CHAPTER - II

EFFECTS OF CORTICOSTERONE TREATMENT ON OXIDATIVE ENERGY METABOLISM OF RAT BRAIN MITOCHONDRIA DURING DEVELOPMENT.

Mitochondrial respiration and ATP synthesis are two pathways lying at the heart of metabolism. Process of respiration comprises the oxidation of mitochondrial NADH by oxygen, which is coupled by the electron transport chain to the pumping of protons out across mitochondrial inner membrane. This generates an electrochemical gradient of protons consisting of membrane potential and a pH gradient. The protons return down their gradient either *via* a proton leak or *via* the ATP synthase. The ATP synthase couples the transport of protons across the membrane to the synthesis of ATP inside the mitochondrial matrix. The phosphate for the phosphorylation reaction is imported into the mitochondria by the phosphate carrier, and the ATP is exported to the cytosol in exchange for ADP by the adenine nucleotide carrier. The main substrate for the respiratory chain (NADH) is supplied by glucose, fatty acids and amino acids *via* three interconnected pathways (1). Mammalian mitochondrial electron transport chain (ETC) and oxidative phosphorylation system is shown in Figure 1. Various dehydrogenases serve as the entry points in mitochondrial ETC. Cytochromes b, c_1, c and aa_3 (cytochrome oxidase) are also important components of the ETC.

Glucocorticoids under both <u>in vitro</u> and <u>in vivo</u> conditions, are reported to have effect on mitochondrial respiration and oxidative phosphorylation in liver, brain, heart and muscles. Review of literature suggests that most of

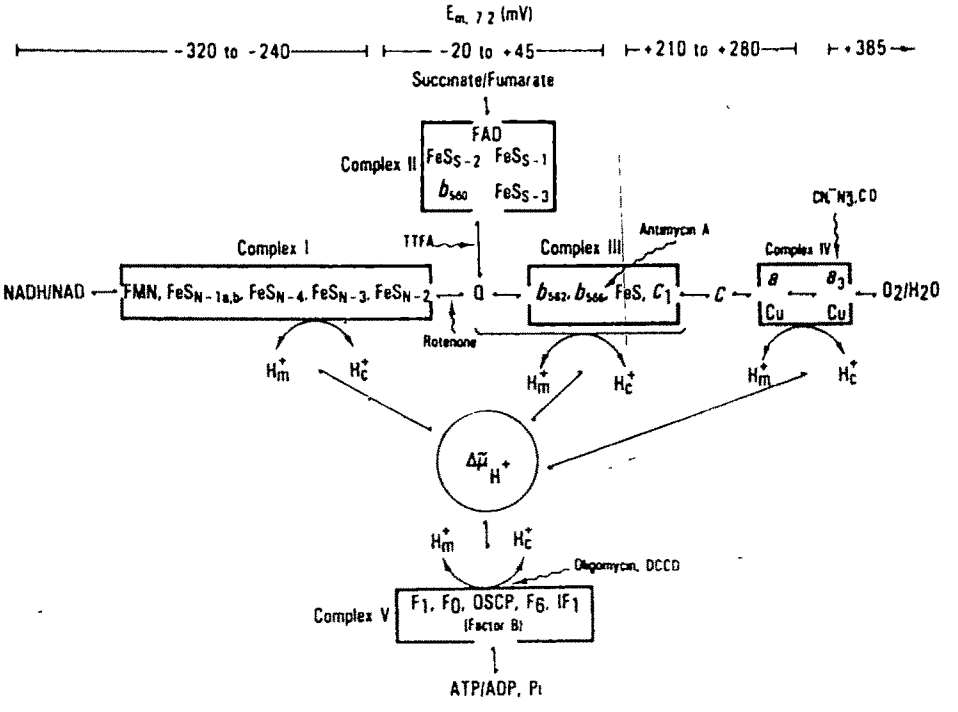

Figure 1

Mitochondrial electron transport chain and oxidative phosphorylation system, showing well-characterized components of complexes I, II, III, IV and V. Fes, iron sulfur cluster, and cytochromes a, b and c.

the researchers have concentrated on rat liver mitochondria as a model system (e.g. see Chapter IV). Reports about glucocorticoid effects on brain mitochondrial oxidative energy metabolism are scarce and these are contradictory (2-4).

Bottoms and Goetsch (2) studied effects of corticosterone treatment of adrenalectomized rats on oxidative metabolism in the brain. Single intramuscular injection of corticosterone produced no effect on oxygen uptake by brain homogenates. Regardless of substrate, corticosterone treatment had no significant effect on oxygen consumption or ATP formation by brain mitochondria, but a slight trend towards an increase in P : O ratio was observed using malate as the substrate (2).

