# Chapter 4

## Effect of Dexamethasone Treatment on Oxidative Energy Metabolism in Rat Brain Mitochondria During Postnatal Development.

## Introduction

Dexamethasone is one of the synthetic glucocorticoid agonist have been used for therapeutic purposes in different age groups (1-5) These agents widely used as an anti-inflammatory agents and also used for the treatment of skin diseases, bronchial asthma, bronchiopulmonary dysplasia (BPD), respiratory distress syndrome in fetus, rheumatoid arthritis and meningitis in practically all age groups including children (1-5) Use of dexamethasone as potent glucocorticoid as well as an anti-inflammatory agent is recommended by American National Institute of Health (6) However adverse side-effects have been reported in as high as 50% of the cases when it is used at pharmacological doses, (7)

As is well recognized that the developmental processes are energy dependent (8) and the mitochondria are the center for energy production in the cells Also , in earlier studies we observed that dexamethasone treatment resulted in impairment of macromolecules synthesis and metabolic activity of the cells of the tissues such as liver and brain (9) It was of interest to find out the effect of dexamethasone treatment on oxidative energy metabolism of the brain mitochondria in rats belonging to different age groups i e 2 week to 5 week and adults

It is known that human brain development is complete between 20-26 weeks of gestational period, this period equivalent to rat brain developmental period which continues postnatally up to the 3rd week (6,7) Although caution has to be exercised while extrapolating the results from animal studies to human, some parallels can be drawn as under guidelines

The present studies were thus undertaken to find out if the chronic treatment with dexamethasone affects energy metabolism in the rat brain mitochondria Measurements on oxidative phosphorylation and on related parameters are carried out to get entire picture of overall effects on rat brain mitochondria during development

## Materials and Methods

### Chemicals

Sodium salt of L glutamic acid was from E Merck, Darmstadt, Germany. Sodium salts of malic acid, pyruvic acid and succinic acid and $NAD^+$, NADH, dichlorophenolindophenol (DCIP), N,N,N',N' tetramethyl-*p*-phenylenediamine (TMPD), rotenone and Triton X-100 were purchased from Sigma, USA ADP was from Fluka All other chemicals were purchased locally and were of highest purity grade available

### Animals and Dexamethasone Treatment

The detail plan is as described in the previous Chapter 2 of the thesis

### Isolation of mitochondria

The animals were killed by decapitation and their brains were quickly removed and placed in a beaker containing chilled (0-4°C) isolation medium The isolation medium contains 0 25M sucrose, 10mM tris-HCl (pH 7 4), 1mM EDTA and 250µg BSA/ml The tissue was repeatedly washed with the isolation medium to remove adhering blood and 10% (w/v) homogenate was prepared using a Potter Elvehjem type glass – teflon homogenizer The nuclei and cell debris were sedimented by centrifuging at 650 x g for

10 min and discarded The supernatant was subjected to a further centrifugation at 6,500 x g for 10 min The synaptosomal – myelin found at the top of the centrifuge tubes were carefully discarded The mitochondria were washed by suspending them gently in the isolation medium and resedimenting at 6500 x g for 10 min Finally the mitochondria were suspended in isolation medium to give a protein concentration about 30-50 mg/ml The mitochondria found to be free of contamination of synaptosomes, microsomes (10)

**Oxidative Phosphorylation**

Measurement of oxidative phosphorylation was carried out using a Clark type oxygen electrode as described previously (10,11) The respiration medium contained in a total volume of 1 6 ml 225mM sucrose, 5mM potassium phosphate buffer, pH 7 4, 10mM tris-HCl buffer, pH 7 4, 20mM KCl, 0 2 mM EDTA and 150 µg BSA/ml Depending on the substrate used, 3-5 mg of mitochondrial protein was added and respiration was initiated by the addition of the substrate The substrates used were glutamate (10mM), pyruvate (10mM) + malate (1mM), succinate (10mM) and ascorbate (10mM) + TMPD (0 1mM) With the latter two substrates 1 µM rotenone was included State 3 respiration rates were initiated by adding 40-150 nmoles of ADP in small aliquots (5-20µl) and the respiration rates in the presence of added ADP (state 3) and after its depletion (state 4) were recorded Calculation of ADP/O ratio and ADP phosphorylation rates was as described previously (12,13)

**Assay of Dehydrogenases**

Glutamate dehydrogenase (GDH) activity was measured spectrophotometrically at 37°C The assay system (total volume 1ml) contained 100mM potassium phosphate buffer pH

7.4, 10mM sodium glutamate, 0 1% Triton X 100 and 100-200 µg of mitochondrial protein as the source of the enzyme After incubating at 37 $^0$C for 1mın the reaction was initiated by the addition of 1.5 mM $NAD^+$ and the linear rate of reaction was recorded at 5 sec interval by monitoring the increase ın the absorbance at 340nm (14)

For the measurement of malate dehydrogenase (MDH) activity the assay medium contained in a total volume of 1ml 100mM potassium phosphate buffer pH 7 4, 2 5mM sodium oxaloacetate, 0 1% Triton X 100 and 5-15 µg of mitochondrial protein as the source of the enzyme After pre-incubatıon at 37 $^0$C for 1mın the reactıon was initiated by the addition of 1 5mM NADH The linear rate of reaction was recorded at 5 sec ınterval by monitoring the decrease in absorbance at 340 nm (15)

