Chapter 7

Effect of Picrotoxin-induced Epileptic Condition and Antiepileptic Drug Treatment on Lysosomal Function in Rat Brain and Liver

Introduction

Epilepsy, a chronic seizure disorder results due to uncontrolled hypersynchronous discharges from the glutamatergic system and leads to neuronal cell loss, e.g. loss of hippocampal pyramidal cells and of granular and hilar cells in dentate gyrus and neurodegeneration (1-3). While the former is believed to be the underlying cause for the lowered seizure threshold, neurodegeneration has been shown to be associated with memory impairment in animals as well as in humans (1-3). It has also been recognized that neurodegeneration results as a consequence of excitotoxicity (2, 3). In the light of the above, it may be anticipated that in the epileptic condition the cerebral function may be affected at the cellular and/or subcellular level.

Interestingly, it has been reported that in cobalt epilepsy the acid phosphatase activity in rat brain was significantly elevated (4). By contrast, in the skin biopsy samples and in the leukocytes from epileptic patients the arylsulfatase A activity decreased (5, 6). In kainate-evoked seizures, the levels of cathepsin D mRNA in rat brain were found to be elevated (7). Enhanced cathepsins activity had also been reported in progressive myoclonus epilepsy (8, 9). These reports are thus the suggestive of the possible alterations in lysosomal functions in epilepsy. However, no in depth studies have been carried out to evaluate the lysosomal function in the epileptic condition. Hence, it was of interest to delineate the effect of chronic epileptic condition induced by PTX on cerebral lysosomal function in rats.

The antiepileptic drugs (AEDs) which are being used for the treatment include carbamazepine (CBZ), valproate (VPA), phenytoin (PHY), clobazam (CLB), lamotrigine (LTG), topiramate (TPR) etc. These AEDs are structurally unrelated and their mechanisms of actions differ (10). The cellular actions of AEDs appear to be related to their capacity to suppress seizures (11). Interestingly, besides being used as antiepileptics these drugs have emerged as potential agents for the treatment of other neuropsychological disorders (12) e.g. CBZ and VPA for bipolar disorders, schizophrenia and peripheral neuropathy (13-15); LTG for effective management of cluster headache and refractory affective disorder (16-18) and CLB against psychosomatic stress and anxiety (19-21). When used for the management of the aforementioned disorders (12-21) it is likely that the actions of the AEDs may be mediated by modulating the cellular / subcellular functions, rather than by regulating neurotransmitters balance.

In vivo studies have shown that AEDs bring about the activation of lysosomal enzymes in various tissues of rats and begal dogs (22). Activation of lysosomal enzymes in liver of phenobarbitone treated rats has been reported (23). Clinical trials in humans with VPA and CBZ treatment showed the presence of high levels of N-acetyl-β-glucosaminidase and β-galactosidase activity in the urine (24). Release of lysosomal enzymes in neutrophils following phenytoin treatment has also been reported (25).

In the view of these reports it was of interest to find out if treatment with AEDs alters the lysosomal functions, when these are given to intact non-epileptic control and epileptic animals. We evaluated the effects of AEDs treatment on the lysosomal enzyme activities in the brain, which is the target tissue for AED action. Additionally, we carried out parallel studies in liver, a tissue where the AEDs are mainly metabolized.

Exposure to PTX is known to induce generalized tonic-clonic seizures in the rats; chronic tonic-clonic seizures are established after 20 day exposure to PTX (26). PTX binds to the chloride channel and benzodiazepine binding site of $GABA_A$ receptor complex in the brain (27). As a consequence, non-competitive inhibition of synaptic GABA transmission results in generalized tonic-clonic convulsions (26, 27).

Whereas the effects of PTX exposure are manifested in terms of generalized tonic-clonic seizures, liver is the major site of PTX metabolism (28, 29); PTX is metabolized to picrotoxinin and picrotin (28-30). It is possible that PTX by itself or its metabolites picrotoxinin and picrotin may affect the liver function especially if the animals are exposed for prolonged duration of 20 days. Hence we examined this possibility in terms of lysosomal membrane integrity and lysosomal function in rat liver following prolonged exposure to PTX.

The studies included measurement of the activities of the marker enzymes viz. acid phosphatase, cathepsin D, acid ribonuclease (RNAse II) and acid deoxyribonuclease (DNAse II). The results of these studies are summarized here.

MATERIALS AND METHODS

Chemicals

Carbamazepine, lamotrigine and clobazam were generous gifts from Sarabhai Piramal Pharmaciuticals Ltd. Vadodara, Glaxo Smith Kline, UK and Aventis Pharma Ltd. Mumbai, respectively. Yeast RNA, calf thymus DNA and hemoglobin were purchased from Sigma Chemical Co. USA. Sodium β-glycerophosphate (BGP) was from Mallinkrodt, USA. All other chemicals were of analytical-reagent grade and were purchased locally.

The treatment with PTX and AEDs were essentially the same as described in Chapter 2 and 3.

Experimental

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). Tissues were quickly dissected out and placed in beakers containing chilled (0-4 °C) 0.25 M sucrose.

Tissue homogenates (10% w/v) were prepared in 0.25 M sucrose using a Potter-Elvehjem type glass – Teflon homogenizer. The homogenate was centrifuged at 2000 rpm for 10 min at 4 °C in a Sorvall RC 5B*plus* centrifuge. The pellet containing nuclei and unbroken cell debris was discarded and the supernatant (S_1 fraction) was used within 15 min for the measurement of the 'free' activity. For the measurement of the 'total' activity the S1 fraction was diluted (1:5) with tris-HCl buffer (10 mM, pH 7.4) containing 0.1% Triton X-100 and subjected to three cycles of freezing and thawing.

Enzyme assays

Acid phosphatase activity was measured in the medium (total volume 0.6 ml) containing 0.1 M sodium acetate buffer, pH 5. After pre-incubating 0.2 ml of the enzyme at 37 °C for 1-2 min the reaction was initiated by adding BGP at the final concentration of 15 mM. The reaction was allowed to proceed for 15 min and was terminated by the addition of 1 ml of 5% (w/v) trichloroacetic acid (TCA). The tubes were kept on ice for 30 min and then centrifuged at 2000 rpm for 10 min. The amount of liberated inorganic phosphorous in the supernatant was estimated by the method of Fiske and Subba Row (31).

Cathepsin D activity was measured in the medium (total volume 0.5 ml) containing 50 mM sodium acetate buffer, pH 3.8 and 0.25 M sucrose. After pre-incubating 0.05 ml of enzyme at 37 °C the reaction was initiated by adding 0.5 mg of hemoglobin. At the end of 10 min of incubation period the reaction was terminated by the addition of 2.5 ml of 5%

(w/v) TCA. The tubes were kept on ice and the contents were filtered using a Whatman filter paper No. 1. The tyrosine positive materials in the filtrate were estimated by the method as described (32).