Roosevelt et al. (3) have reported the effect of cortisol treatment of adrenalectomized adult female rats on brain mitochondrial energy metabolism. Cortisol treatment 1 hour prior to killing showed a significant increase over adrenaletomized comtrols in state 3 respiration, respiratory control ratio and ADP/O ratio with NAD^+ -linked substrates. When succinate was used as the substrate, the treatment group showed decreased state 3 respiration and respiratory control ratios suggesting that effects of glucocorticoid on mitochondrial energy metabolism are site specific. The observed changes involved synthesis of specific mRNA and proteins as cycloheximide and actinomycin D abolished the

cortisol induced changes in brain mitochondrial respiration and oxidative phosphorylation (3). The authors also reported that cortisol treatment increased the rotenone sensitive factor of NADH dehydrogenase, which could be one of the reasons for increase in state 3 respiration rates with NAD^+ - linked substrates, since the cytochrome content of mitochondrial respiratory chain remained unaltered (3). Bedetti et al. (4) have reported that adrenalectomy caused increase in D 3 hydroxybutyrate dehydrogenase (OHBD) activity in rat liver and brain mitochondria. Corticosterone depressed OHBD activity in liver mitochondria of normal rats and restored the normal level of OHBD in adrenalectomized rats.

Since young neonates are more susceptible to deleterious effects of glucocorticoid overload on brain development (Chapter I), it is possible that these effects could be mediated *via* impairment in mitochondrial ATP synthesis. Brain has got very high requirement for ATP for its proper functioning. During development the ATP requirement would further increase for synthesis of variety of biomolecules. However, survey of literature reveals that not much information is available on glucocorticoid effects on mitochondrial energy metabolism in developing brain (2 - 4). Therefore it is of interest to study the effects of corticosterone treatment on brain mitochondrial energy metabolism during postnatal development.

The objective of the present study was to examine the effects of in vivo corticosterone treatments - acute and chronic - on following parameters of brain mitochondria isolated from animals belonging to different age groups i.e. 14-, 21-, 35-day-old and adults: 1) Mitochondrial state 3 and state 4 respiration rates, ADP/O ratios and ADP phosphorylation rates using different substrates i.e. glutamate, pyruvate + malate, succinate and ascorbate + TMPD and 2) Specific activities of mitochondrial primary dehydrogenases i.e. glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate-DCIP reductase (SDR).

MATERIALS AND METHODS

Chemicals

Corticosterone, Sodium salts of pyruvic acid and L - malic acid, N, N, N',N'- tetramethyl *p* phenylenediamine (TMPD), rotenone, 2, 6 - dichlorophenolindophenol (DCIP), nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide reduced from (NADH) were purchased from Sigma Chemical Co. USA. Sodium salts of succinic and L - glutamic acid were purchased from British Drug Houses, U.K. Sodium salts of ascorbic and oxaloacetic acid were from Sarabhai Chemicals and SRL India respectively. Adenosine 5' diphosphste (ADP) was purchased from Boehringer Mannheim.

Germany. Tris(hydroxymethyl)aminomethane (tris) and bovine serum albumin (BSA) were from SRL, India.

All other chemicals and solvents used were of analytical-reagent grade, purchased locally.

All the experiments were carried out using double distilled water distilled in all glass distillation unit.

Animals

Male albino rats of Charles - Foster strain of different age groups i.e. 14 days, 21 days, 35 days and 8 to 10 weeks old (adults) were used. The animals had free access to food and water.

Corticosterone treatment

Two types of corticosterone treatments acute and chronic-were employed. The animals animals in both the treatment groups received corticosterone suspension in 0.3% carboxymethyl cellulose (CMC) at a dose of 40 mg/Kg body weight (5,6). Since serum levels of corticosterone are lowest in the early morning (7), care was taken to inject the animals between 6.00 to 7.00 A.M.

In the acute treatment group, the animals were injected corticosterone suspension subcutaneously (s.c.) in the neck

fold, two hours prior to killing. Controls were given only the vehicle (0.3% CMC).

For chronic treatment, the animals were injected corticosterone suspension for three consecutive days prior to the day of killing when they reached proper age groups ; e.g. for the studies on 21-day-old animals, the injections were given on day 18, 19 and 20 and animals were killed on day 21. Corticosterone suspension was prepared fresh by weighing a known amount of corticosterone and then homogenizing it with small quantity of 0.3% CMC solution using glass - teflon homogenizer. The homogenous slurry was then made up to desired volume with addition of 0.3% CMC solution. The final corticosterone suspension was vortexed vigorously before injection.

Isolation of brain mitochondria

Procedure for the isolation of brain mitochondria was based on that of Ozawa et al.(8) and Bangur et al. (9).