Succinate DCIP reductase (SDR) actıvity was measured in the assay system (total volume 1ml) contaıning 100mM potassium phosphate buffer, pH 7 4, 1 5mM freshly prepared KCN, 15mM sodium succinate and 100-300 µg of mitochondrıal protein as the source of the enzyme After ıncubating at 37 $^0$C for 1min the reactıon was ınitiated by addıng 10 µM DCIP and the decrease in absorbance at 600 nm was recorded at 5 sec ıntervals (16)

**Measurement of cytochrome content**

Mıtochondrıa were solubilized in phosphate buffered ısolation medium with suitable aliquots of Triton X 100 (10%v/v) and sodium desoxycholate(10%w/v) and difference spectra of sodıum dithionate reduced cytochromes were recorded against potassıum ferrıcyanıde oxidized cytochromes Contents of cytochromes were calculated by usıng wavelengths pairs as described previously (17) The $EmM^{-1}cm^{-1}$ were 24, 23 4 and 18 7 respectıvely for cytochromes $aa_3$, b and $c+c_1$(18)

**ATPase activity**

The ATPase activity in the brain mitochondria was determined in a medium containing 250mM sucrose, 10mM KCl, 0 2mM EDTA, 2 mM ATP, 2mM $MgCl_2$ and/or 50μM DNP were added as indicated previously (10) After pre-incubating the mitochondrial protein (Ca 100μg) in the assay medium at 37 °C, the reaction was initiated by the addition of ATP at a final concentration of 2 mM (10) The reaction was terminated with 5% w/v TCA Tubes were centrifuged at 3000 rpm in a table top centrifuge and 1 0ml of aliquots of the supernatant were taken for the estimation of the inorganic phosphorus liberated which was estimated by method as described by Fiske and Subba Row (19)

Protein estimation was according to the method of Lowry et al using bovine serum albumin as the standard (20)

## Results

### General

In the control group, the rates of respiration with glutamate, pyruvate + malate were more or less steady up to 5th week of life but increased in adult animals by about 2 fold When succinate was used as the respiratory substrate, the respiration rate almost doubled by 3rd week, remained so up to 5th week after which there was a further 4 fold increase For ascorbate + TMPD oxidation the rates increased linearly from the 4th week onwards to reach highest value in the adult animals The ADP/O ratios were in the expected ranges The pattern is consistent with the previously reported observations (Tables 1-4)

Table 1

Effect of dexamethasone treatment on oxidative phosphorylation in rat brain mitochondria with glutamate as the substrate*

| Age | Treatment | ADP/O ratio | Rate of respiration (n mole $O_2$/min/mg protein) | | ADP phosphorylation ra (n mole/min/mg protei |
|---|---|---|---|---|---|
| | | | +ADP | - ADP | |
| 2 week | Control (20) | 2 67 + 0 21 | 10 70 ± 0 58 | 5 06 ± 0 66 | 56 65 ± 3 58 |
| | Dex (12) | 3 26 ± 0 18[a] | 13 44 ± 0 97 [a] | 3 16 ± 0 62 [a] | 87 63 ± 5 38 [d] |
| 3 week | Control (24) | 3 01 ± 0 13 | 12 16 ± 0 86 | 2 91 ± 0 29 [c] | 71 88 ± 4 96 |
| | Dex (11) | 1 34 ± 0 09 [d] | 9 50 ± 1 34 | 4 56 ± 0 49 | 25 46 ± 4 12 [d] |
| 4 week | Control (22) | 3 04 ± 0 18 | 7 57 ± 0 46 | 2 69 ± 0 46 | 44 98 ± 3 32 |
| | Dex (11) | 3 24 ± 0 11 | 12 56 ± 0 87 [d] | 4 12 ± 0 50 [a] | 80 23 ± 4 82 [d] |
| 5 week | Control (6) | 3 17 ± 0 38 | 11 82 ± 0 95 | 5 02 ± 0 24 | 75 87 ± 14 27 |
| | Dex (28) | 2 52 ± 0 07 | 40 80 ± 2 74 [d] | 8 39 ± 1 32 [b] | 202 60 ± 11 00 [d] |
| Adult | Control (8) | 2 68 ± 0 11 | 22 10 ± 1 53 | 10 33 ± 0 63 | 117 86 ± 8 04 |
| | Dex (10) | 2 37 ± 0 26 | 7 01 ± 1 00 [d] | 3 16 ± 0 63 [d] | 31 72 ± 3 30 [d] |

The experimental conditions are as described in the text The results are expressed as mean ± SEM of the number of observations indic in the parenthesis

[a] $p< 0\ 05$, [b] $p<0\ 02$, [c] $p<0\ 01$ and [d] $p<0\ 001$ as compared with the corresponding control