RNAse II activity was measured in the assay medium (total volume 0.2 ml) containing 0.15 M sodium acetate buffer, pH 5.0. After pre-incubating 0.05 ml of enzyme for 1-2 min at 37 °C the reaction was initiated by the addition of 300 µg of RNA. The reaction was carried out for 30 min and was terminated by the addition of 2 ml of ice-cold 10% perchloric acid (PCA) containing 2.5 mg of uranyl acetate per ml. The tubes were kept on ice for one hr and then centrifuged at 2000 rpm for 10 min. The amount of nucleotides released in the supernatant was determined by measuring the absorption at 260 nm (32).

DNAse II activity was determined in the assay medium (total volume 0.2 ml) containing 0.15 M sodium acetate buffer, pH 5.0, and 0.15 M KCl. After pre-incubating the enzyme (0.05 ml) at 37 °C for 1-2 min the reaction was initiated by the addition of 150 µg of DNA. The reaction was allowed to proceed for 30 min and was terminated by the addition of 2 ml of ice-cold 10% PCA. The tubes were kept on ice for one hr and then centrifuged at 2000 rpm for 10 min. The amount of nucleotides released in the supernatant was determined by recording the absorbance 260 nm (32).

The ratio of Total activity / Free activity (T/F ratio) is taken as the index of lysosomal membrane integrity (32-34).

Protein was estimated according to the method of Lowry *et al.* (35) using bovine serum albumin as the standard. Results are presented as mean ± SEM. Statistical analysis of the data was done by Students' *t*-test.

Results

Effect of treatment with PTX and AEDs on body, brain and liver weight are shown in Table 1 and 2. As can be noted, there was no appreciable change after PTX and/or AEDs treatment. Table 3 and 4 represents the effect of either PTX or AEDs treatment on protein content in various subcellular fractions in brain and liver respectively. Treatment with CBZ to the epileptic animals caused about 30 to 42% decrease in mitochondrial protein content in both the tissues (Table 3 and 4). However, in general, 1.2 to 1.8 folds elevation in the total protein content of liver homogenate was seen after AEDs treatment to the PTX-induced epileptic animals (Table 3).

Table 1. Effect of treatment with PTX, CBZ, LTG and CLB on body weight of rats.

Group	Body weight (g)
Control	236.7 ± 6.38
PTX	238.3 ± 6.07
CBZ	259.8 ± 4.78
LTG	221.9 ± 5.40
CLB	244.8 ± 4.52
PTX-CBZ	247.3 ± 5.00
PTX-LTG	239.5 ± 7.01
PTX-CLB	283.5 ± 3.95

Results are given as mean ± S.E.M. of eight animals in each group.

Table 2. Effect of treatment with PTX, CBZ, LTG and CLB on brain and liver weights of rats.

Group	Brain weight		Liver weight	
	Gram weight	% of body wt	Gram weight	% of body wt
Control	1.56 ± 0.07	0.72 ± 0.03	6.68 ± 0.34	3.17 ± 0.12
PTX	1.56 ± 0.02	0.66 ± 0.02	7.67 ± 0.20	3.22 ± 0.05
CBZ	1.75 ± 0.07	0.57 ± 0.02	8.73 ± 0.42	3.36 ± 0.17
LTG	1.61 ± 0.03	0.73 ± 0.03	6.50 ± 0.20	2.91 ± 0.08
CLB	1.63 ± 0.03	0.66 ± 0.01	7.42 ± 0.09	3.03 ± 0.06
PTX-CBZ	1.58 ± 0.03	0.64 ± 0.01	8.63 ± 0.31	3.49 ± 0.08
PTX-LTG	1.60 ± 0.04	0.67 ± 0.01	6.98 ± 0.35	2.91 ± 0.08
PTX-CLB	1.65 ± 0.03	0.58 ± 0.01	8.90 ± 0.24	3.14 ± 0.08

Results are given as mean ± S.E.M. of eight animals in each group.

Table 3. Effect of treatment with PTX, CBZ, LTG and CLB on protein content in various sub-cellular fractions of rat brain.

Group	Protein (mg/ml)		
	Homogenate	S_1 fraction	Mitochondria
Control	9.12 ± 0.44	7.50 ± 0.24	17.20 ± 0.69
PTX	9.87 ± 0.27	8.30 ± 0.23	15.11 ± 0.56
CBZ	9.82 ± 0.20	8.62 ± 0.16	12.50 ± 0.14
LTG	10.54 ± 0.32	7.93 ± 0.12	13.67 ± 0.31
CLB	8.17 ± 0.26	7.81 ± 0.48	14.37 ± 0.29
PTX-CBZ	11.41 ± 0.51	9.01 ± 0.32	10.96 ± 0.44*[a]
PTX-LTG	9.43 ± 0.19	7.75 ± 0.27	17.86 ± 0.24
PTX-CLB	10.27 ± 0.26	8.67 ± 0.19	18.12 ± 0.59

Results are given as mean ± S.E.M. of eight animals in each group.

*, $p<0.001$ as compared to the control.
a, $p<0.001$ as compared to the PTX-induced epileptic group.

Table 4. Effect of treatment with PTX, CBZ, LTG and CLB on protein content in various sub-cellular fractions of rat liver.

Group	Protein (mg/ml)		
	Homogenate	S_1 fraction	Mitochondria
Control	14.49 ± 0.35	11.80 ± 0.43	51.14 ± 1.25
PTX	15.02 ± 0.55	12.81 ± 0.26	40.95 ± 2.58
CBZ	15.46 ± 0.56	12.24 ± 0.34	39.92 ± 0.90
LTG	15.86 ± 0.97	12.53 ± 0.32	40.48 ± 2.04
CLB	13.18 ± 0.49	10.07 ± 0.26	38.63 ± 1.14
PTX-CBZ	18.32 ± 0.60*	14.47 ± 0.51	29.43 ± 1.47** a
PTX-LTG	25.81 ± 0.89** a	14.43 ± 0.52	57.51 ± 0.81
PTX-CLB	20.61 ± 0.50* a	13.90 ± 0.34	48.77 ± 1.17

Results are given as mean ± S.E.M. of eight animals in each group.

*, $p<0.05$ and **, $p<0.001$ as compared to controls.
a, $p<0.001$ as compared to the PTX-induced epileptic group.

Effect of PTX-induced convulsions on BRAIN and LIVER lysosomal enzymes:

As can be noted from data in Table 5, in the control group the free and total activities of the four enzymes and the ratio of Total activity / Free activity in the brain was in the expected range (33, 34). The latter values were in the range of 2.8 to 12.4 depending on the enzyme under consideration.

