Chapter 2

Effect of Picrotoxin-induced Convulsions on Oxidative Energy Metabolism in Rat Brain and Liver Mitochondria

Introduction

Mitochondrial oxidative phosphorylation is the primary source of energy for neuronal metabolism. About 40% of the total ATP is consumed by Na^+,K^+-ATPase and Ca^+,Mg^+-ATPase, which maintains the neuronal plasma membrane potential and intracellular Ca^{2+} sequestration (1, 2, 3). Mitochondrial function is crucial determinant of cell death and oxidative stress, thus it act as the "stress sensor", and in extreme circumstances "executioner" of the cell (4). Additionally mitochondria play an important role in neurotransmitter metabolism (5). There are accumulated evidences suggesting that epileptic seizures can occur as a presenting sign of mitochondrial dysfunction in the central nervous system (6, 7).

Neuronal activity can cause short-term changes of mitochondrial membrane potential which serves as an important factor for regulating mitochondrial permeability transition (MPT) (8). Additionally, long-term changes of oxidative phosphorylation resulting from modulation of mitochondrial gene expression in response to increased synaptic activity have been described (9). Intense seizure activity causes massive influx of Ca^{2+} through voltage-gated and N-methyl-D-aspartate (NMDA)-dependent ion channels (10), which results in elevated intracellular and intra-mitochondrial Ca^{2+} thus leading to mitochondrial membrane depolarization. Thereby resulting in energy failure and superoxide production (11, 12, 13). This could trigger the acute neuronal cell death that occurs after status epilepticus (14). Free radicals like superoxide, hydroxyl radical are

highly reactive oxygen species, which, unopposed, can damage cell structures including lipids, proteins and DNA (15).

Alterations in neurotransmitter concentrations in synapse (16, 17, 18), reduced plasma membrane Na^+,K^+-ATPase activity, cellular pH and residual ATP content in subcellular fractions and lysosomal dysfunction in rat brain have also been reported in some animal models of epilepsy (19, 20, 21). In broad variety of mitochondrial cytopathies, epileptic phenotype is observed, for example myclonus epilepsy with 'ragged red fibers' (MERRF) syndrome, Leigh syndrome, progressive external opthalmoplegia (PEO) or chronic progressive external opthalmoplegia (CPEO) and so on (22, 23, 24). Deficiency of complex I of respiratory chain is documented in patients with temporal-lobe epilepsy (25).

In contrast to the short-term alterations of mitochondrial function by epileptoform activity, it remains unclear whether, and how exactly cerebral mitochondrial structure-function is altered under the long-term epileptic condition.

Many species of animals do develop spontaneous seizures; however, these cases are sporadic, and not usually suitable for experimentation (26). Several animal models are being used to study epilepsy (26). This includes use of various chemical epileptogens such as kainic acid, picrotoxin (PTX), pentylenetetrazole, bicuculine etc., electrically

kindled and genetic models like photosensitive baboons, DBA/2J, E1 and totterer mice, genetically epilepsy prone rats (GEPRs) etc.

Picrotoxin (PTX) is a non-competitive $GABA_A$ receptor antagonist that provokes seizures by reducing inhibitory action of GABA. In the present study PTX-induced animal model was used to investigate long-term changes in the mitochondrial oxidative energy metabolism in epileptic rat brain. Parallel studies were also carried out in liver – a peripheral tissue and major metabolic organ – that served as internal control. Mitochondrial function was characterized by assessing oxidative energy metabolism by employing various respiratory substrates, cytochromes content and activity of ATP-synthesizing enzyme F_oF_1-ATPase and dehydrogenases. The results of these investigations are summarized in this chapter.

Materials and Methods

Chemicals

Picrotoxin (PTX), sodium salts of succinic, pyruvic, L-malic and oxaloacetic acids; ATP, ADP, rotenone, Triton X-100, NADH, NAD^+, dichloroindophenol (DCIP), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, USA). L-glutamic acid was obtained from E. Merck (Dramstadt, Germany). *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) and 2,4-dinitrophenol (DNP) was purchased from British Drug Houses (Dorset, Poole, England). Ascorbic acid was from Sarabhai

Chemicals (Vadodara, India). All other chemicals were purchased locally and were of analytical-reagent grade.

Animals and treatment with PTX

Male albino rats of Charles-Foster strain (200-250 g) were used. The animals had free access to food and water. PTX solution was prepared fresh daily in saline and was injected intraperitoneally (ip) at the dose of 1.5 mg/kg body weight for 20 consecutive days (27). Initially, the animals developed seizures within 30 min of PTX administration. Tonic-clonic convulsions were well established within 8-10 days of treatment. At the later stages, seizures developed within 10-20 min of PTX administration. The controls were given equivalent volume of saline. There was no mortality of rats.