**Table 2** Effect of dexamethasone treatment on oxidative phosphorylation in rat brain mitochondria with pyruvate and malate as the substrates

| Age | Treatment | ADP/O ratio | Rate of respiration (n mole $O_2$ /min/mg_protein) | | ADP phosphorylation rate (n mole/min/mg protein) |
|---|---|---|---|---|---|
| | | | +ADP | - ADP | |
| 2 week | Control (19) | 2 85 ± 0 18 | 14 68 ± 0 60 | 6 85 ± 0 68 | 82 15 ± 5 43 |
| | Dex (14) | 3 19 ± 0 17 | 13 85 ± 1.12 | 4 31 ± 0 63 [a] | 87 85 ± 7 05 |
| 3 week | Control (24) | 3 64 ± 0 13 | 14 98 ± 0 98 | 4 16 ± 0 26 | 108 02 ± 5 01 |
| | Dex (11) | 1 65 ± 0 17 [c] | 13 67 ± 1 43 | 5 03 ± 0 52 | 46 82 ± 0 7.81 [c] |
| 4 week | Control (32) | 3 19 ± 0 19 | 10 13 ± 0 48 | 3 48 ± 0 39 | 62 54 ± 4 29 |
| | Dex (22) | 3 63 ± 0 11 | 21 96 ± 1 19 [c] | 5 29 ± 0 58 [a] | 142 18 ± 9 62 [c] |
| 5 week | Control (13) | 3 14 ± 0 14 | 15 95 ± 0 80 | 7 62 ± 0 50 | 99 70 ± 6.85 |
| | Dex (21) | 2 72 ± 0 09 [a] | 32 35 ± 2 39 [c] | 11 80 ± 1 01 [c] | 173 08 ± 12 34 [c] |
| Adult | Control (21) | 2 67 ± 0 17 | 35 03 ± 4 45 | 15 12 ± 1 96 | 185 67 ± 20 57 |
| | Dex (11) | 1 44 ± 0 08 [c] | 9 59 ± 1 94 [c] | 4 27 ± 0 79 [c] | 28 57 ± 7 16 [c] |

*The experimental conditions are as described in the text The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis

[a] $p< 0\ 02$, [b] $p<0\ 01$, and [c] $p<0\ 001$ as compared with the corresponding control

Table 3

Effect of dexamethasone treatment on oxidative phosphorylation in rat brain mitochondria with succinate as the substrate*

| Age | Treatment | ADP/O ratio | Rate of respiration (n mole $O_2$/min /mg protein) | | ADP phosphorylation rate (n mole/min/mg protein) |
|---|---|---|---|---|---|
| | | | +ADP | - ADP | |
| 2 week | Control (7) | 1 77 ± 0 13 | 12 38 ± 1 35 | 8 05 ± 1 15 | 42 51 ± 4 37 |
| | Dex (16) | 2 23 ± 0 16 [c] | 18 90 ± 1 80 [d] | 7 01± 0 88 | 86 28 ± 8 00 [c] |
| 3 week | Control (25) | 1 81 ± 0 07 | 21 74 ± 0 99 | 10 77 ± 0 83 | 77 10 ± 3 60 |
| | Dex (12) | 0 45 ± 0 05 [c] | 13 75 ± 1 07 [c] | 9 52 ± 0 66 | 13 02 ± 2.38 [c] |
| 4 week | Control (12) | 1 93 ± 0 11 | 16 43 ± 0 77 | 6 33 ± 0 59 | 64 20 ± 5 14 |
| | Dex (21) | 2 21 ± 0 09 | 36 72 ± 2 08 [c] | 16 93 ± 1.66 [c] | 159 80 ± 9 62 [c] |
| 5 week | Control (19) | 1 69 ± 0 14 | 22 87 ± 0 91 | 14 23 ± 1 08 | 75 80 ± 6 43 |
| | Dex (19) | 1 59 ± 0 10 | 46 81 ± 2 09 [c] | 24 76 ± 2 02 [c] | 146 93 ± 6 40 [c] |
| Adult | Control (9) | 1 85 ± 0 06 | 85 57 ± 4 49 | 45 26 ± 4 50 | 313 60 ± 21 86 |
| | Dex (17) | 0 84 ± 0 09 [b] | 21 49 ± 1 59 [b] | 14 86 ± 1 51 [c] | 35 09 ± 2 77 [c] |

*The experimental conditions are as described in the text The results are expressed as mean ± SEM of the number of observat indicated in the parenthesis

[d] $p< 0\ 01$, [b] $p< 0\ 002$, and [c] $p<0\ 001$ as compared with the corresponding control

**Table 4.** Effect of dexamethasone treatment on oxidative phosphorylation in rat brain mitochondria with ascorbate + TMPD as the substrates

| Age | Treatment | ADP/O ratio | Rate of respiration (n mole $O_2$ /min /mg protein) | | ADP phosphorylation rate (n mole/min/mg protein) |
|---|---|---|---|---|---|
| | | | +ADP | - ADP | |
| 2 week | Control (12) | 0 35 ± 0 02 | 11 04 ± 1 28 | 6 42 ± 0 97 | 7 59 ± 0 84 |
| | Dex (10) | 0 15 ± 0 03 [d] | 29 27 ± 8 02 [a] | 8 92 ± 2 17 | 8 60 ± 0 60 |
| 3 week | Control (24) | 0 34 ± 0 02 | 12 27 ± 0 92 | 7 59 ± 0 68 | 7 82 ± 0 61 |
| | Dex (8) | 0 08 ± 0 01 [d] | 16 35 ± 2 00 | 11 49 ± 1 89 | 2 67 ± 0.44 [d] |
| 4 week | Control (10) | 0 26 ± 0 02 | 15 52 ± 1 40 | 8 71 ± 1 52 | 8 00 ± 0 39 |
| | Dex (18) | 0 33 ± 0 03 | 25 70 ± 2 55 [c] | 12 55 ± 1 62 [a] | 15 97 ± 1 72 [d] |
| 5 week | Control (11) | 0 32 ± 0 04 | 30 27 ± 5 33 | 16 17 ± 2 49 | 18 71 ± 3 31 |
| | Dex (13) | 0 08 ± 0 01 [d] | 56 38 ± 4 45 [d] | 37 34 ± 4 72 [d] | 8 70 ± 0 71 [d] |
| Adult | Control (12) | 0 23 ± 0 02 | 72 13 ± 11 75 | 45 17 ± 8 28 | 32 68 ± 3 82 |
| | Dex (14) | 0 14 ± 0 02 [b] | 55 88 ± 7 85 | 27 57 ± 4 97 | 15 97 ± 1 83 [d] |