The animals were killed by decapitation and the brains were quickly removed, weighed and transferred to the beakers containing chilled (0 - 4°C) isolation medium consisting of 0.25 M sucrose, 10mM tris. HCl, 1mM EDTA and 250 µg BSA/ml; pH 7.4. The brain tissue was washed several times with chilled isolation medium to remove adhering blood. Ten percent (w/v)

homogenates were prepared in the isolation medium using Potter-Elvehjem type glass-teflon homogenizer. The homogenates were centrifuged at 650 g for 10 minutes to sediment nuclei and cell debries. Fluffy coat of myelin formed on the top was carefully discarded. The post-nuclear supernatant was then subjected to further centrifugation at 10,000 g for 10 minutes to sediment mitochondria. The loose pellet of synaptosomes was discarded after gentle swirling without disturbing the mitochondrial tight pellet. The mitochondrial pellet was washed once by resuspending in isolation medium and resedimenting by centrifugation at 10,000 g for 10 minutes. Finally the washed pellets were resuspended in the isolation medium to give about 10-15 mg mitochondrial protein/ml. All the operations were carried out at 0-4°C. This procedure gives pure brain mitochondria which are practically free from contamination of synaptosomes, microsomes and cytosol (9).

For studies on mitochondrial lipids the mitochondrial pellets were washed thrice.

Measurement of oxidative phosphorylation in brain mitochondria.

Measurements of oxidative phosphorylation were carried out polarographically using a Clark-type oxygen electrode as described by Satav and Katyare (10). The respiration medium (1.5 ml) contained 225 mM sucrose, 5 mM phosphate buffer, 10

mM tris.HCl, 10 mM KCl, 0.02 mM EDTA and 100 µg BSA/ml ; all at pH 7.4. 2 to 4 mg of mitochondrial protein was added. The concentration of substrates used were: glutamate (10 mM), pyruvate (10 mM) + malate (1.0 mM), succinate (10 mM) and ascorbate (10 mM) + TMPD (0.1 mM). With succinate and ascorbate + TMPD, 1 µM rotenone was also included in the reaction medium .

Small aliquots of ADP (about 200 n moles in 15-20 µl) were added, and rates of respiration in the presence of ADP (state 3) and after its depletion (state 4) were recorded. The ADP/O ratios were calculated using the formula : n moles of ADP phosphorylated / n atoms of oxygen consumed. The rates of ATP synthesis (ADP phosphorylation rates) were calculated using the formula : ADP phosphorylation rate = ADP/O ratio x 2 x state 3 respiration rate. The ratio of state 3/state 4 respiration rates gives respiratory control index (RCI) (11).

Assays of mitochondrial dehydrogenases

Glutamate dehydrogenase (GDH) activity was assayed at 37°C spectrophotometrically in the direction of glutamate to a ketoglutarate (12). The assay system in the final volume of 1.0 ml contained 120 mM potassium phosphate buffer pH 7.8 , 5 mM sodium L - glutamate and 0.5 mg mitochondrial protein as a source of enzyme. The reaction mixture was pre-incubated for

a minute and the reaction was started by addition of 1.5 mM NAD^+. The increase in absorbance at 340 nm was measured at 5 second intervals. The specific activity was calculated using E mM^{-1} cm^{-1} = 6.22 for NADH.

Malate dehydrogenase was assayed spectrophotometrically (13) in the direction of oxaloacetate to malate. The assay system in the final volume of 1.0 ml contained 120 mM phosphate buffer pH 7.4, 2.5 mM sodium oxaloaceatate, 1% triton X - 100 and 10 µg of mitochondrial protein as a source of enzyme. The reaction mixture was pre-incubated at 37°C for a minute and the reaction was started by addition of 1.5 mM NADH. The decrease in absorbance at 340 nm was measured at 5 second intervals. The specific activity was calculated using extinction coefficient of NADH as given above.

Succinate - DCIP reductase was assayed spectrophotmetrically (14) at 37°C. The assay system in the final volume of 1.0 ml contained 120 mM potassium phoshphate buffer, pH 7.4, 1.5 mM KCN, 20 mM sodium succinate and 0.5 mg mitochondrial protein as a source of enzyme. The decrease in absorbance at 600 nm was measured at 5 second intervals. The specific activity was calculated using E mM^{-1} cm^{-1} = 21.0 for DCIP. KCN solution was prepared fresh prior to use.

Estimation of proteins

Protein estimation was carried out according to the method of Lowry et al. (15) with some modifications.

1. **Standatd BSA (100 μg/ml)**

Known amount of BSA was weighed and dissolved in minimum amount of distilled water. Few drops of 0.1 N NaOH were added to facilitate solublization and finally the volume was made up with distilled water in such a way that it should give 0.07 O.D. at 280 nm (1 mg BSA/ml gives 0.7 O.D. at 280 nm). This precaution is necessary since BSA is hygroscopic and could give underestimates in slope if one relied only on the weight.

2. **Reagent - A (5X)**

Dissolve 20 g anhydrous sodium carbonate , 4.0 g sodium hydroxide and 200 mg sodium potassium tartarate in distilled water and finally make up the volume to 200 ml. This is a stock solution; it has to be diluted 1 : 5 times before use .

3. **Reagent-B**

0.5% copper sulfate ($CuSO_4.7H_2O$) solution (w/v)in distilled water.

4. Reagent-C

50 ml of 1:5 diluted Reagent - A to which 1.0 ml of

Reagent -B was added and mixed properly. This Reagent - C has to be prepared freshly.