As is evident from Table 5 that in the brain PTX treatment resulted in 2.3 fold increase in the free RNAse II activity, while the free DNAse II activity decreased by 20%. The total acid phosphatase, RNAse II and DNAse II activities decreased from 35 to 71%, with maximum effect being seen for DNAse II activity. The ratios of Total activity / Free activity were significantly lower suggesting that there is loss of lysosomal membrane integrity, which makes it more permeable following PTX treatment. The cathepsin D activity, both free and total was completely abolished.

In liver, exposure to PTX resulted in 43% and 217% elevation in the free RNAse II and DNAse II activities respectively (Table 9). The free acid phosphatase activity was unaffected. The total acid phosphatase activity was decreased by 28%, whereas the total DNAse II activity was 2.2 fold higher in the liver after PTX treatment. Consequently the ratios of Total activity / Free activity were low implying loss of lysosomal membrane integrity (Table 9). Additionally, changes in the latent activity suggested that PTX

treatment resulted in 35% decrease in acid phosphatase activity whereas the latent DNAse II activity increased substantially by 86% (data not shown). In the case of cathepsin D, both free and total activities were completely abolished (Table 9).

Since the effect on cathepsin D was so strikingly different, we decided to find out if the observed changes occurred as a consequence of prolonged exposure to PTX or were the effects of PTX *per se*. To ascertain this possibility, S_1 fraction was pre-incubated with PTX (1 to 1000 μM) prior to estimating the free and total cathepsin D activities for both the tissues. It was observed that, in brain and liver, the free and total activities were unaffected by added PTX thereby ruling out the direct effect of PTX on cathepsin D activity (Fig 1).

Figure 1. Effect of incubation in vitro with PTX on the Cathepsin D activity. The enzyme samples were incubated for 5 min with 1, 10, 100 and 1000 μM PTX prior to measuring the activities. Other experimental details are as described in text. Results are given as mean ± SEM of 4 independent observations. Cathepsin D activity is given as μg tyrosine positive materials / 10min / mg protein

---●--- Free activity ---■--- Total activity

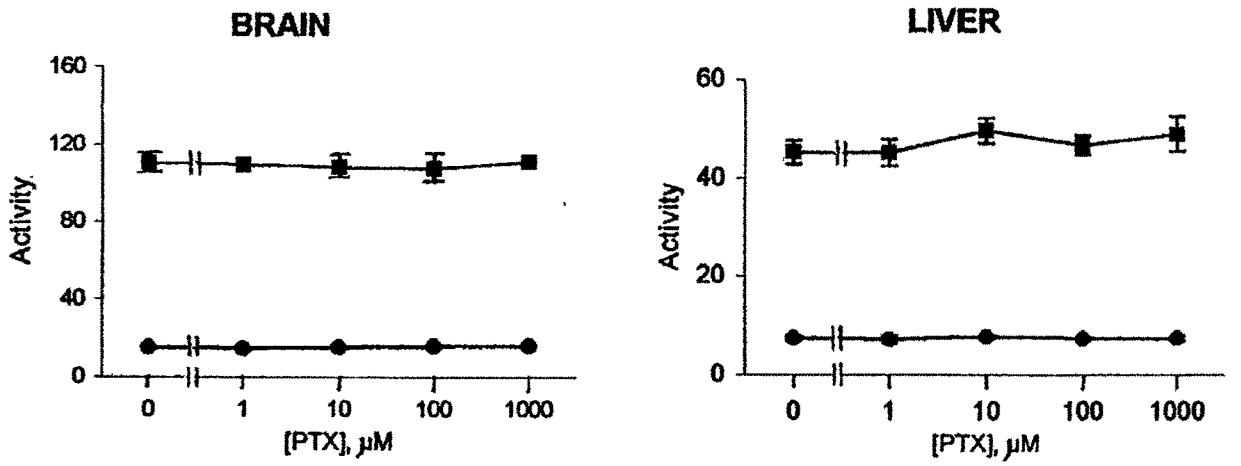

Table 5. Effects of Picrotoxin treatment on brain lysosomal enzyme activities.

Enzyme	Treatment	Activity		Total activity
		Free	Total	Free activity
Acid phosphatase	Control	104.0 ± 3.0	339.0 ± 11.0	3.26 ± 0.06
	Epileptic	94.0 ± 5.0	220.0 ± 15.0**	2.34 ± 0.14**
Cathepsin D	Control	17.98 ± 1.2	96.53 ± 6.9	5.37 ± 0.38
	Epileptic	ND	ND	-
RNAse II	Control	31.0 ± 1.5	320.0 ± 14.0	10.39 ± 0.31
	Epileptic	70.0 ± 4.0**	190.0 ± 11.0**	2.77 ± 0.16**
DNAse II	Control	42.0 ± 1.3	520.0 ± 51.0	12.38 ± 0.81
	Epileptic	34.0 ± 2.0*	150.0 ± 17.0**	4.41 ± 0.38**

The experimental details are as described in the text. Results are given as mean ± S.E.M. of 16 independent observations in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as µg tyrosine positive materials / 10min / mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as nmole nucleotides / 10min / mg protein.
ND, not detectable; *, $p < 0.01$ and **, $p < 0.001$ compared with the corresponding control.

Table 6. Effects of Carbamazepine (CBZ) treatment on brain lysosomal enzymes activities in control and PTX-induced epileptic condition

Enzyme	Activity	Control (8)	PTX treated (8)	CBZ treated (8)	PTX-CBZ (8)
Acid phosphatase	Free activity	91.8 ± 4.50	105 ± 6.44	87.0 ± 7.94	107 ± 4.90*
	Total activity	334 ± 9.0	250 ± 16.0****	176 ± 14.10****	256 ± 2.20****
	T/F ratio	3.41 ± 0.18	2.40 ± 0.15****	2.02 ± 0.170****	2.39 ± 0.16****
Cathepsin D	Free activity	20.83 ± 0.83	ND	37.45 ± 1.69****	ND
	Total activity	77.38 ± 6.013	ND	103.7 ± 1.71****	ND
	T/F ratio	3.71 ± 0.218	-	2.77 ± 0.09***	ND
DNAse II	Free activity	40.50 ± 1.56	33.10 ± 1.66**	45.0 ± 2.40	ND
	Total activity	96.02 ± 7.00	49.50 ± 1.90****	74.0 ± 2.20**	ND
	T/F ratio	2.37 ± 0.26	1.49 ± 0.07	1.64 ± 0.11*	ND
RNAse II	Free activity	35.0 ± 1.70	65.03 ± 5.60****	41.0 ± 3.40	21.70 ± 1.40$^{****, a}$
	Total activity	335 ± 23.4	150 ± 8.64****	130 ± 1.10****	134 ± 7.70****
	T/F ratio	9.57 ± 0.56	2.31 ± 0.12****	3.17 ± 0.27****	6.18 ± 0.38$^{****, a}$

The experimental details are as described in the text. Results are given as mean ± S.E.M. of no. of observations indicated in parentheses in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as μg tyrosine positive materials / 10min / mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as n mole nucleotides / 10min / mg protein.
PTX-CBZ treated: PTX-induced epileptic animals treated with CBZ; T/F ratio: Ratio of Total activity over Free activity.
ND, not detectable; *, $p<0.05$; **,$p<0.01$; ***,$p<0.002$, ****,$p<0.001$ compared with Control group;
a, $p<0.001$ compared with PTX-treated group.