The animals were kept in individual cages and observed for incidences, character and intensity of epileptic manifestations. The animals were scored according to the scale described by Kubova et al. (1992) (28) as indicated below:

0 No changes

1 Uneasiness, scratching tremor, single myoclonic jerks

2 Atypical minimal seizures

3 Minimal seizures consisting of clonic convulsions involving the head and forelimb muscles and leaving righting reflexes intact

4 Major seizures without the tonic phase

5 Complete major seizures i.e. generalized tonic-clonic convulsions with loss of righting reflexes.

Isolation of mitochondria

The animals were killed by decapitation on day 21 of PTX treatment. Brain and liver were quickly dissected out and placed in beakers containing chilled (0-4 °C) isolation medium (0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4, 1mM EDTA and 250 µg BSA/ml) (29). The tissue was thoroughly washed with isolation medium to make it free from blood and 10% (w/v) homogenate was prepared using a Potter Elvehjem type glass-Teflon homogenizer. The nuclei and cell debris were sedimented by centrifugation at 650 × g for 10 min and discarded. The supernatant was subjected to a further centrifugation at 10,000 × g for 10 min. From the sedimented fraction in brain, the loosely packed synaptosomal-myelin fraction was discarded after gentle swirling, taking care not to disturb the tightly packed mitochondrial pellet. The mitochondria were washed by gently suspending them in the isolation medium and resedimenting at 6500 × g for 10 min. Finally the mitochondria were suspended in the isolation medium to give a protein concentration of ca. 10-15 mg/ml and 25-30 mg/ml for liver. All the steps in the isolation procedure were carried out at 0-4 °C in a Sorvall RC 5B*plus* refrigerated centrifuge using SS34 rotor. It was shown previously in the lab that this procedure yields pure

mitochondrial preparations which are practically free from synaptosomal, microsomal and cytosolic contaminations (30).

Measurement of oxidative phosphorylation

Measurement of oxidative phosphorylation was carried out at 25 °C using Clark type oxygen electrodes as described previously (29, 30). For brain mitochondria, the respiration medium contained, in a volume of 1.6 ml, 225 mM sucrose, 5 mM potassium phosphate buffer, pH 7.4, 10 mM Tris-HCl buffer, pH 7.4, 10 mM KCl, 0.2 mM EDTA and 100 µg BSA/ml. For liver, total 1.6 ml of respiration medium consisted of 225 mM sucrose, 20 mM KCL, 10 mM Tris-HCl buffer pH 7.4, 5 mM potassium phosphate buffer pH 7.4, 0.2 mM EDTA and 160 µg of BSA (i.e. 0.1 mg/ml). Depending on the substrate used, 2-5 mg of mitochondrial protein was added and respiration was initiated by the addition of substrate. The substrate used were glutamate (10 mM), pyruvate (10 mM) + malate (1 mM), succinate (10 mM), and ascorbate (10 mM) + TMPD (0.1 mM). With the latter two substrate 0.1 µM rotenone was included. State 3 respiration rates were initiated by the addition of about 75 to 200 nmol of ADP in small aliquots (10-20 µl) and the respiration rates in the presence of added ADP (state 3) and after its depletion (state 4) were recoded. Calculations of the ADP/O ratio and ADP phosphorylation rates were as described previously (31, 32).

Assay of dehydrogenases

Malate dehydrogenase activity was measured in the assay medium contained, in a total volume of 1 ml, 120 mM potassium phosphate buffer, pH 7.8, 2.5 mM sodium oxaloacetate, 0.1% Triton X-100 and 10-20 μg of mitochondrial protein as the source of enzyme. After preincubation at 37 °C for 1 min the reaction was initiated by the addition of 1 mM NADH. The liner rate of reaction was recorded at 5-s intervals by monitoring the decrease in absorbance at 340 nm in a Shimadzu UV 160 UV-VIS spectrophotometer (33).

Succinate dehydrogenase (succinate DCIP reductase) activity was measured spectrophotometrically, in the assay medium (total volume 1 ml) contained 120 mM potassium phosphate buffer, pH 7.4, 1.5 mM freshly prepared KCN, 20 mM sodium succinate, and 200 μg mitochondrial protein as the source of the enzyme. After incubation at 37 °C for 1 min the reaction was initiated by the addition of 10 μM DCIP and the decrease in absorbance at 600 nm was recorded at 5-s intervals (34).

Glutamate dehydrogenase activity was assayed spectrophotometrically at 37 °C. The assay system (total volume 1 ml) contained 120 mM potassium phosphate buffer, pH 7.8, 5 mM sodium glutamate, 0.1% Triton X-100, and 200 μg of mitochondrial protein as the source of enzyme. After incubation at 37 °C for 1 min the reaction was initiated by the

addition of NAD^+ at a final concentration of 1.5 mM and the linear rate of reaction was recorded at 5 s intervals by monitoring the increase in absorbance at 340 nm (35).

Assay of ATPase

The brain mitochondrial ATPase activities were determined in the assay medium (total volume 0.1 ml) containing 250 mM sucrose, 10 mM Tris-HCl buffer, pH 7.4, 10 mM KCl, 0.2 mM EDTA. The assays were performed in the absence and presence of $MgCl_2$ (2 mM) and DNP (50 μM), or a combination thereof. After pre-incubating the mitochondrial protein (Ca. 50-70 μg) in the assay medium at 37 °C, the reaction was initiated by addition of ATP at a final concentration of 2 mM. The reaction was carried out for 10 min and then terminated by the addition of 1.1 ml of 5% (w/v) trichloroacetic acid (TCA). The amount of liberated inorganic phosphorous was estimated by the method of Katewa and Katyare (2003) (36).