*The experimental conditions are as described in the text The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis

[a] $p< 0\ 05$, [b] $p< 01$, [c] $p<0\ 002$ and [d] $p<0\ 001$ as compared with the corresponding control

**Effects of dexamethasone Treatment**

Dexamethasone treatment resulted in generalized increase in the state 3 respiration rates in the growing animals however in the adults dexamethasone treatment caused about 70% decrease Dexamethasone also resulted in uncoupling of the mitochondria in 3 week and 5 week old animals where ADP/O ratio decreased by 56 to 21% respectively For pyruvate and malate the increase in the respiratory activity was seen in the 4 and 5 week old animals As in the case of glutamate the state 3 respiration decreased by 73% in adults after dexamethasone treatment The ADP/O ratios were low in the 3 week and 5 week animals and in the adults With succinate as the substrate the state 3 respiration rates were high in 2, 4 and 5 week animals whereas 37% to 74% decrease was noted in the 3 week group and adults Uncoupling was noted in the 3 week group and adults When ascorbate + TMPD was the respiratory substrates the state 3 respiration rates increased from 66 to 165% in the developing animals, the adult showed the marginal decrease which was statistically significant Except for 4 week group the ADP/O ratio decreased from 39 to 76 % in different age groups (Tables 1-4 )

Consistent with the pattern of respiration the glutamate dehydrogenase activity increased in the growing animals but was low in the adults Malate dehydrogenase activity showed marginal changes while pattern of SDR was comparable to that of glutamate dehydrogenase (Fig 1 )

The content of cytochrome $aa_3$ decreased in all age groups after dexamethasone treatment from 15 to 50 % almost similar trend seen for cytochrome b content (Fig 2 ) The content of cytochrome c + $c_1$ increase in 3 week animals but decrease in adults (Table 5 ) The ATPase activities were affected in an age dependent manner (Table 6 )

**Fig. 1** Effect of dexamethasone treatment on rat brain mitochondrial dehydrogenase activity, (A) Glutamate dehydrogenase, (B) Malate dehydrogenase and (C) Succinate dehydrogenase activity The activity is given as nmoles /min /mg protein ▭ bars represents for control whereas ▬ represents dexamethasone treated age groups Error bar represents the SEM of 12 independent observations

[a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.001$ as compared with the corresponding control