5. Folin Ciocalteau Reagent (FC Reagent)

FC-Reagent was diluted 1:1 with distilled water before use.

Procedure

Different aliquots of BSA standard were taken to give concentration of protein in the range of 4 to 40 µg and then volume was made up to 0.5 ml with distilled water. Reagent blanks contained 0.5 ml of distilled water. Samples were first diluted and then aliquots were taken in such a way that sample protein should fall within the range of this method. Volume was made up to 0.5 ml with distilled water. To all the tubes 2.5 ml of Reagent-C was added. After mixing the contents, the tubes were kept at room temperature for 15 minutes. Finally 0.25 ml of FC-Reagent was added to each tube, and after vigorous vortexing the tubes were kept at room temperature for 30 minutes for the development of blue color. Readings were taken against reagent blanks in a colorimeter using a filter of 660 nm. Slope was calculated from the standard graph and used to find out protein content in samples. This method gives a slope of 0.006 (1 µg protein gives 0.006 O.D.).

RESULTS

The results on the effects of *in vivo* acute and chronic corticosterone treatments on oxidative energy metabolism in rat brain mitochondria from four different age groups are summarized in Tables 1 - 4. Four different electron donor systems were employed in these studies with a view to obtaining a comprehensive picture of the effects of the glucocorticoid overload.

As can be noted, in the control group the respiration rates with NAD^+ - linked substrates i.e. glutamate and pyruvate + malate in general were lower compared to those obtained either with succinate or ascorbate + TMPD. It is also evident that the respiration rates for individual substrate oxidation were low in the initial stages of development e.g. 14 days, but increased by several folds in the adult stage; the increase amounted from 5.3 to 19.5 fold depending on the substrate used. The maximum increase was seen for ascorbate + TMPD (19.5 fold) followed by succinate (9.6 fold). Possibly this reflects the preferential substrate oxidation with maturation and increased oxidative potential which is consistent with the observed rates of ADP phosphorylation (Tables 1 - 4).

In mitochondria from young animals the ADP/O ratios obtained with succinate were lower, but by the adult stage the

value increased to normal level suggesting that in young animals, site II of ATP synthesis may not be fully functional. However, the mitochondria become tightly coupled with advancement of age. With ascorbate + TMPD, there was no significant change in ADP/O ratios in different age groups suggesting that site III of ATP synthesis may be fully functional even in young animals (Tables 1 - 4).

From the data presented one could discern that the effects of acute and chronic treatments with corticosterone were differential. Table 1 Shows effects of corticosterone treatment on respiration and oxidative phosphorylation in rat brain mitochondria with glutamate as the substrate. Acute treatment with corticosterone resulted in 40 to 70% decrease in state 3 respiration rates (depending on the age group) with significant reduction of ADP/O ratios. When animals were given chronic treatment, the ADP/O ratios decreased by upto 45% in case of 21-day-old animals. State 3 respiration rates were substantially low in 21-day-old group but returned to the values close to controls in in 35-day-old and adult rats. The changes in state 3 respiration rates and ADP/O ratios were reflected in the decreased ADP phosphorylation rates. Acute treatment caused 50 to 75% decrease in rates of ATP synthesis and the effects of chronic treatment were less severe on ATP synthesis in general except for 21-day-old rats.

Table 1

Effect of corticosterone treatment on oxidative phosphorylation in rat brain mitochondria with glutamate as the substrate.

Age	Treatment	ADP/O ratio	Respiration rate		ADP Phosphorylation rate
			State 3	State 4	
	Control	2.51 ± 0.16	3.3 ± 0.18	1.3 ± 0.10	16.4 ± 0.72
14 Day	Acute	2.33 ± 0.27	1.9 ± 0.09^{c}	1.3 ± 0.07	8.8 ± 0.60^{c}
	Chronic	2.04 ± 0.10^{a}	3.4 ± 0.19	1.3 ± 0.15	13.8 ± 0.84^{a}
	Control	2.61 ± 0.12	7.2 ± 0.36	2.9 ± 0.18	35.7 ± 1.73
21 Day	Acute	1.81 ± 0.11^{c}	4.4 ± 0.33^{c}	2.4 ± 0.27	14.9 ± 1.39^{c}
	Chronic	1.48 ± 0.09^{c}	4.2 ± 0.48^{c}	2.9 ± 0.32	12.3 ± 1.39^{c}
	Control	3.01 ± 0.12	2.2 ± 0.48	3.2 ± 0.36	72.4 ± 5.08
35 Day	Acute	2.46 ± 0.21^{a}	3.6 ± 0.37^{c}	1.2 ± 0.12^{c}	17.9 ± 2.23^{c}
	Chronic	1.91 ± 0.10^{c}	9.1 ± 0.85^{b}	1.5 ± 0.34^{b}	34.7 ± 3.73^{c}
	Control	3.31 ± 0.08	17.6 ± 1.36	2.9 ± 0.96	117.4 ± 8.57
Adult	Acute	2.23 ± 0.11^{c}	9.5 ± 0.61^{c}	2.7 ± 0.23	44.5 ± 2.09^{c}
	Chronic	2.95 ± 0.14^{a}	14.2 ± 0.41^{a}	5.7 ± 0.32	87.0 ± 4.65^{b}

Results are given as mean ± SEM of 12 independent observations in each group.