Liver mitochondrial ATPase activity was measured in the assay medium (total volume 0.4 ml) containing 50 mM Tris-HCl buffer pH 7.4, 75 mM KCl and 4 mM EDTA. The assays were performed in the absence and presence of $MgCl_2$ (6 mM) and 100 μM DNP, or a combination of thereof. After pre-incubating the liver mitochondrial protein (Ca. 1 mg) in the assay medium at 37 °C, the reaction was initiated by the addition of ATP at a final concentration of 5 mM. The reaction was carried out for 10 min and then terminated by the addition of 0.1 ml of 5% (w/v) SDS (37) and the amount of liberated inorganic phosphorus was estimated by the method of Katewa and Katyare (2003) (36).

Cytochrome content

The contents of cytochromes (aa_3, b and $c+c_1$) were calculated from the difference spectra as described previously (38, 39). Briefly, 8-10 mg mitochondrial protein was taken up in potassium phosphate buffered sucrose and solubilized by adding 0.4 ml 10% Triton X-100. The total volume was made up to 2.5 ml. The sample was then transferred to two 1 ml cuvettes. The sample in the reference cuvette was oxidized by adding small amount of potassium ferricyanide and the sample in the experimental cuvette was reduced by adding a few mg of sodium dithionite. From the difference spectra, contents of $c+c_1$, b and aa_3 were calculated using the wavelength pairs 540-552, 562-575 and 605-625 nm respectively (38, 39). The spectra were recorded in a Shimadzu UV 160 spectrophotometer.

Protein estimation was done by the method of Lowry et al. (1951) (40) with BSA as the standard. Statistical evaluation of the data was performed using the Students' *t*-test.

Results

Effect of PTX treatment on brain mitochondrial function

The results on the effects of PTX-induced seizures on rat brain mitochondrial oxidative phosphorylation are summarized in Table 1. From the data presented, it can be noted that PTX treatment resulted in overall 27-41% inhibition of state 3 and state 4 respiration rates with glutamate as the substrate. The ADP phosphorylation rate was lower by 44%. With pyruvate + malate as the substrate pair, the extent of inhibition of state 3 respiration rate was 16%, while state 4 respiration rate was higher by 1.3 fold. The ADP/O ratio was decreased by 33%. As a consequence the ADP phosphorylation rate also decreased to a similar extent as observed for glutamate. When succinate was used as substrate, PTX treatment affected state 3 and state 4 respiration rates to a lesser extent and the inhibition ranged from 13-18%. However, the uncoupling of mitochondria was much higher compared to the former two substrates. Thus, the ADP/O ratio was decreased by 47%. The overall effect was a decrease in ADP phosphorylation rate by 65%. With ascorbate + TMPD as the substrate, the respiration rates were not affected after PTX treatment. However, ADP/O ratio was higher by 1.3 fold. Consequently, the ADP phosphorylation rate was elevated by about 1.4 fold.

Table 1. Effect of PTX-induced seizures on oxidative phosphorylation in rat brain mitochondria

Substrate	Animals	APD/O ratio	Respiration rate (nmole O_2/min/mg protein)		ADP-phosphorylation rate (nmole/min/mg protein)
		+ADP	-ADP		
Glutamate	Cont (16)	3.49 ± 0.16	33.75 ± 1.36	12.83 ± 0.69	233.19 ± 12.44
	PTX (24)	3.18 ± 0.17	19.85 ± 0.59***	9.42 ± 0.46***	130.28 ± 9.46***
Pyruvate	Cont (16)	4.50 ± 0.21	32.04 ± 1.60	9.59 ± 0.62	284.47 ± 17.61
+ malate	PTX (24)	3.00 ± 0.07***	26.76 ± 1.34*	12.43 ± 0.58**	158.40 ± 7.82***
Succinate	Cont (16)	1.79 ± 0.11	55.95 ± 2.21	32.88 ± 1.77	228.82 ± 16.88
	PTX (24)	0.96 ± 0.07***	45.74 ± 2.42**	28.77 ± 1.66	80.85 ± 4.97***
Ascorbate	Cont (16)	0.25 ± 0.01	43.06 ± 2.98	28.75 ± 2.62	21.83 ± 1.22
+ TMPD	PTX (24)	0.34 ± 0.02**	49.02 ± 3.33	30.14 ± 2.33	31.76 ± 2.14***

The experimental conditions are as described in the text. The results are expressed as mean ± SEM of the number c observation indicated in the parentheses.

*, $p<0.025$; **, $p<0.005$; ***, $p<0.001$.

The effect of PTX-induced seizures on the dehydrogenases activities in brain is shown in Table 2. It is evident that malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) activity were lowered by 20% and 80% respectively.