Table 7. Effects of Lamotrigine (LTG) treatment on brain lysosomal enzymes activities in control and PTX-induced epileptic condition

Enzyme	Activity	Control (8)	PTX treated (8)	LTG treated (8)	PTX-LTG (8)
Acid phosphatase	Free activity	106 ± 5.40	98.8 ± 7.20	101 ± 6.20	92.40 ± 2.40*
	Total activity	321± 13.5	246 ± 13.0***	262 ± 10.2**	196 ± 10.0$^{****, a}$
	T/F ratio	3.03± 0.141	2.49 ± 0.16*	2.59 ± 0.13*	2.12 ± 0.08****
Cathepsin D	Free activity	26.18 ± 0.53	ND	20.35 ± 1.09****	21.67 ± 2.32^{b}
	Total activity	70.40 ± 4.75	ND	12.00 ± 0.48****	233.8 ± 9.17$^{****, b}$
	T/F ratio	2.69 ± 0.12	-	0.60 ± 0.03****	10.79 ± 0.79$^{****, b}$
DNAse II	Free activity	40.0 ± 2.05	34.10 ± 1.61*	48.0 ± 4.40	ND
	Total activity	80.0 ± 6.60	51.43 ± 2.14****	75.0 ± 2.23	ND
	T/F ratio	2.01± 0.13	2.53 ± 0.06**	1.56 ± 0.09**	ND
RNAse II	Free activity	32.03 ± 1.80	57.02 ± 3.70****	80.2 ± 5.67****	ND
	Total activity	291 ± 18.0	144 ± 7.50****	102 ± 5.50****	ND
	T/F ratio	9.09 ± 0.54	2.53 ± 0.15****	1.27 ± 0.08****	ND

The experimental details are as described in the text. Results are given as mean ± S.E.M. of no. of observations indicated in parentheses in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as μg tyrosine positive materials / 10min /mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as nmole nucleotides / 10min / mg protein; PTX-LTG treated: PTX-induced epileptic animals treated with LTG; T/F ratio: Ratio of Total activity over Free activity; ND, not detectable;
*, $p<0.05$; **,$p<0.01$; ***,$p<0.002$ ****,$p<0.001$ compared with Control group;; a, $p<0.01$ and b, $p<0.001$ compared with PTX-treated group.

Table 8. Effects of Clobazam (CLB) treatment on brain lysosomal enzymes activities in control and PTX-induced epileptic condition

Enzyme	Activity	Control (8)	PTX treated (8)	CLB treated (8)	PTX-CLB (8)
Acid phosphatase	Free activity	102 ± 2.90	102 ± 5.10	75 ± 6.40***	138 ± 4.70$^{****, b}$
	Total activity	351 ± 15.8	250 ± 9.90****	221 ± 8.23****	312 ± 19.0^{a}
	T/F ratio	3.44 ± 0.08	2.45 ± 0.10****	3.07 ± 0.21	2.26 ± 0.11****
Cathepsin D	Free activity	23.77 ± 1.19	ND	31.70 ± 2.01**	ND
	Total activity	70.97 ± 4.97	ND	227 ± 11.9****	ND
	T/F ratio	3.03 ± 0.17	-	7.19 ± 0.42****	ND
DNAse II	Free activity	41.26 ± 2.00	33.07 ± 1.89**	33.0 ± 1.50**	ND
	Total activity	88.01 ± 5.28	50.31 ± 2.59****	186 ± 14.8****	ND
	T/F ratio	2.15 ± 0.12	1.52 ± 0.08****	5.45 ± 0.34****	ND
RNAse II	Free activity	33.52 ± 1.34	61.03 ± 3.36****	59.0 ± 3.64****	91.40 ± 6.40$^{****, b}$
	Total activity	313 ± 18.8	147 ± 7.64****	428 ± 18.6****	293 ± 20.3^{b}
	T/F ratio	9.33 ± 0.37	2.42 ± 0.07****	7.25 ± 0.38***	3.21 ± 0.22$^{****, a}$

The experimental details are as described in the text. Results are given as mean ± S.E.M. of no. of observations indicated in parentheses in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as µg tyrosine positive materials / 10min /mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as nmole nucleotides / 10min / mg protein.
PTX-CLB treated: PTX-induced epileptic animals treated with CLB; T/F ratio: Ratio of Total activity over Free activity.
ND, not detectable; *, $p<0.02$; **,$p<0.01$; ***,$p<0.002$ ****,$p<0.001$ compared with Control group;
a, $p<0.01$ and b, $p<0.001$ compared with PTX-treated group.

Table 9. Effects of PTX treatment on liver lysosomal enzyme activities.

Enzyme	Treatment	Activity		Total activity
		Free	Total	Free activity
Acid phosphatase	Control	124 ± 5.0	373 ± 14.0	3.01 ± 0.12
	Epileptic	110 ± 5.0	270 ± 16.0**	2.45 ± 0.14*
Cathepsin D	Control	7.70 ± 1.2	58.03 ± 7.6	7.54 ± 1.12
	Epileptic	ND	ND	-
RNAse II	Control	37.0 ± 2.0	190 ± 8.0	5.41 ± 0.25
	Epileptic	53.0 ± 1.4**	200 ± 8.0	3.77 ± 0.19**
DNAse II	Control	18.0 ± 0.4	73.0 ± 7.0	4.29 ± 0.25
	Epileptic	57.0 ± 1.1**	160 ± 6.0**	2.81 ± 0.08**

The experimental details are as described in the text. Results are given as mean ± S.E.M. of 16 independent observations in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as μg tyrosine positive materials / 10min / mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as n mole nucleotides / 10min / mg protein.
ND, not detectable; *, $p < 0.01$ and **, $p < 0.001$ compared with the corresponding control.