Respiration rates are expressed as n moles of O_2 consumed/min/mg protein and ADP phosphorylation rates as n moles of ADP phosphorylated/min/mg protein.

$^{a}P < 0.05$; $^{b}P < 0.01$ and $^{c}P < 0.001$ compared to the corresponding controls.

Table 2

Effect of corticosterone treatment on oxidative phosphorylation in rat brain mitochondria with pyruvate + malate as the substrate.

Age	Treatment	ADP/O ratio	Respiration rate		ADP Phosphorylation rate
			State 3	State 4	
	Control	2.19 ± 0.12	4.3 ± 0.17	2.6 ± 0.14	18.8 ± 1.28
14 Day	Acute	1.80 ± 0.10[a]	2.9 ± 0.09[b]	1.3 ± 0.07[b]	10.9 ± 0.76[b]
	Chronic	1.83 ± 0.10[a]	4.9 ± 0.24	2.6 ± 0.17	17.9 ± 1.25
	Control	2.14 ± 0.03	10.4 ± 0.29	6.5 ± 0.31	43.3 ± 1.68
21 Day	Acute	1.61 ± 0.11[b]	7.0 ± 0.60[b]	1.9 ± 0.40[b]	23.7 ± 1.45[b]
	Chronic	1.27 ± 0.10[b]	8.9 ± 0.52[a]	2.7 ± 0.50[b]	22.1 ± 1.91[b]
	Control	2.98 ± 0.14	17.4 ± 2.25	8.8 ± 0.32	104.3 ± 16.9
35 Day	Acute	1.92 ± 0.10[b]	4.8 ± 0.36[b]	1.9 ± 0.14[b]	18.5 ± 1.10[b]
	Chronic	1.73 ± 0.15[a]	19.2 ± 1.44	7.2 ± 0.46[a]	65.3 ± 5.77[a]
	Control	3.32 ± 0.10	24.7 ± 1.03	9.2 ± 0.91	164.7 ± 8.77
Adult	Acute	2.34 ± 0.07[b]	10.2 ± 0.73[b]	4.3 ± 0.20[b]	47.4 ± 3.88[b]
	Chronic	2.78 ± 0.32	22.9 ± 1.38	11.6 ± 0.97	127.9 ± 15.1[a]

Results are given as mean ± SEM of 12 independent observations in each group.

Other details are same as given in Table 1.

[a] $P < 0.05$ and [b] $P < 0.001$ compared to the corresponding controls.

Table 3

Effect of corticosterone treatment on oxidative phosphorylation in rat brain mitochondria with succinate as the substrate.

Age	Treatment	ADP/O ratio	Respiration rate		ADP Phosphorylation rate
			State 3	State 4	
14 Day	Control	0.83 ± 0.08	7.5 ± 0.30	4.5 ± 0.20	12.4 ± 1.47
	Acute	0.82 ± 0.07	5.5 ± 0.22[c]	4.5 ± 0.31	9.1 ± 0.76
	Chronic	0.95 ± 0.03	9.8 ± 0.39[c]	6.1 ± 0.21[c]	18.6 ± 1.57[b]
21 Day	Control	0.79 ± 0.08	20.2 ± 1.08	14.3 ± 1.12	32.8 ± 3.39
	Acute	0.21 ± 0.05[c]	22.9 ± 1.00	15.4 ± 0.87	9.6 ± 1.40[c]
	Chronic	0.14 ± 0.02[c]	22.1 ± 1.73	15.6 ± 1.94	6.1 ± 0.68[c]
35 Day	Control	1.11 ± 0.14	39.0 ± 4.31	17.5 ± 2.13	87.2 ± 9.96
	Acute	1.06 ± 0.13	12.2 ± 1.23[c]	6.0 ± 0.50[c]	25.8 ± 1.74[c]
	Chronic	0.79 ± 0.09	37.1 ± 2.45	23.6 ± 4.12	58.4 ± 4.57[b]
Adult	Control	1.49 ± 0.09	72.2 ± 4.96	39.1 ± 2.08	216.3 ± 25.2
	Acute	0.79 ± 0.14[c]	22.3 ± 0.98[c]	15.4 ± 0.57[c]	37.0 ± 7.62[c]
	Chronic	1.51 ± 0.04	48.8 ± 2.83[c]	30.0 ± 1.06[c]	145.8 ± 14.5[a]

Results are given as mean ± SEM of 12 independent observations in each group.

Other details are as given in Table 1.

[a] $P < 0.05$; [b] $P < 0.02$ and [c] $P < 0.001$ compared to the corresponding controls.