Table 2. Effect of PTX-induced seizures on rat brain mitochondrial dehydrogenases activities

Animals	MDH	SDH	GDH
Control (12)	2610.9 ± 82.44	11.16 ± 0.57	27.57 ± 1.78
PTX (20)	2058.5 ± 125.7*	2.19 ± 0.22*	133.4 ± 7.18*

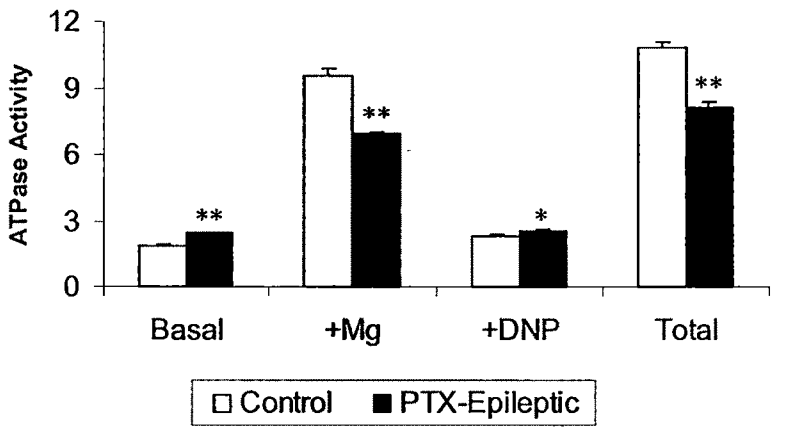
The experimental details are as given in text. MDH (malate dehydrogenase), SDH (succinate dehydrogenase) and GDH (glutamate dehydrogenase). The enzyme activities are in nmoles/min/mg protein. The results are given as mean ± SEM of the number of observation indicated in the parentheses.

* $p<0.001$

Since F_oF_1ATPase (complex V) plays an important role in ATP synthesis (41), the effect of PTX-induced epileptic condition on brain mitochondrial ATPase activity was estimated under different conditions. The results are presented in Fig 1. As can be noted, in the controls addition of Mg^{2+} showing the stimulation of ATPase activity while DNP

did not have any effect. PTX treatment caused elevation of basal and DNP stimulated activities, whereas Mg^{2+} stimulated activity was lower. Therefore composite decrease in the total activity ($+Mg^{2+}$ and +DNP) was observed.

Figure 1. Effect of PTX-induced seizures on rat brain mitochondrial ATPase activity.



The experimental conditions are as described in text. The results are given as mean ± SEM of 8 independent observations. The ATPase activity is given in μmole P_i liberated /h/mg protein. *, $p<0.05$ and **, $p<0.001$ compared with control.

The effect of PTX-induced convulsions on cytochrome content in the brain mitochondria was evaluated. The typical cytochrome spectra are shown in Fig. 2, and the contents of the respective cytochromes are given in Table 3. PTX-treatment resulted in 20% decrease in cytochrome b content without any appreciable changes in cytochrome aa_3 and cytochrome $c+c_1$ content (Table 3).

Figure 2. Typical difference spectra of cytochromes of rat brain mitochondria from control (A) and PTX-treated (B) animals. The experimental details are as described in text.