Table 10. Effects of Carbamazepine (CBZ) treatment on liver lysosomal enzymes activities in control and PTX-induced epileptic condition

Enzyme	Activity	Control (8)	PTX treated (8)	CBZ treated (8)	PTX-CBZ (8)
Acid phosphatase	Free activity	111 ± 5.60	106 ± 4.80	95 ± 1.50***	127 ± 5.50$^{****, a}$
	Total activity	358 ± 25.0	275 ± 16.5****	333 ± 22.0***	277 ± 10.5
	T/F ratio	3.23 ± 0.20	2.61 ± 0.14****	3.51 ± 0.14**	2.18 ± 0.09$^{*, a}$
Cathepsin D	Free activity	14.42 ± 0.47	ND	9.08 ± 0.26**	ND
	Total activity	56.62 ± 5.51	ND	52.35 ± 5.32****	ND
	T/F ratio	3.92 ± 0.28	-	5.77 ± 0.34****	ND
DNAse II	Free activity	15.01 ± 0.38	58.01 ± 2.20**	43.3 ± 2.60**	43.3 ± 3.20$^{****, b}$
	Total activity	114 ± 6.00	164 ± 7.70****	125 ± 8.70****	130 ± 11.1^{a}
	T/F ratio	7.60 ± 0.30	2.83 ± 0.12****	2.89 ± 0.19****	3.00 ± 0.24
RNAse II	Free activity	21.0 ± 1.63	51.03 ± 2.50****	414 ± 5.90****	21.40 ± 1.93^{b}
	Total activity	165 ± 5.20	217 ± 4.60****	390 ± 13.0****	81.0 ± 1.47$^{****, b}$
	T/F ratio	4.28 ± 0.29	4.25 ± 0.15****	9.51 ± 0.23***	3.79 ± 0.21^{b}

The experimental details are as described in the text. Results are given as mean ± S.E.M. of no. of observations indicated in parentheses in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as μg tyrosine positive materials / 10min /mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as nmole nucleotides / 10min / mg protein.
PTX-CBZ treated: PTX-induced epileptic animals treated with CBZ; T/F ratio: Ratio of Total activity over Free activity.
ND, not detectable; *, $p<0.02$; **,$p<0.01$; ***,$p<0.002$ ****,$p<0.001$ compared with Control group;
a, $p<0.02$ and b, $p<0.001$ compared with PTX-treated group.

Table 11. Effects of Lamotrigine (LTG) treatment on liver lysosomal enzymes activities in control and PTX-induced epileptic condition

Enzyme	Activity	Control (8)	PTX treated (8)	LTG treated (8)	PTX-LTG (8)
Acid phosphatase	Free activity	110 ± 9.30	84.0 ± 9.40	116 ± 10.3***	.0 ± 1.18
	Total activity	373 ± 14.0	230 ± 8.24****	280 ± 23.2***	140 ± 6.10$^{****, b}$
	T/F ratio	3.39 ± 0.18	2.74 ± 0.13**	2.41 ± 0.21*	1.49 ± 0.13****
Cathepsin D	Free activity	14.55 ± 0.41	ND	17.53 ± 1.51	7.31 ± 0.69$^{****, b}$
	Total activity	52.35 ± 5.33	ND	11.32 ± 0.96****	67.15 ± 3.34$^{*, b}$
	T/F ratio	3.60 ± 0.23	-	0.65 ± 0.06****	9.18 ± 0.66$^{****, b}$
DNAse II	Free activity	13.00 ± 0.20	55.0 ± 1.50****	46.0 ± 2.60****	15.3 ± 0.86$^{*, b}$
	Total activity	110.1± 5.40	158 ± 6.20****	193 ± 6.20****	117 ± 5.10^{b}
	T/F ratio	8.47 ± 0.13	2.87 ± 0.10****	4.14 ± 0.02****	7.65 ± 0.38
RNAse II	Free activity	22.2 ± 1.74	53.20 ± 1.60****	24.2 ± 1.50	23.40 ± 0.89^{b}
	Total activity	174 ± 8.00	221 ± 6.90****	183 ± 13.0	120.0 ± 11.2$^{***, b}$
	T/F ratio	7.56 ± 0.44	4.15 ± 0.13****	7.56 ± 0.77	5.13 ± 0.34$^{****, a}$

The experimental details are as described in the text. Results are given as mean ± S.E.M. of no. of observations indicated in parentheses in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as μg tyrosine positive materials / 10min / mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as nmole nucleotides / 10min / mg protein.
PTX-LTG treated: PTX-induced epileptic animals treated with LTG; T/F ratio: Ratio of Total activity over Free activity.
ND, not detectable; *, $p<0.02$; **,$p<0.01$; ***,$p<0.002$ ****,$p<0.001$ compared with Control group;
a, $p<0.02$ and b, $p<0.001$ compared with PTX-treated group.

Table 12. Effects of Clobazam (CLB) treatment on liver lysosomal enzymes activities in control and PTX-induced epileptic condition

Enzyme	Activity	Control (8)	PTX treated (8)	CLB treated (8)	PTX-CLB (8)
Acid phosphatase	Free activity	108 ± 7.40	95.0 ± 4.80	53.5± 2.23****	148 ± 6.80$^{****, c}$
	Total activity	345 ± 13.1	273 ± 16.4**	119 ± 15.4****	364 ± 14.2^{b}
	T/F ratio	3.19 ± 0.11	2.84 ± 0.11*	2.22 ± 0.19****	2.46 ± 0.11$^{****, a}$
Cathepsin D	Free activity	13.45 ± 0.54	ND	22.04 ± 0.68****	ND
	Total activity	53.47 ± 2.67	ND	76.89 ± 3.43****	ND
	T/F ratio	3.71 ± 0.22	-	3.49 ± 0.13	ND
DNAse II	Free activity	14.01 ± 0.28	56.52 ± 1.70****	43.7 ± 2.31****	66.01± 1.76$^{****, b}$
	Total activity	112.1 ± 3.63	161 ± 5.15****	319 ± 7.64****	192 ± 12.4$^{****, a}$
	T/F ratio	8.04 ± 0.24	2.86 ± 0.09****	7.30 ± 0.28	2.91 ± 0.13****
RNAse II	Free activity	22.61± 0.90	52.12 ± 1.46****	52.6 ± 1.61****	57.30 ± 3.20****
	Total activity	170 ± 8.51	219 ± 5.48****	300 ± 1.03****	197.0 ± 10.5
	T/F ratio	7.52 ± 0.27	4.21 ± 0.12****	5.70 ± 0.98	3.44 ± 0.19$^{****, a}$

The experimental details are as described in the text. Results are given as mean ± S.E.M. of no. of observations indicated in parentheses in each group. Acid phosphatase activity is expressed as nmole P_i / 10min / mg protein and the Cathepsin D activity is given as µg tyrosine positive materials / 10min / mg protein. The nucleases (RNAse II and DNAse II) activity is expressed as nmole nucleotides / 10min / mg protein.
PTX-CLB treated: PTX-induced epileptic animals treated with CLB; T/F ratio: Ratio of Total activity over Free activity.
ND, not detectable; *, $p<0.05$; **,$p<0.01$; ***,$p<0.001$ compared with Control group;
a, $p<0.05$; b, $p<0.002$ and c, $p<0.001$ compared with PTX-treated group.