Fig.1

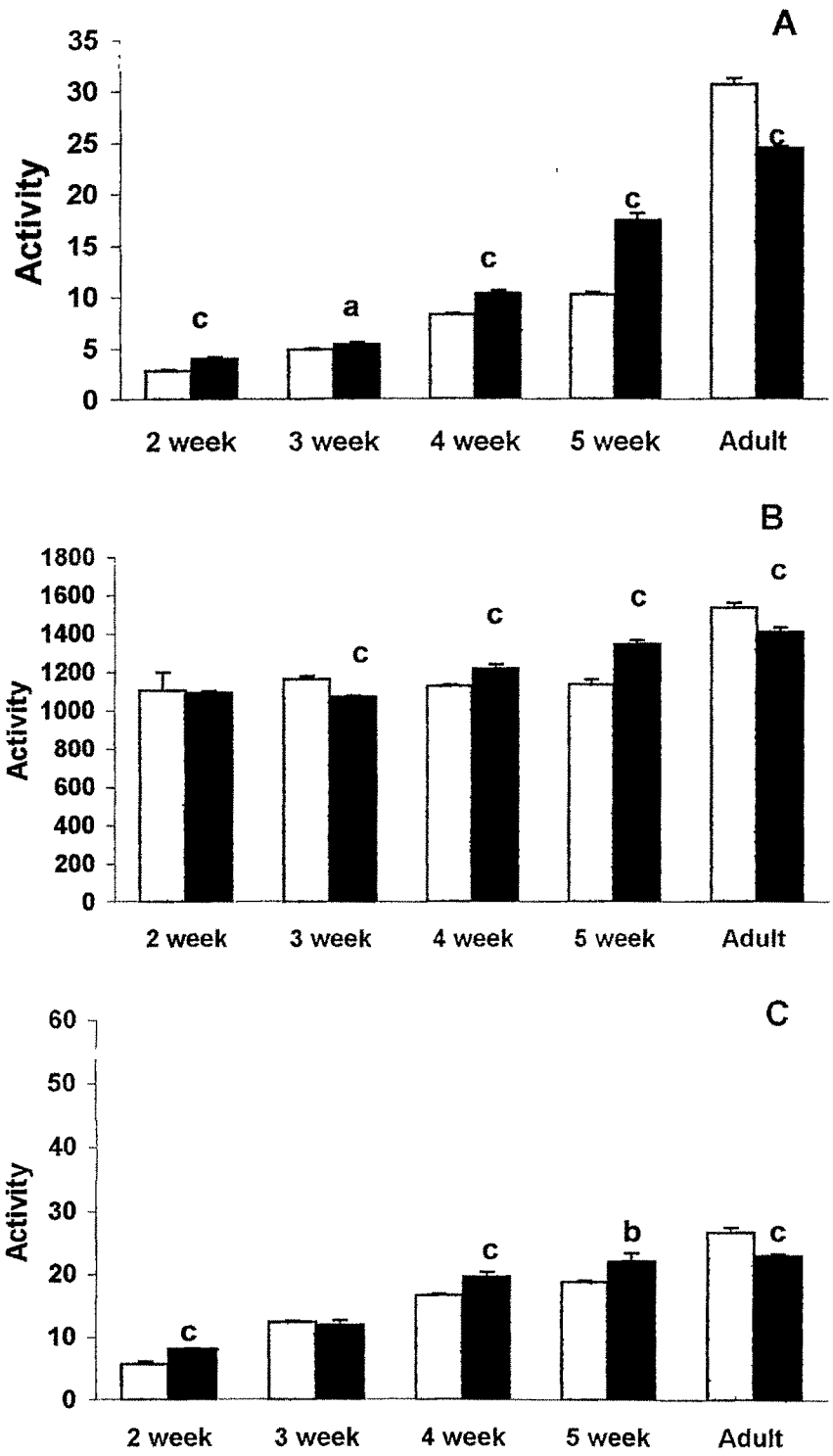
A
Activity
35
30
25
20
15
10
5
0
c
a
c
c
c
2 week
3 week
4 week
5 week
Adult
B
Activity
1800
1600
1400
1200
1000
800
600
400
200
0
c
c
c
c
2 week
3 week
4 week
5 week
Adult
C
Activity
60
50
40
30
20
10
0
c
c
b
c
2 week
3 week
4 week
5 week
Adult

**Fig. 2** Typical cytochrome spectra of brain mitochondria form individual age-groups The spectra of control group (A to E for 2,3,4,5, week and adult respectively) are given on the left hand side panel and those for dexamethasone treated group (F to J for corresponding age groups) are given on the right hand side panel The ordinate represents absorption units while the abscissa represents wavelengths in nm

Protein concentrations for determination of cytochrome spectrum are 8 53, 8 27, 8 32, 5 04 and 4 46 mg/ml in the control group (A to E) These values for the comparing dexamethasone treated groups are 9 15, 7 6, 6 80, 6 08 and 5 07 mg/ml (F to J)

g. 2

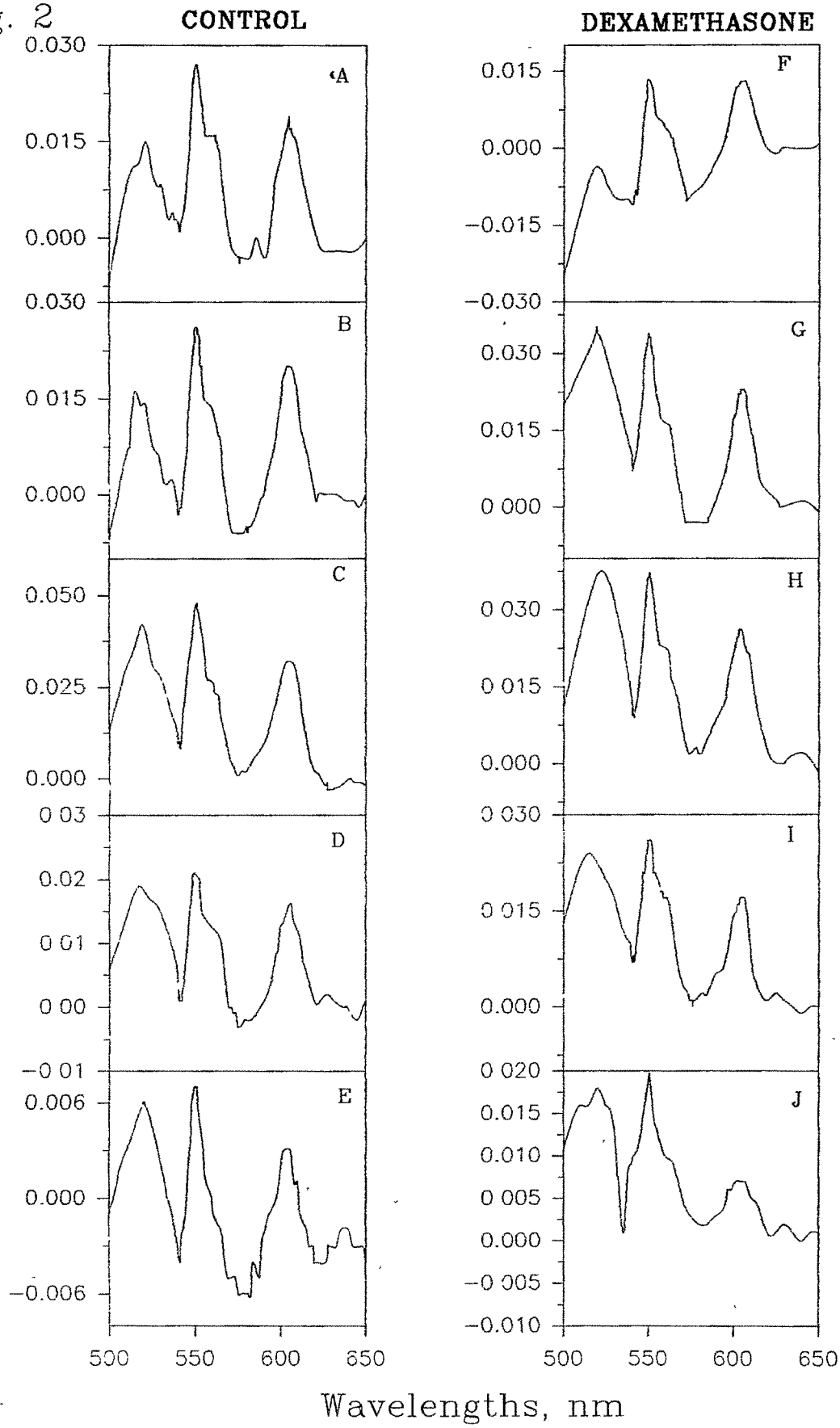
CONTROL
DEXAMETHASONE
A
B
C
D
E
F
G
H
I
J
0.030
0.015
0.000
0.030
0 015
0.000
0.050
0.025
0.000
0 03
0.02
0 01
0 00
−0 01
0.006
0.000
−0.006
0.015
0.000
−0.015
−0.030
0.030
0.015
0 000
0 030
0 015
0.000
0 030
0 015
0.000
0 020
0.015
0.010
0 005
0.000
−0 005
−0.010
500
550
600
650
Wavelengths, nm