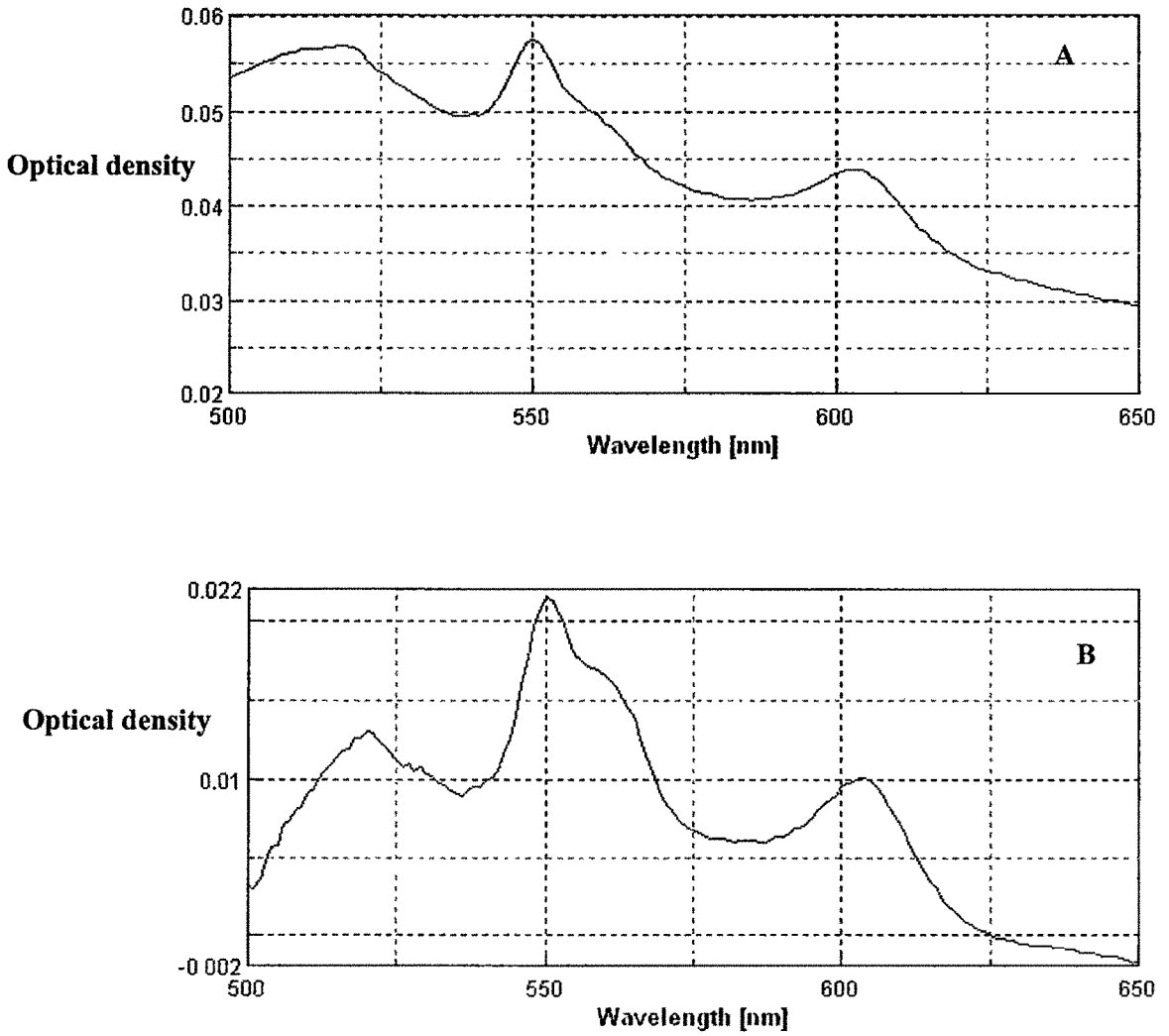


Table 3. Effect of PTX-induced seizures on mitochondrial cytochrome content in rat brain

Animals	Cytochrome content (pmoles/mg protein)		
	Cytochrome aa3	Cytochrome b	Cytochrome c+c1
Control (16)	222.21 ± 16.01	241.98 ± 12.41	241.86 ± 16.13
PTX (17)	222.75 ± 10.08	192.54 ± 7.48*	220.34 ± 10.42

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

* $p<0.002$

Effect of PTX treatment on liver mitochondrial function

The results on the effects of PTX-induced seizures on rat liver mitochondrial oxidative phosphorylation are summarized in Table 4. From the data presented, it can be noted that PTX treatment resulted in overall 15-57% inhibition of state 3 and state 4 respiration rates with glutamate and pyruvate+malate as the substrate. The ADP/O ratio was decreased by 32-44% with glutamate, pyruvate+malate and succinate as the substrate. As a consequence decrease in ADP phosphorylation rate was seen by 50-72% with the above three substrate. The extent of inhibition was higher (72%) with pyruvate+malate as the substrate pair. With ascorbate+TMPD, the opposite trend was observed with 1.3 to 1.7 fold increase in state 3 and state 4 respiration rates. Consequently, the ADP phosphorylation rate was elevated by about 1.6 fold.

The effect of PTX-induced seizures on the dehydrogenases activities in is shown in Table 5. As can noted that glutamate dehydrogenase (GDH) and succinate dehydrogenase (SDH) activity were increased (1.5 to 4 fold) while malate dehydrogenase activity decreased in the PTX treated group.

The results on effect of PTX treatment on rat liver ATPase activity are presented in Fig 3. PTX treatment caused 1.9 fold elevation of basal and Mg^{2+} stimulated activities, whereas DNP stimulated activity was slightly lower. Therefore composite decrease (33%) in the total activity (+Mg^{2+} and +DNP) was observed.

Table 4. Effect of PTX treatment on oxidative phosphorylation in rat liver mitochondria

Substrate	Animals	APD/O ratio	Respiration rate (nmole O_2/min/mg protein)		ADP-phosphorylation rate (nmole/min/mg protein)
		+ADP	-ADP		
Glutamate	Cont (16)	3.45 ± 0.17	35.42 ± 1.99	11.72 ± 0.42	243.46 ± 18.88
	PTX (24)	1.94 ± 0.12***	22.12 ± 1.18***	9.99 ± 0.54*	76.63 ± 3.40**
Pyruvate	Cont (16)	2.85 ± 0.14	29.66 ± 1.82	13.56 ± 1.05	166.24 ± 11.05
+ malate	PTX (24)	1.93 ± 0.11***	12.72 ± 0.93***	8.04 ± 0.42***	46.66 ± 3.43***
Succinate	Cont (16)	2.01 ± 0.07	65.16 ± 2.50	28.63 ± 1.32	260.60 ± 11.22
	PTX (24)	1.33 ± 0.09***	56.80 ± 3.33	29.47 ± 1.54	129.57 ± 8.07***
Ascorbate	Cont (16)	0.19 ± 0.009	42.82 ± 3.03	21.40 ± 1.29	15.50 ± 1.00
+ TMPD	PTX (24)	0.18 ± 0.009	53.97 ± 2.59**	35.72 ± 1.89***	24.47 ± 1.51***

The experimental conditions are as described in the text. The results are expressed as mean ± SEM of the number of observation indicated in the parentheses.