Effect of treatment with AEDs to the non-epileptic animals on BRAIN lysosomal enzymes:

Effects of AED treatments on brain lysosomal enzyme activities are shown in Tables 6-8.

The acid phosphatase activities, both 'free' and 'total' decreased significantly but disproportionately following CBZ and LTG treatments (20-50% decrease), except for the 'free' activity in the LTG treated group, which was unchanged (Table 6, 7). Consequently, the T/F ratio decreased. CLB treatment, on the other hand, caused significant decrease of similar magnitude in both 'free' as well as 'total' activities (30-35% decrease), because of which the T/F ratio was unchanged (Table 8).

The effect of the three AEDs on cathepsin D activity was variable. CBZ treatment showed about equal (47-49%) increase in 'free' as well as 'total' activity while LTG treatment caused disproportionate decrease in the 'free' and 'total' activities (19% and 83% decrease respectively, Table 6, 7). Also the 'free' and 'total' activities were almost comparable which indicates total leakage of cathepsin D. Thus T/F ratio decreased in the CBZ and LTG treated rats (Table 6, 7). CLB treatment, on other hand, caused a small increase (26% increase) in 'free' activity while elevating the 'total' activity by 3.2 fold. This resulted in a very significant increase in the T/F ratio (Table 8).

The 'free' activity of RNAse II increased significantly after LTG and CLB (1.5 and 2 fold respectively) treatments (Table 7, 8); CBZ treatment had only marginal effect (Table 6). The 'total' activity decreased in CBZ and LTG groups (60-67% decrease) but was somewhat high (1.4 fold) in the CLB treated group. Consequently, the T/F ratio decreased in all the groups (Table 6-8).

CBZ and LTG treatments had no effect either on DNAse II 'free' or 'total' activities (Table 6, 7). These treatments also did not affect the T/F ratios. CLB treatment resulted in lowering of 'free' (20% decrease) but elevation of 'total' activitiy (2.4 fold higher activity). Thus the T/F ratio was significantly high (Table 8).

Thus in general, in the brain, CBZ and LTG treatments tended to decrease T/F ratio for practically all the lysosomal enzymes indicating damage to lysosomal integrity, whereas following CLB treatment lysosomal membrane integrity was better preserved.

Effect of treatment with AEDs to the non-epileptic animals on LIVER lysosomal enzymes:

Effects of AED treatments on liver lysosomal enzyme activities are shown in Tables 10-12.

The 'free' acid phosphatase activity was practically not affected upon AED treatments (Table 10-12) except for the CLB group where the activity showed 51% decrease (Table 12). The 'total' activity showed decrease following LTG and CLB administration (19 and 66% decrease respectively), whereas CBZ treatment had no effect (Table 10-12). Thus in the LTG and CLB groups the T/F ratio decreased.

The 'free' cathepsin D activity increased by 1.6 to 2 folds in the LTG and CLB treated rats. The 'total' activity was significantly reduced in the LTG treated rats and was comparable to 'free' activity once again indicating damage to membrane integrity and complete leakage of the enzyme; opposite effect was seen with CLB treatment where both 'free' and 'total' activities increased (Table 11, 12). CBZ treatment had no effect either on 'free' or on 'total' activity (Table 10). The T/F ratio decreased in both LTG and CLB groups (Table 11, 12).

The effects of AED treatment on 'free' RNAse II activity were variable; CBZ and CLB caused 2 to 2.5 fold increase with similar increase in the 'total' activities (Table 10, 12). LTG had no effect (Table 11). The T/F ratio increased in the CBZ group but was significantly low in the CLB group (Table 10, 12).

All the AEDs brought about significant reduction (overall 65% decrease) in the 'free' as well as 'total' DNAse II activity (Table 10-12). Thus T/F ratios decreased after CBZ and

LTG treatment; the ratio increased in CLB treated rats due to greater reduction in the 'free' activity.

In general, in the liver, LTG treatment showed decrease in 'total' activity of practically all the lysosomal enzymes except for RNAse II and a consequent decrease in the T/F ratio (Table 11), whereas the effects of CBZ and CLB were of variable nature (Table 10, 12).

Effect of treatment with AEDs on BRAIN and LIVER lysosomal enzymes in the PTX-induced epileptic animals:

The effect of CBZ treatment on lysosomal enzyme activities in brain and liver of epileptic animals are shown in Tables 6 and 10 respectively.

The results clearly indicate that, in the brain, CBZ treatment was not able to restore the acid phosphatase and cathepsin D activities in the epileptic animals (Table 6). In the brain, free and total DNAse II activities were completely lost. RNAse II free activity was decreased by 64% with 1.5 fold increase in the T/F ratio (Table 6).

In the liver, CBZ treatment followed the same trend as in brain (Table 10). Here also, acid phosphatase and cathepsin D activities were not recovered back to normal; in-fact 1.3 fold increase in free acid phosphatase activity was observed. Free as well as total

activities of DNAse II and RNAse II were decreased significantly (20-63% decrease) thereby T/F ratio was remained unaffected after CBZ treatment to the epileptic animals (Table 10).

The effect of LTG treatment on lysosomal enzyme activities in brain and liver of epileptic animals are shown in Tables 7 and 11 respectively.

In the brain, LTG treatment resulted in complete loss of DNAse II and RNAse II free as well total activities (Table 7). The free activities of these enzymes have a tendency to decrease in epileptic condition (Table 5). The total acid phosphatase activity was decreased by 21% without affecting the free activity, thus resulting in the lowering of T/F ratio. Cathepsin D free activity was completely restored as compared to control with tremendous elevation in total activity, thus resulted into 2.6 fold increase in T/F ratio (Table 7).

In the liver, acid phosphatase and cathepsin D activities followed similar pattern to the brain (Table 11). Whereas, the free DNAse II activity was increased by 1.3 folds with 27% decrease in the total activity, thereby reflecting a 1.7 fold increase in T/F ratio. In the case of RNAse II, both free and total activities decreased significantly (55% and 45% respectively), thus resulting in increased T/F ratio. Hence, DNAse II and RNAse II

activity were nearly restored in the liver with LTG treatment as compared to control (Table 11).