Table 5

Effect of Dexamethasone treatment on brain mitochondrial cytochrome contents

| Age | Treatment | Cytochrome content ( pmole / mg protein ) | | |
|---|---|---|---|---|
| | | $aa_3$ | b | $c+c_1$ |
| 2 week | Control (13) | 113 09 ± 3 24 | 116 5 ± 5 75 | 170 76 ± 6 29 |
| | Dex (10) | 62 70 ± 3 45 [c] | 80 39 ± 6 31 [c] | 192 16 ± 13 13 |
| 3 week | Control (13) | 141 55 ± 16 33 | 126 78 ± 10 13 | 194 33 ± 7 89 |
| | Dex (10) | 102 26 ± 4 33 [a] | 104 94 ± 4 65 | 230 39 ± 12 65 [a] |
| 4 week | Control (16) | 170 27 ± 6 26 | 144 22 ± 3 43 | 212 59 ± 7 26 |
| | Dex (9) | 139 91 ± 9 24 [b] | 103 13 ± 6 82 [c] | 211 26 ± 16.19 |
| 5 week | Control (14) | 173 87 ± 10 75 | 168 99 ± 10 75 | 227 98 ± 7 17 |
| | Dex (12) | 148 27 ± 5 22 [b] | 144 12 ± 8 07 | 220 33 ± 5 95 |
| Adult | Control (14) | 195 15 ± 7 91 | 196 39 ± 7 77 | 244 80 ± 6 05 |
| | Dex (16) | 97 32 ± 3 91 [c] | 124 84 ± 4 50 [c] | 197 22 ± 7 05 [c] |

*The experimental conditions are as described in the text The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis

[a] $p< 0.05$, [b] $p<0.02$, and [c] $p<0\ 001$ as compared with the corresponding control

**Table 6.** Effect of Dexamethasone treatment on brain mitochondrial ATPase

| Age | Treatment | Basal | +Mg | +DNP | +Mg +DNP |
|---|---|---|---|---|---|
| 2 week | Control (8) | 1 07 ± 0 055 | 6 07 ± 0 314 | 1 08 ± 0 063 | 6 45 ± 0 372 |
| | Dex (4) | 0 00 ± 0 000 [e] | 2 39 ± 0 102 [e] | 0 00 ± 0 000 [e] | 4 15 ± 0 395 [d] |
| 3 week | Control (8) | 1 50 ± 0 153 | 10 27 ± 0 725 | 1 95 ± 0 279 | 9 84 ± 0 668 |
| | Dex (8) | 1 65 ± 0 203 | 7 22 ± 0 624 [c] | 1 44 ± 0 176 | 7 45 ± 0 543 [b] |
| 4 week | Control (8) | 2 45± 0 196 | 10 97± 0 585 | 2 46 ± 0 192 | 12 64 ± 0 769 |
| | Dex (8) | 1 10 ± 0 110 [e] | 6 41 ± 0 242 [e] | 0 98 ± 0 053 [e] | 9 08 ± 0 390 [e] |
| 5 week | Control (14) | 2 45 ± 0 202 | 12 63 ± 0 869 | 2 61 ± 0 286 | 13 76 ± 0 693 |
| | Dex (20) | 2 30 ± 0 207 | 10 97 ± 0 305 | 2 78 ± 0 204 | 11 62 ± 0 394 [b] |
| Adult | Control (12) | 3 62 ± 0 261 | 14 34 ± 0 421 | 3 33 ± 0 177 | 17 23 ± 0 720 |
| | Dex (8) | 2 67 ± 0 326 [a] | 10 43 ± 0 691 [e] | 2 96 ± 0 238 | 12 13 ± 0 550 [e] |