*, $p<0.025$; **, $p<0.01$; ***, $p<0.001$.

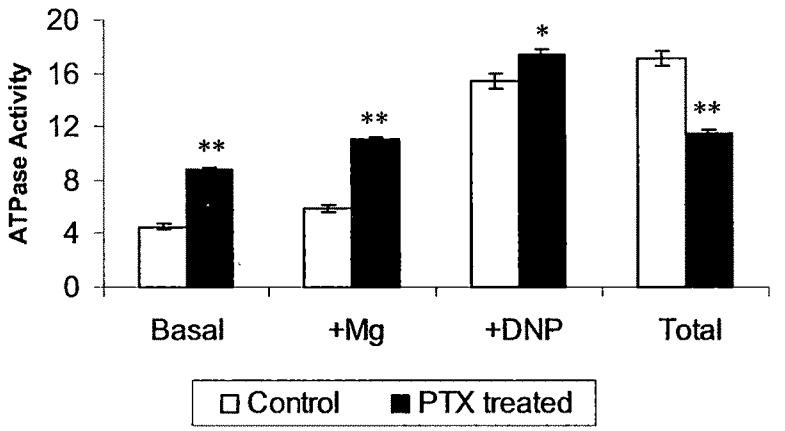
Table 5. Effect of PTX treatment on rat liver mitochondrial dehydrogenases activities

Animals	MDH	SDH	GDH
Control (12)	2828.6 ± 75.28	16.09 ± 0.97	24.48 ± 1.15
PTX (20)	1802.7 ± 108.2*	25.97 ± 1.33*	97.80 ± 5.34*

The experimental details are as given in text. MDH (malate dehydrogenase), SDH (succinate dehydrogenase) and GDH (glutamate dehydrogenase). The enzyme activities are in nmoles/min/mg protein. The results are given as mean ± SEM of the number of observation indicated in the parentheses.

* $p<0.001$

Figure 3. Effect of PTX treatmenton rat liver mitochondrial ATPase activity.



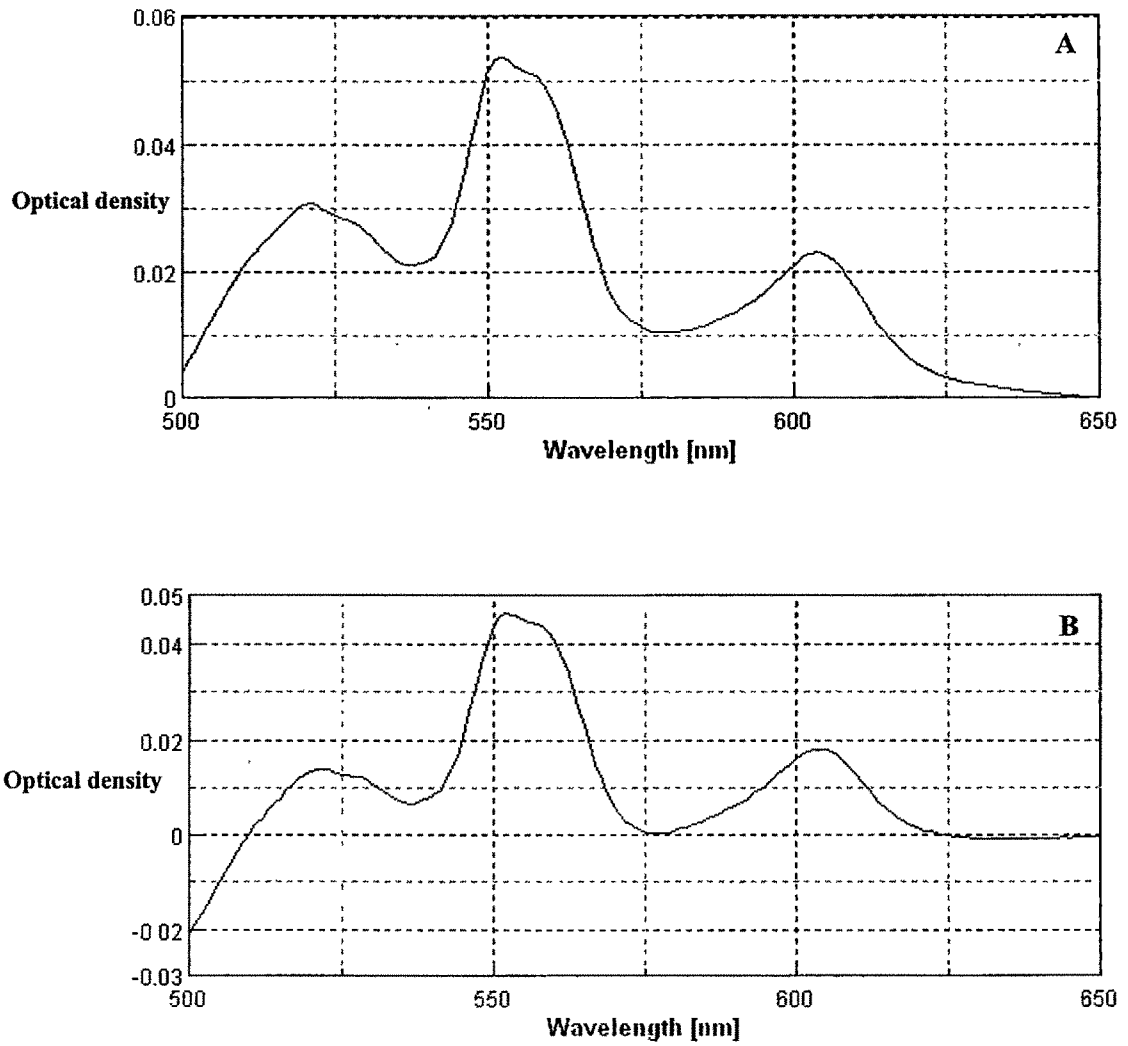
The experimental conditions are as described in text. The results are given as mean ± SEM of 8 independent observations. The ATPase activity is given in µmole P_i liberated /h/mg protein. *, $p<0.05$ and **, $p<0.001$ compared with control.