Table 4

Effect of corticosterone treatment on oxidative phosphorylation in rat brain mitochondria with ascorbate + TMPD as the substrate.

Age	Treatment	ADP/O ratio	Respiration rate		ADP Phosphorylation rate
			State 3	State 4	
	Control	0.38 ± 0.04	7.4 ± 0.84	3.3 ± 0.39	5.5 ± 0.51
14 Day	Acute	0.36 ± 0.03	5.7 ± 0.74	3.5 ± 0.53	4.1 ± 0.35[a]
	Chronic	0.35 ± 0.03	3.3 ± 0.41[f]	1.4 ± 0.24[f]	2.3 ± 0.40[f]
	Control	0.31 ± 0.03	26.7 ± 1.34	15.5 ± 0.93	16.3 ± 1.23
21 Day	Acute	0.17 ± 0.01[f]	33.7 ± 1.52[d]	15.3 ± 1.68	11.5 ± 0.90[c]
	Chronic	0.18 ± 0.02[e]	30.8 ± 1.23[a]	15.5 ± 1.78	11.1 ± 0.98[c]
	Control	0.49 ± 0.07	73.7 ± 14.7	48.4 ± 3.44	72.2 ± 7.34
35 Day	Acute	0.23 ± 0.02[e]	26.7 ± 2.46[d]	14.9 ± 1.55[f]	12.4 ± 1.26[f]
	Chronic	0.45 ± 0.03	34.8 ± 3.67[b]	13.9 ± 1.35[f]	31.2 ± 2.14[f]
	Control	0.36 ± 0.02	144.1 ± 7.90	59.4 ± 3.03	104.3 ± 6.69
Adult	Acute	0.38 ± 0.02	78.6 ± 5.55[f]	37.9 ± 2.39[f]	59.6 ± 4.32[f]
	Chronic	0.35 ± 0.02	146.7 ± 13.9	62.1 ± 7.23	102.8 ± 7.79

Results are given as mean ± SEM of 12 independent observations in each group.

Other details are given in Table 1.

[a]$P < 0.05$; [b]$P < 0.02$; and [c]$P < 0.01$; [d]$P < 0.005$; [e]$P < 0.002$ and [f]$P < 0.001$ compared to the corresponding controls.

When pyruvate and malate were used as the substrate couple (Table 2), the effects of corticosterone treatment were more or less similar to those mentioned above for glutamate; both are NAD^+ - linked substrates. However, there were some clear cut differences. Unlike in case of glutamate, here on acute treatment state 4 respiration rates also decreased parallel to state 3 respiration rates. Chronic treatment more severely affected the coupling efficiency as evidenced from significant reduction in ADP/O ratios, than the state 3 respiration rates. Only in 21-day group, state 3 respiration rate decreased significantly on chronic treatment.

With succinate as the substrate (Table 3), acute treatment caused 50 and 75% decrease in ADP/O ratios in adults and 21-day-old animals respectively. Decrease in state 3 respiration rate ranging from 27 to 70% was observed in all age groups except the 21-day-old animals. Chronic corticosterone treatment decreased the ADP/O ratio by 80% only in 21-day-old animals; in other age groups there was no effect. State 3 respiration decreased in 14-day-old animals there was a significant increase (+ 31%). Both the treatments significantly decreased ADP phosphorylation rates in all the age groups except the 14 - day - old animals.

Table 4 summarizes the results obtained using ascotbate and TMPD as a substrate pair. 35-day-old animals were maximally affected by acute treatment as evidenced by 50% and

65% decrease in ADP/O ratio in 21-day-old group. Both the treatments in general significantly decreased the ADP phosphorylation rates in almost all the age groups; in the 35-day-old animals the effects on rates of ATP synthesis were more pronounced.

As the primary dehydrogenases form the entry ports for transfer of electrons from substrates to the electron transport chain, it was of interest to study the effects of corticosterone treatments on their activites. Table 5 shows the effects of corticosterone treatments on glutamate dehydrogenase (GDH) activity in brain mitochondria. In 14-day-old animals the GDH activity was low and gradually increased with age, registering 22 fold increase in the adults. In 35-day-old animals and adults, both the treatments significantly decreased the GDH activity with the extent of decrease being higher in acute treatment group. In case of 21-day group the corticosterone treatments actually *increased* the GDH activity.

Developmental changes in mitochondrial malate dehydrogenase (MDH) activity (Table 6) showed totally different pattern compared to that for GDH. 14-day-old animals showed highest MDH activity which decreased gradually with advancement of age. Acute treatment with corticosterone led to sigificant decrease (28 to 46%) in MDH activity in all the age groups. Chronic treatment had no effect on MDH activity except in 14-day-old animals where decreased by 17%.

Table 5

Effect of corticosterone treatment on glutamate dehydrogenase (GDH) activity in rat brain mitochondria during development.

