of metabolic processes in liver.

As evident from the data given in Table 13-16 that, drastic alterations various phospholipids parameters and ratios after AEDs treatment are marginally affected in the epileptic condition alone. Additionally, substantial alterations in acidic to basic phospholipids ratio, SPM to PC or PE ratios indicated that, membrane environment required for the proper functioning of respiratory chain and ATPase is disturbed that could result in to distorted mitochondrial membrane permeability transition.

Biochemical studies in patients with epileptic phenotype revealed decreased activities of all respiratory chain complexes in isolated liver mitochondria and decreased amounts of respiratory chain complexes I, III, IV and ATP-synthase in liver and frontal cortex, but not in muscle, heart, and fibroblasts (10). Depletion of CoQ that - transfers electrons to complex I and II of respiratory chain – is associated with CoQ responsive oxidative phosphorylatoin deficiency in Leigh syndrome (6). Thus, stress condition like epilepsy or insults like AEDs treatment could lead to accumulation of mitochondrial respiratory chain disorders that are established cause of liver failure (4).

Diazepam treatment induces significant changes in concentration of individual phospholipid classes especially PC, PS, PI and DPG in liver mitochondria (9). Phenobarbital treatment aggravate diverse spectrum of effects on mitochondrial and microsomal function by altering antioxidant system and Cyt-P450 activity in rat liver (7). Nevertheless, valproic acid and phenytoin are known to be directly interacting with crucial mitochondrial structures like enzymes, respiratory chain components and membrane (10-12, 13, 16).

The liver is possibly the most important site of lipid synthesis and storage (21, 22). It is well documented that metabolism of individual classes of phospholipids play a vital role in maintaining the integrity of biological membranes by affecting their turnover rate. Moreover, it is well known that phospholipids are essential structural and functional component of liver plasma and subcellular membranes like mitochondria and act as enzyme activators and precursors of bioactive substances (23, 24).

The observed changes in composition of membrane and content of individual phospholipids like PC, PS, PI and DPG after AEDs treatment could have major effects on the energy state, the signal transduction pathways as well as the membrane stability and permeability of liver cells. PS is required for activation of Na^+,K^+-ATPase, while PI activates phosphodiesterase (25, 26). Both of these phospholipid classes play a role as a modulator in the receptor dependent activation of a variety of cell surface enzymes. DPG is located in the inner mitochondrial membrane where, along with PC, it has been shown

to play an important role in the activity of mitochondrial proteins such as cytochrome c oxidase (27), the ADP/ATP carrier protein (28) and the mitochondrial phosphate transporter (29). Other phospholipid classes are associated with the activities of enzymes like Ca^{2+},Mg^{2+}-APTase, adenylate cyclase and glucose-6-phosphatase (16, 27-29).

The common side effects of AEDs treatment are drowsiness, ataxia, dizziness, nausea, memory impairment etc (30). Therefore, in general, AEDs can exert alterations in the liver mitochondrial lipid composition in control animals. However, despite of marginal change in PTX treatment group, the AED treatment to the epileptic animals differentially affected the PL profile, which is clearly pinpointing the sole cause of AED treatment in liver mitochondria that could be attributed to precipitation of above mentioned side effects.

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Summary

Effect of PTX-induced epileptic condition and treatment with AEDs (CBZ, LTG and CLB) on lipid/phospholipid profile of rat liver mitochondria was evaluated. PTX treatment resulted in marginal changes in the percentage composition of individual phospholipids. CHL content was increased by 1.2 folds with 14% decrease in TPL/CHL molar ratio. Membrane fluidization was prominent in PTX treatment. TPL content and TPL/CHL ratio were decreased in either treatment with AEDs to control or to the epileptic animals. Lyso, PS and PI components was elevated by 1.3 to 4 folds with AEDs treatment to the control animals. When epileptic animals were treated with AEDs, Lyso, SPM, PS and PI components remained elevated with general decreased in PE as compared to controls. TPL content and TPL/CHL ratios remained low in the epileptic animals treated with AEDs. In general, AEDs can exert alterations in the liver mitochondrial lipid composition in control animals. However, despite of marginal change in PTX treatment group, the AED treatment to the epileptic animals differentially affected the phospholipid profile that could be the possible reason for mitochondrial respiratory chain dysfunction and reported side effects of AEDs.