Table 6. Effect of PTX-induced seizures on mitochondrial cytochrome content in rat brain

Animals	Cytochrome content (pmoles/mg protein)		
	Cytochrome aa3	Cytochrome b	Cytochrome c+c1
Control (16)	222.99 ± 13.11	279.90 ± 5.66	251.91 ± 9.72
PTX (17)	137.53 ± 6.59*	290.54 ± 11.12	345.37 ± 15.01*

Results are given as mean ± SEM of the number of observation indicated in the parentheses. * $p<0.001$

Figure 4. Typical difference spectra of cytochromes of rat liver mitochondria from control (A) and PTX-treated (B) animals. The experimental details are as described in text.



The effect of PTX-induced convulsions on cytochrome content in the liver mitochondria are shown in Fig. 4 (typical cytochrome spectra), and the contents of the respective cytochromes are given in Table 6. PTX-treatment resulted in 38% decrease in cytochrome aa3 content while cytochrome $c+c_1$ content increased by 1.4 fold (Table 6).

Discussion

Defects in oxidative phosphorylation in the central nervous system are the characteristic sign of mitochondrial encephalopathies, which is observed in variety of diseases with epileptic phenotype (24, 42). The present investigations were done to find out the effects of chronic epileptic condition induced by PTX on brain and liver mitochondrial function. The results clearly indicate that brain mitochondrial respiratory rates were inhibited by the PTX-induced epileptic condition. The major lesions were impairment of ADP/O ratios and respiration rates with pyruvate + malate, glutamate and succinate as the respiratory substrates (Table 1). Interestingly the extent of inhibition was relatively higher when succinate was used as the substrate. This evidence is also supported by decreased activity of dehydrogenases (MDH and SDH) after PTX treatment (Table 2). These would suggest that the extent of inhibition was common for both NAD^+ and flavins linked systems. The contrasting feature was elevated rates of respiration in brain with ascorbate + TMPD as the electron donor. The possible explanation would be, ascorbate + TMPD does not include any dehydrogenase systems. One interesting observation was that the mitochondrial oxidative phosphorylation was uncoupled in the epileptic condition, which can be easily derived from the data given in Table 1. It is worth to note in this context that, expression of mitochondrial uncoupling protein 2 (UCP2) was increased during seizure activity that dissociates the cellular energy metabolism (43). Because decline of respiratory chain activities caused a rate limitation of mitochondrial

respiration, it could also affect the generation of the mitochondrial membrane potential that is linked to respiration by proton pumping.

Analysis of the cytochrome content revealed that cytochrome b - that is encoded by mitochondrial DNA (mtDNA) (39) - was significantly decreased under PTX-induced epileptic condition in brain (Table 3). Interestingly, 2-3 fold decrease in the mtDNA copy number has been reported in pilocarpine-induced convulsions (44). Mitochondrial DNA damage has also been reported in experimental models of epilepsy (12, 45). Possible mutation of mtDNA, could also lead to decreased expression of mitochondrial-encoded subunits of respiratory chain. Multiple deficiency of mitochondrial respiratory chain (MRC) complex I, IV and cytochrome oxidase have been documented for MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) and MERRF (myoclonus epilepsy with 'ragged red fibers') syndromes (24).

Additionally, the basal and DNP stimulated ATPase activities had increased following PTX treatment (Fig. 1) which is the suggestive of increased mitochondrial membrane fragility. When mitochondrial ATPase become compromised, a fall in cellular ATP levels results in decreased Ca^{2+} and Mg^{2+} pump activity and thus subsequent decrease in neuronal energy and increased membrane excitability could be implemented (3, 46).