The effect of CLB treatment on lysosomal enzyme activities in brain and liver of epileptic animals are shown in Tables 8 and 12 respectively.

Thus, in the brain, DNAse II activity was completely abolished with CLB treatment, whereas, cathepsin D activity was not restored (Table 8). Free acid phosphatase activity was increased by 1.3 fold with the restoration of total activity (1.3 fold increase), thus T/F ratio was unaffected. RNAse II activity showed further increase in both free and total activities (1.5 and 2 fold increase respectively); with 1.3 fold elevation in T/F ratio (Table 8).

In the liver, free as well total acid phosphatase activity was elevated by 1.3 to 1.5 folds, with slight decrease (13%) in T/F ratio (Table 12). Here also, cathepsin D activity was not restored. DNAse II free and total activities were increased marginally (about 1.2 fold). Whereas RNAse II activity remains consistent with compared to PTX treatment (Table 12).

Discussion

Effects of PTX treatment on brain and liver lysosomal function:

The process of neurodegeneration in epilepsy as a consequence of excitotoxicity (2, 3) can result in the accumulation of the cell debris. One may hence anticipate that the lysosomal enzymes in general and cathepsin D in particular may play a role in the removal of the debris. Neurodegeneration and accumulation of lipofuchsin pigments in brains of cathepsin D deficient CD -/- mice has been reported (36). However, the mechanism of clearance of cell debris remains unclear.

Deficiency of lysosomal enzymes leading to pathological conditions has been reported by several investigators (5, 6, 36-42). The cathepsin D deficient (CD-/-) mice manifest seizures and become blind near the terminal stage i.e. around postnatal day 26 (36). Mutation in ovine cathepsin D gene causes congenital lysosomal storage disease with profound neurodegeneration (40). Earlier, Hetman *et al.* reported increased cathepsin D mRNA and increased cathepsin D immunoreactivity in the rat brain 3 days after kainate treatment (7). In contrast to this, we found that the cathepsin D activity was completely abolished after PTX treatment in brain and liver (Table 5 and 9). However, it may be pointed out here that in their experiment Hetman *et al.* did not measure the enzyme activity; their estimates were restricted only to the measurement of mRNA and immunoreactivity as determined by histochemistry (7). It is possible that in their

experiments the authors detected the cathepsin D peptide which had the immunoreactive epitope but not enzyme activity. In other words, despite the increased mRNA levels, either the enzymatically active protein was not synthesized and/or a truncated cathepsin D protein was synthesized. Complete loss of cathepsin D activity as we observe here possibly suggest that cathepsin D may not play any role in clearing of cell debris which originate due to excitotoxicity. Interestingly, reports reveal that cathepsins B, L and S activities are found to be enhanced in progressive myoclonus epilepsy (8, 9).

Also, of interest to note in this context is the reported presence of proteases other than cathepsin D e.g. cathepsin A and calcium activated neutral protease (CANP) in the brain (42). These proteases act on neurofilament proteins in humans and mouse brain (42). Also, the elevation of cathepsin A and carboxypeptidase in neuropathological conditions has been reported (42). Neurodegeneration in epilepsy due to excitotoxicity is believed to result in increased intracellular concentration of Ca^{2+}. This could possibly bring about the activation of CANPs. Taken together the results would thus suggest that these proteases (42) along with other cathepsins (8, 9) rather than cathepsin D might play an important role in clearing the cell debris. However, this possibility needs to be verified by more direct experiments.

Cerebral lysosomal dysfunction is noteworthy as reflected in terms of decreased ratios of Total activity / Free activity for acid phosphatase, DNAse II and RNAse II (Table 5). It may also be noted that the free RNAse II activity in the brain increased by 1.3 fold (Table

5). Earlier we have noted similar increase in free RNAse II activity in paracetamol-induced hepatotoxicity (34). The increased free nuclease activities in the brain can result in indiscriminate degradation of nucleic acids thereby hampering the metabolic activity ultimately leading to necrosis or neuronal death.

Studies on the liver were carried out to find out the effects of PTX exposure on the liver lysosomal functions, since liver is the site of PTX metabolism, while the epileptogenic effects are manifested due to the binding of PTX to $GABA_A$ receptor complex (27). Interestingly, GABA related disturbances are also found to alter the feeding behaviors (13). However, apparently $GABA_A$ receptors are not present in the liver (44, 45). Hence, with respect to liver the effects we report here are not related to $GABA_A$ receptor mediated action of PTX and it may be suggested that the observed effects could be due to prolonged exposure to PTX and/or its metabolites picrotoxinin and picrotin.

The generalized feature of PTX treatment was the increased free nuclease activities with increase only in the total DNAse II activity in liver (Table 9). As a consequence the ratio of Total activity / Free activity decreased significantly, implying that the PTX treatment adversely affected the lysosomal permeability. In the case of liver acid phosphatase the total activity was low; once again the ratio of Total activity / Free activity decreased (Table 9).

Increased free nucleases activity is a matter of concern since these enzymes can lead to nucleic acids breakdown and DNA fragmentation. The lysosomal DNAse II is known to be Ca^{2+} dependent and levels of free cytosolic Ca^{2+} are known to increase in excitotoxicity. If similar changes are seen in liver then this would further enhance the damage ultimately leading to necrosis. In earlier studies in our lab, it was shown that in paracetamol-induced hepatotoxicity the nucleases levels increased (34); necrosis in paracetamol hepatotoxicity is well recognized (46).

Effect of treatment with AEDs to the non-epileptic animals on brain and liver lysosomal function:

The results on AEDs treatment on our present studies showed that indeed the treatment with AEDs affected the lysosomal function even in non-epileptic rats and that the effects of the AEDs are diverse and tissue and enzyme specific (Tables 6-8 and 10-12). Nevertheless, some similarities and differences in their action on the lysosomal enzymes in the two tissues i.e. liver and brain were evident.

Thus CBZ treatment lowered the acid phosphatase and cathepsin D activities in liver and brain with loss of membrane integrity (Table 6, 10). Similar effects were seen even for RNAse II activity for brain; opposite effects were noted in liver. CBZ treatment also resulted in decreased DNAse II activity and loss of membrane integrity only in liver (Table 10).

LTG treatment resulted in lowering of the acid phosphatase and cathepsin D activities in both the tissues (Table 7, 11). The effects on cathepsin D were of more drastic nature. LTG treatment also affected in a similar manner the RNAse II activity in the brain and DNAse II activity in liver (Table 7, 11).

CLB treatment increased the cathepsin D and RNAse II activities in both the tissues and similar trend was seen for DNAse II activity in the brain (Table 8, 12). The acid phosphatase activities in liver and brain and DNAse II activity in the liver showed opposite trend (Table 8, 12).