The experimental conditions are as described in the text ATPase activity in µmole Pi liberated / hr / mg protein The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis

[a] $p< 0\ 05$, [b] $p<0\ 02$, [c] $p<0\ 01$, [d] $p<0\ 002$, and [e] $p<0\ 001$ as compared with the corresponding control

## Discussion

The results of the present studies indicate that the dexamethasone treatment in general stimulated the state 3 respiration rates in brain mitochondria in growing animals whereas the reverse was true for the adults Dexamethasone treatment also resulted in uncoupling of oxidative phosphorylation in substrate and age-dependent manner From computation of data on ADP/O ratio (Tables 1-4) it may be inferred that in the 2 week group uncoupling effect of dexamethasone was restricted to the 3rd site In the 3 week group the 2nd and 3rd site affected to the extent of 75% whereas no uncoupling occured in the 4 week animals In the 5 week and adults the uncoupling occurred only in the 3rd site Therefore it was suggested in brain mitochondria uncoupling effect seem marginally in the 3rd site of phosphorylation

In the earlier study we observe that in the liver mitochondria dexamethasone treatment caused inhibition of state 3 respiration in general and site specific and age-dependant uncoupling of oxidative phosphorylation The results of the present studies therefore show that the action of dexamethasone in brain mitochondria is opposite to that seen in the liver mitochondria atleast as far as the respiratory activity is concerned Also only 3rd site of phosphorylation seems to be susceptible to uncoupling This paradoxical situation may be explained as follows

In the brain the mineralocorticoid receptors are saturated to the extent of 80% irrespective of diurnal variation in plasma corticosterone levels (21) The saturation of glucocorticoid receptor is related to the circadian rhythm of corticosterone release (21) Further it has been shown that corticosterone binds to the mineralocorticoid receptors and has toxic

inhibitory effect on HPA axis (21) Further it has also been shown that dexamethasone is freely permeable into the brain by a pathway which bypasses mdr1aP-glycoprotein (22) However under in vivo condition dexamethasone does not bind with mineralocorticoid receptor

It would therefore seem that only condition of chronic dexamethasone treatment the hormone reach to the brain and may bind to the unoccupied glucocorticoid receptor and exert a positive gene activation response Indeed that seems to be the case that judged from that data that increased glutamate dehydrogenase activity and succinate cytochrome c reductase activity This mechanism would be operative especially in the young animals where plasma corticosterone levels reported to be low during stress non responsive period (SNRP) (23) In the adults where the corticosterone levels are normal a regular negative gene activation response, as noted previously for liver is seen

It has also been reported that dexamethasone direct interacts with mitochondrial genome and the HRE is located at COX 1 elements (24,25,26) Our results shown that upon dexamethasone treatment the contents of cytochrome $aa_3$ and cytochrome b decreased in general in an age specific manner whereas changes in cytochrome c + $c_1$ content were only marginal The ATPase activity in general was affected in age-dependent manner in the dexamethasone treated groups It is known that the crucial peptides of cytochrome oxidase, cytochrome b and ATPase coded by mitochondrial DNA (27) Hence it may be suggested that dexamethasone may exert age-dependent effect on the expression of these crucial peptides coded by mitochondrial DNA (27) In this aspects the effects of dexamethasone on liver and brain mitochondria are very similar

## Summary

Dexamethasone treatment in general stimulated the state 3 rates in brain mitochondria in growing animals, whereas in adults opposite effects were seen Dexamethasone treatment also resulted in uncoupling of mitochondria in an age-dependent and tissue-specific manner. The glutamate dehydrogenase activity increased in growing animals but was low in the adults After dexamethasone treatment malate dehydrogenase activity showed marginal changes while pattern of succinate cytochrome c reductase was comparable to that of glutamate dehydrogenase Contents of cytochrome aa3 and b decreased in all age groups after dexamethasone treatment, the content of cytochrome $c+c_1$ increased in 3 week animals but decreased in the adults The ATPase activities showed age-specific changes after dexamethasone treatment

## References


1 Tsuneishi, S , Takada, S , Motoike, T , Ohashi, T , Sano, K , Nakamura, H , 1991 Effects of dexamethasone on the expression of myelin basic protein, proteolipid protein,and glial fibrillary acidic protein genes in developing rat brain Exp Brain Res 61, 117-123

2 Rastogi, A , Akintorin, S M.., Bez, M L , Morales, P , Pildes, R S , 1996 A controlled trial of dexamethasone to prevent bronchopulmonary dysplasia in surfactant-treated infants Pediatrics 98, 204-210

3 Gilbertson, E O , Spellmann, M C , Piacquidio, D J , Mulford, M I , 1998 Super potent topical corticosteroid use associated with adrenal suppression Clinical considerations J Am Acad Dermatol 38, 318-321

4 Romagnoli, C , Zecca, E , Vento, G , Maggio, L , Papacci, P , Tortorolo, G , 1999 Effect on growth of two different dexamethasone courses for preterm infants at risk of chronic lung diseases a randomized trial Pharmacology 59, 266-274