Requirement of phospholipids for the optimum functioning of mitochondrial membrane proteins is well documented (47). In parallel studies (Chapter 5) it was also found that

PTX-induced convulsions alter the cerebral mitochondrial lipid/phospholipid composition. The level of acidic phospholipids i.e. phosphatidylserine and phosphatidylinositol decreased while lysophosphatidylglycerol levels increased. The altered phospholipid composition could have a bearing on the decreased respiration rates, which are observed here (Table 1).

The changes in energy metabolism suggest that during seizure activity, residual production does not keep up with the increased energy demands. ATP is involved in many physiological processes such as maintaining the ion / cellular pumps, mitochondrial Ca^{2+} homeostasis, various phosphorylation processes, regulation in cell membrane permeability, protein and neurotransmitter biosynthesis and exocytosis (48). Nevertheless, significant decrease in nicotinamide adenine dinucleotide (NAD) in brain regions by kainate-induced seizures has been reported (13). For every mole of NAD that is consumed, four free energy equivalent of ATP are required to generate NAD. This high utilization of energy when coupled with inhibition of oxidative phosphorylation and dehydrogenases, which is apparent here, compromises the cell's capability to maintain energy levels causing mitochondrial and neuronal damage. Neuropathological investigations have repeatedly pointed seizure related alterations of neurons characterized by swollen and often disrupted mitochondria (49).

Drastic alterations in respiration rates, ADP phosphorylation rates (Table 4) and ATPase activity (Fig 3) in liver mitochondria is an indicator of compromised oxidative energy

metabolism. Cytochrome content and dehydrogenases activities showed differential effects that suggests tissue specific alterations in mitochondrial components. Mitochondrial respiratory chain (MRC) is long being recognized as the major source of reactive oxygen species (ROS) in the cells (50), and one of the most important being the production of highly damaging superoxide (O_2^-) and nitric oxide (NO). Mammalian mitochondria are sensitive targets for cytotoxic effects of ROS, especially because higher PUFA content. NO is reported to specifically interact with mitochondrial function by competing with oxygen for cytochrome c oxidase, interacting with complex II and III to irreversibly blocking respiration and ATP synthesis (51, 52, 53). Complex I is extremely sensitive to oxidative stress because of irreversible oxidative modification of [4Fe-4S] clusters (53). Similarly, mtDNA is known to be extremely vulnerable due to lack of protective histones and inadequate repair mechanism against oxidative damage (54).

Damage is not only limited to the cerebral structures during seizures (12, 42, 55) but it could also aggravate in to peripheral system like blood plasma, RBCs, fibroblasts, muscle fibers etc. (56, 57, 58).Recent evidences mount that chronic epilepsy and prolonged use of antiepileptic drugs (AEDs) are associated with multiple risk factors that are critically implicated in pathobiology and dysfunction of the vessel wall through complex molecular mechanisms that promote atherogenesis (59). Moreover, increased ROS production and insufficiency of the antioxidant system was found in erythrocytes of epileptic patients that is proposed to play an essential role in the development of oxidative stress in epileptic patients (60). Therefore effects of PTX-induced epileptic condition on liver

mitochondrial oxidative energy metabolism are coherent with the above reports with respect to functional alterations in peripheral tissues as well.

Lipid peroxidation induced mitochondrial dysfunction and reduced energy levels have been reported by several researchers, suggesting that neuronal injuries are caused by excessive generation of ROS (42, 55, 61). Likewise, increased ROS production and decreased scavenging enzyme activity has also been documented in long-term seizure activity (42, 62, 63). Nevertheless, MRC dysfunction along with elevated free radicals could also trigger the cell death pathways in the epileptic condition (8, 42, 64, 65).

To conclude, mitochondrial respiratory chain dysfunction could directly affect the phosphorylation potential dependent Na^+,K^+-ATPase and thus cause a lowered resting membrane potential at the plasma membrane, which could contribute to hyper-excitability and decreased threshold for the long-term seizure activity.

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Summary

Mitochondrial function is a key determinant of both excitability and viability of neurons. Studies in this chapter were carried out to decipher the status of mitochondrial oxidative energy metabolism in the chronic condition of generalized seizures induced by picrotoxin (PTX) in rat brain and liver. In both the tissues, PTX-induced convulsions resulted in decreased respiration rates with glutamate, pyruvate+malate and succinate as the substrate. Consequently, the ADP-phosphorylation rates were drastically reduced by 44-72%. An opposite trend was observed with ascorbate+TMPD as the substrate. In general, uncoupling of the mitochondrial electron transport was observed after PTX treatment. Glutamate dehydrogenase activity increased by 3-4 fold in brain and liver whereas malate dehydrogenase and succinate dehydrogenase activities showed tissue specific alterations. There was significant reduction in cytochrome b content in brain after PTX treatment. In liver, cytochrome aa3 decreased whereas cytochrome c+c1 increased without affecting cytochrome b content. The basal F_oF_1ATPase (complex V) activity increased in both the tissues indicating increased membrane fragility. Possible consequences of mitochondrial respiratory chain (MRC) dysfunction could be related to excessive generation of ROS that could affect both cerebral and peripheral system. In conclusion, impairment of MRC function suggests novel pathophysiological mechanism important for chronic epileptic condition.