	GDH activity (n moles/min/mg protein)		
Age	Control	Acute	Chronic
14 Days	1.13 ± 0.06	1.03 ± 0.01	0.95 ± 0.05[a]
21 Days	3.09 ± 0.49	5.40 ± 0.28[c]	10.40 ± 0.35[d]
35 Days	6.51 ± 0.48	3.14 ± 0.24[d]	5.15 ± 0.09[b]
Adult	24.87± 3.76	6.35 ± 0.30[d]	12.97 ± 0.85[b]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.002$ and [d]$P < 0.001$ compared to the corresponding controls.

Table 6

Effect of corticoterone treatment on malate dehdrogenase (MDH) activity in rat brain mitochondria during development.

	MDH activity (n moles/min/mg protein)		
Age	Control	Acute	Chronic
14 Days	10563 ± 372	6962 ± 283[c]	8756 ± 631[a]
21 Days	9734 ± 211	6997 ± 699[b]	10189 ± 570
35 Days	9569 ± 302	6273 ± 484[c]	9811 ± 389
Adult	8916 ± 623	4855 ± 266[c]	9226 ± 254

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.05$; [b]$P < 0.002$ and [c]$P < 0.001$ compared to the corresponding controls.

Table 7

Effect of corticoterone treatment on succinate-DCIP reductase (SDR) activity in rat brain mitochondria during development.

	SDR activity (n moles/min/mg protein)		
Age	Control	Acute	Chronic
14 Days	4.55 ± 0.48	3.93 ± 0.33	6.71 ± 0.67[a]
21 Days	15.88 ± 0.87	23.29 ± 1.17[b]	28.88 ± 1.17[b]
35 Days	13.88 ± 0.66	16.13 ± 2.14	17.81 ± 0.58[b]
Adult	20.73 ± 0.90	17.81 ± 0.58[a]	20.58 ± 1.34

Results are given as mean ± SEM of 8 independent observations in each group.

[a] $P < 0.02$ and [b] $P < 0.001$ compared to the corresponding controls.

In case of succinate - DCIP reductase (SDR), 14-day-old animals displayed very low activity which increased by more than 3 fold by the age of 21-days. A further increase was seen in the adults. Acute treatment with corticosterone led to significant increase (+47%) in SDR activity in 21-day-old animals but in adults the activity was decreased marginally by 14%. In 14- and 35-day-old animals acute treatment had no effect. Chronic treatment brought about 28 to 82% increase in the SDR activity in all the age groups except the adults.

DISCUSSION

The results on developmental changes in mitochondrial oxidative metabolism are in general agreement with those of Rajan and Katyare (16) and Milstein et al. (17), but differ from those of Holtzman and Moore (18). From the results presented it is clear that effects of corticosterone on oxidative metabolism in brain mitochondria were age - dependent and treatment specific. There were some differences in corticosterone effects even for substrates like glutamate and pyruvate + malate, which donate electrons at NAD^+ - level i.e. at the same site.

Both the treatments - acute and chronic - caused significant reduction in state 3 respiration rates and also the uncoupling of oxidative phosphorylation in brain

mitochondria as evidenced from lowered ADP/O ratios. Roosevelt et al. (3) have also reported decreased state 3 respiration rates with succinate as a substrate in brain mitochondria from cortisol treated adrenalectomized rats. In vivo corticosterone treatment is also known to cause decrease in state 3 respiration rates and ADP/O ratios in liver mitochondria in age dependent manner (6). As a result of significant decrease in state 3 respiration and ADP/O ratios, the ADP phosphorylation rates were lowered to a greater extent. Acute tratment caused a greater reduction in state 3 respiration rates compared to the chronic treatment. Chronic treatment mainly affected the coupling efficiency of brain mitochondria.

When the results were analyzed further to find out the effects of corticosterone on different sites of ATP synthesis in brain mirochondria, it was found that the treatment did not affect any one site in particular but affected all the three sites of ATP synthesis. However the extent of uncoupling of oxidative phosphorylation at three different sites was variable depending on age of the animals and the treatment. In 14-day-old and adult rats mainly the first and second sites of ATP synthesis were affected; in 21-day group the major effect of corticosterone treatment was on the second and third site. In case of 35-day-old animals, all the three sites were affected. In general the extent of decrease in ATP production

by all the three sites was greater following chronic treatment than the acute treatment.

Decreased ATP synthesis could have serious consequences on the proper functioning and development of the brain, as brain has high energy requirement for variety of specialized functions including electrical activity, and the cellular ATP demand would be enhanced during development for biosynthesis of various macromolecules such as nucleic acids, proteins and lipids (19).

The effects of corticosterone on mitochondrial energy metabolism in brain were age-dependent. The 21- and 35-day-old rats were most affected by corticosterone overload. This is a critical age for brain development; the period of massive postnatal cell formation in the rat brain is restricted to first three weeks after birth (20). After that, the neuronal connectivities are formed and brain development becomes nearly complete. Decreased ATP synthesis upon corticosterone administration to young animals could help in understanding molecular mechanisms of reported negative effects of glucocorticoid overload on brain development. Reduction in ATP synthesis in brain mitochondria from corticosterone-treated young animals could explain the reported decrease in cellular DNA and protein content, thymidine incorporation into DNA, glucocorticoid overload to young developing rats (Chapter-I).