5 Schimmer, B P , Parker, K L , 2001 Adrenocorticotropic hormone, adrenocortical steroids and their synthetic analogs, inhibitors of the synthesis and actions of adrenocortical hormones In Gilman, A G , Hardman, J G , Limbird, L E (Eds ), The pharmacological basis of therapeutics . 10th edition. The McGraw-Hill Companies, pp 1635-1648

6 Whitelaw, A , Thorensen, M (2000) Antenatal steroids and the developing brain Arch Dis Child Fetal Neonatal Ed 83, F154 – F157

7 Flagel, S B , Vazquez, D M , Watson, Jr S J , Neal, Jr C R (2002) Effects of tapering neonatal dexamethasone on rat growth, neurodevelopment, and stress response Am J Physiol Integrtative Comp Physiol 282, R55-R63

8 O'Neill, J, 1974. Carbohydrate metabolism in the developing nervous system In Himwich, W. ( Eds ), Biochemistry of the developing Brain, 2, Marcel Dekker, New York pp 341-348

9 Balazs, R and Cotterrell, M. (1972) Effect of hormonal state on cell number and functional maturation of the brain Nature 236, 348-350

10 Swegert, C V, Dave, K R, Katyare, S S, 1999 Effect of aluminium-induced Alzheimer like condition on oxidative energy metabolism in rat liver, brain and heart mitochondria Mech Age Dev 112, 27-42

11 Satav, J G, Katyare, S S, 1982 Effect of experimental thyrotoxicosis on oxidative phosphorylation in rat liver, kidney and brain mitochondria Molec Cell Endocrinol 28, 173-189

12 Katyare, S S, Joshi, M V, Fatterpaker, P, Sreenivasan, A, 1977, Effect of thyroid deficiency on oxidative phosphorylation in rat liver, kidney and brain mitochondria Arch Biochem Biophys 182, 155-163

13 Ferreira, J, Gil, L, 1984 Nutritional effects on mitochondrial bioenergetics alterations in oxidative phosphorylation by rat liver mitochondria Biochem J 218, 61-67

14 Leighton, F, Poole, B, Beaufay, H, Baudhuin, P, Coffer, J W, Flower, S, De Duve, C, 1968 The large scale separation of peroxisomes, mitochondria and lysosomes from livers of rats injected with triton X-100 Improved isolation procedures, analysis and biochemical properties of fractions J Cell Biol 37, 482-513

15 Ochoa, S, 1995 Malic dehydrogenase from pig heart, In Colowick, S P, Kaplan, N O (Eds ), Methods Enzymol 1, Academic Press, New York, pp 735-739

16 King, T E , 1967 Preparation of succinate-cytochrome c- reductase and the cytochrome b-c1 particle and the reconstitution of cytochrome c-reductase. In Methods Enzymol , 10, (Estabrook, R W , Pullman, M E , Eds ,) Acadamic Press, New York, pp. 216-225

17 Katyare, S S , Rajan, R R , 1988 Enhanced oxidative phosphorylation in rat liver mitochondria following prolonged *in vivo* treatment with imipramine Br J Pharmacol 95, 914-922

18 Subramanian, M , Katyare, S S , 1990 Oxidative phosphorylation in mouse liver mitochondria during weaning. Mech Age Dev 54, 121-129

19 Fiske, C H , Subba Row, Y , 1925 Colorimetric determination of phosphorus J Biol Chem 66, 375-400

20 Lowry, O H , Rosebrough, N J , Farr, A L , Randall, R J , 1951 Protein measurement with the Folin phenol reagent J Biol Chem 193, 265-275

21 Reul, J M H M et al (2000) The brain mineralocorticoid receptor greedy for ligand, mysterious in function Eur J Pharmacol 405,235-249

22 Cordon-Cardo, C , O'Brien, J P , Casals, D , Rittman-Graues, L , Bielder, J L , Melamed, M R , Bertine, J R , (1989) Multidrug-resistance gene (P-glycoprotien) is expressed by endothelialcells at blood-brain barrier sites Proc. Natil Acad Sci , 86, 695-698

23 Saplolsky, R M and Meaney, M J (1986) Maturation of the adrenocorticoid stress response Neuroendocrine control mechanisms and the stress hyporesponsive period Brain Res Rev 11, 65-76

24 Demonacos , C , Djordjevic,-Markovic, R , Tsawdaroglou, N , Sekeris, C E , (1995) The mitochondrion is a primary site of action of glucocorticoids the interaction of the glucocorticoids receptor with mitochondrial DNA sequences

showing partial similarity to the nuclear glucocorticoid responsive elements Steroids 55 (1) 43-55

25. Tsiriyotis, C , Sandidos, D A , Sekeris, C E , 1997 The mitochondrion is a primary site of action of glucocorticoids mitochondrial nucleotide sequences, showing similarity to hormone response elements, confer dexamethasone inducibility to chimaeric genes transfected in LATK-cells Biochem Biophys Res Commum 235, 349-354

26 Simon, N , Jolliet, P , Moric, C , Zini, R , Urien, S , Tillement, J P , (1998) Glucocorticoids decrease cytochrome c oxidase activity of isolated rat kidney mitochondria FEBS lett 435, 25-28

27. Poyton, R O , McEwen, J E , 1996 Crosstalk between nuclear and mitochondrial genomes Annu Rev Biochem 65, 563-607