The results thus suggest that the CBZ and LTG in general adversely affect the lysosomal functions and the membrane integrity. CLB in general tends to maintain or improve the membrane integrity.

The similarities in the effects of CBZ and LTG are not surprising because these two AEDs are respectively non-specific and specific Na^+ channel blockers (10, 47). It would be therefore seen that the CBZ and LTG effects at least on the brain may be mediated through changes in Na^+ concentrations. CLB on the other hand is $GABA_A$ receptor agonist and opens Cl^- channels (48); opposite effects of CLB may be attributed to this fact.

Taken together, although there are similarities and differences, the effects of the three AEDs are not only diverse but also seem to be tissue specific and also specific for the enzymes. Also, the 'free' and 'total' activities and their ratios were affected in a tissue specific and enzyme specific manner. This would imply that the AEDs may not only act by altering the membrane integrity but might have effects at genomic level and/or may directly activate/inactivate the individual enzymes. However, these possibilities need to be verified experimentally.

Effect of treatment with AEDs to the PTX-induced epileptic animals on brain and liver lysosomal function:

The result of present studies showed that treatment with AEDs differentially affected the lysosomal functions in epileptic rats and the effects are tissue and enzyme specific.

Therefore, CBZ treatment in the brain, lowered acid phosphatase and RNAse II activities, whereas membrane integrity was lost for these enzymes in both the tissues (Table 6, 10). Cathepsin D activity was not restored as compared to control in brain and liver. DNAse II activity was completely abolished upon CBZ treatment. In liver, DNAse II free activity increased 3 folds, indicating membrane leak out of the enzyme (Table 10). Thus, clearly reflecting loss of membrane integrity.

LTG treatment resulted in lowering of acid phosphatase activity in both the tissues with increased membrane fragility (Table 7, 11). Cathepsin D activity was completely restored in both the tissues with positive improvement in the membrane integrity. DNAse II and RNAse II activity in brain was completely abolished after LTG treatment (Table 7), thus eliminates possible adverse effects of nucleases in epileptic condition. Whereas in liver, LTG treatment was able restore the lysosomal nuclease activities with improvement in T/F ratio, thus maintained the lysosomal membrane integrity (Table 11).

CLB treatment on other hand increased the membrane fragility for acid phosphatase and RNAse II in the brain; and DNAse II, RNAse II, acid phosphatase in the liver (Table 8, 12). DNAse II activity was abolished in the brain and increased significantly in liver with decrease in T/F ratio. Cathepsin D activity was not recovered in both the tissues. Additionally, membrane integrity was not restored back to normal after CLB treatment (Table 8, 12).

The results thus suggest that CBZ and CLB treatment in general failed to maintain or improve the lysosomal function and lysosomal membrane integrity in the epileptic animals. In fact, CLB adversely affected the lysosomal function by elevating the free nuclease activity. On the other hand, LTG treatment tends to improve the lysosomal function by retaining cathepsin D activity and by maintaining or lowering the lysosomal nucleases activities, which may be responsible for cell death or necrosis in the epileptic condition.

The reason for the difference in the effects of these AEDs is their mode of actions. As mentioned in the above section, CBZ is a non-specific sodium ion channel blocker (49) and it also known to act through varying amine and $GABA_A$ neurotransmitter levels (50, 51). Thus, conclusive molecular basis for its antiepileptic action is not clear. CLB is a benzodiazepine (BZ) class of drug, which acts as a $GABA_A$ receptor agonist, exhibit broad spectrum of pharmacological activities (48, 49). LTG on the other hand is a specific-voltage gated sodium ion channel blocker, which inhibits presynaptic release of glutamate (52).

However, in the present studies, LTG treatment was able to restore the lysosomal function to some extent as compared to CBZ and CLB treatment. In the earlier reports, LTG was proven to be a drug of choice amongst the other conventional AEDs tested (53). The adverse effect of CLB can be explained by the fact that BZ receptors exists as supramolecular complex of $GABA_A$ receptor / BZ receptor / chloride ionophore (48, 54). PTX in contrast to BZ binds to Cl^- channels directly (54). PTX is also shown to inhibit binding of BZs to membranes (54). However, reports regarding interaction of PTX and CLB with these types of receptors are lacking. Thus, possible insufficiency of CLB interactions with membrane receptors may contribute to the accumulation of adverse action of CLB on the subcellular membranes.

In conclusion, the results of present studies indicate that the lysosomal dysfunction was manifested especially in terms of increased membrane permeability following prolonged

exposure to PTX. This in turn could possibly lead to process of neurodegeneration. The adverse cellular / subcellular functions in epilepsy are differentially corrected by different kind of AEDs tested. As such in the present studies, LTG improves the adverse lysosomal functions in the epileptic condition as compared to CBZ and CLB treated group. This would imply that, inefficiency of these AEDs in correcting the disturbances in the lysosomal functions and lysosomal membrane integrity that we observe here in epilepsy could not only lead to the repercussion of drug intolerance and side effects, but also deteriorate the lysosomal functions, which were altered under the epileptic condition. It is possible that the disturbances in the lysosomal enzyme activities and membrane integrity which we observe here, could account at least partly for the intolerance and side effects of the AEDs (55).

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Summary

Sudden burst of electrical activity in epilepsy could lead neurodegeneration because of excitotoxicity and thus neuronal death, which may involve the role of lysosomes as a cause or consequence. Hence, studies on the lysosomal functions were carried out in PTX-induced convulsions and in AED treatment by checking activities of the marker enzymes viz. acid phosphatase, cathepsin D, acid ribonuclease (RNAse II) and acid deoxyribonuclease (DNAse II). It was evident that lysosomal dysfunction was manifested especially in terms of increased membrane permeability following prolonged exposure to PTX. This in turn could possibly lead to process of neurodegeneration. AEDs treatment differentially altered the lysosomal function in either control or epileptic animals in both the tissues. PTX and/or AED treatment resulted in to loss of cathepsin D activity in brain and liver. In general, AEDs were exerting their own effects and therefore further deteriorating the lysosomal function in the epileptic animals. LTG improves the adverse lysosomal functions to some extent in the epileptic condition as compared to CBZ and CLB treated group. This would imply that, inefficiency of these AEDs in correcting the disturbances in the lysosomal functions and lysosomal membrane integrity in epilepsy could not only lead to the repercussion of drug intolerance and side effects, but also depreciate the lysosomal functions, which were altered under the epileptic condition. Thus, disturbances in the lysosomal enzyme activities and membrane integrity, could account at least partly for the intolerance and side effects of the AEDs.