Mitochondrial state 3 respiration represents a steady state of electron flow in which substrate, phosphate acceptor and oxygen are all in excess. The maximum capacity for electron transfer is in the presence of an adequate supply of electrons and oxygen. It is usually difficult to reduce cytochrome chain to saturation (21), so that respiration is limited by the velocity of hydrogen transfer from the mitochondrial dehydrogenases (3).

Results on corticosterone effects on mitochondrial dehydrogenase activities (Tables 5 to 7) show that both the corticosterone treatments in general caused significant decrease in activities of pyridine nucleotide - dependent primary dehydrogenases i.e. GDH and MDH. These findings assume importance in the context of the observations reported by Gallaghar et al. (22). These authors have shown that the glucocorticoid treatment causes alterations in the permeability of mitochondrial membrane, thus resulting in selective loss of the pyridine nucleotides, which in turn can cause inhibition of the pyridine nucleotide - dependent mitochondrial dehydrogenases. Similarly, Bedetti et al. (4) reported that adrenalectomy increased and corticosterone treatment depressed OHBD activity which is also a pyridine nucleotide - dependent mitochondrial dehyrogenase involved in oxidation of ketone bodies.

As the mitochondrial dehydrogenases are rate limiting in the respiration, the significant decrease observed in GDH and MDH specific activity explains the inhibition of state 3 mitochondrial respiration when glutamate and pyruvate + malate were used as the respiratory substrates.

Activity of succinate - DCIP reductase (SDR) shows a generalized increase upon corticosterone treatments. This could be a physiologic adjustment to counterbalance the decreased NAD^+ - linked dehydrogenases and substrate oxidation in brain mitochondria. However, even the increased SDR activity was not able to correct the mitochondrial respiration and oxidative phosphorylation, as the mitochondria from corticosterone-treated animals still showed significant reduction even in the succinate supported respiration rates.

Increased SDR activity upon corticosterone administration could not lead to increase in mitochondrial respiration and oxidative phosphorylation, possibly because other components of mitochondrial ETC might have decreased. One interesting possibility is that the observed changes may be due to altered content of cytochromes, which are important components of ETC. However Roosevelt et al. (3) have reported that acute cortisol treatment had no effect on concentration of the cytochromes b, c, a and a3 in brain mitochondria from adrenalectomized adult female rats. Similarly, Allan et al. (23) reported that short-term dexamethasone treatment to adult

rats did not affect the content of cytochromes a, b, c*1* and c in liver mitochondria. Although these glucocorticoids had no effect on cytochrome contents in mitochondria from adult animals (2,23), it is possible that the treatments may affect the cytochrome content in young animals; maximum deleterious effects on mitochondrial respiration and oxidative phosphorylation were observed in young age groups. Secondly, it is known that the effects of glucocorticoid administration are age dependent. Young animals are more sensitive and adults are resistant to corticosterone treatments (6,24). Effects of corticosterone treatments on cytochrome content of brain mitochondria during development is the interesting possibility which needs to be varified experimentally in future.

Additionally, in the studies showing that glucocorticoids have no effect on mitochondrial cytochrome content (3,23), either synthetic or unnatural glucocorticoids were employed Hence the physiologic relevence of such studies becomes questionable. Studies with corticosterone, the natural glucocorticoid hormone of the rat can only give meaningful results.

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SUMMARY

1. Oxidative energy metabolism of brain mitochondria follows a specific developmental Pattern. In general, the rates of state 3 respiration with succinate and ascorbate + TMPD were higher than those obtained with NAD^+ -linked substrates. Respiration rates with the individual substrates were low in young rats but increased several folds in the adults.

2. Second site of ATP synthesis in brain mitochondria from young animals was not fully functional, but by the adult stage the mitochondria became fully coupled as evidenced from increase in ADP/O ratios to normal values. The third site of ATP synthesis seemed to be fully functional even in young animals.

3. The effects of corticosterone treatment on mitochondrial oxidative energy metabolism in brain were age-dependent and treatment specific. In general, both the corticosterone treaments caused significant decease in state 3 respiration rates and uncoupling of mitochondria as evidenced from decreased ADP/O ratios. As a result, the rates of ADP phosphrylation were also lowered significantly. Among all the age groups studied, 21-and 35-day-old rats are most adversely affected by the corticosterone treatments.

4. In general, both the corticosterone treatments caused significant reduction in mitochondrial GDH and MDH activities. This could be one of the reason for decreased state 3 respiration rates with glutamate and pyruvate + Malate. The SDR activity incrased significantly upon corticosterone treatment. This may be a physiologic adjustment to correct the state 3 respiration rates but in spite of the increase in the SDR activity, the state 3 respiration rates with succinate remained low in corticosterone treatment groups.