Chapter 5

Effect of Dexamethasone Treatment on Oxidative Energy Metabolism in Rat Liver Mitochondria during Postnatal Periods and in Adults

Introduction

The regulatory role of adrenal glucocorticoids on carbohydrate metabolism and in maintaining blood glucose level is well recognized(1-3) Several synthetic glucocorticoids have since been prepared with the aim of amplifying the glucocorticoid and anti-inflammatory properties(1,4) These synthetic glucocorticoids have wide therapeutic applications not only as anti-inflammatory agents but they are also being used for treatment of skin diseases, bronchial asthma, bronchopulmonary dysplasia (BPD), respiratory distress syndrome in fetus, rheumatoid arthritis and meningitis in practically all the age groups including children(4-7) The use of repeated courses of corticosteroids is prescribed in 98% of cases by Obstetricians (8,9) The synthetic glucocorticoids are believed to have lesser or no side-effects or toxicity (4) Use of dexamethasone as potent glucocorticoid as well as an anti-inflammatory agent is recommended by American National Institutes of Health (9) However, when used in pharmacological doses, adverse side-effect are seen in as high as 50 % of the cases (10)

In earlier studies we noted that dexamethasone treatment resulted in impairment of macromolecular synthesis and metabolic activity of the cells in tissues such as liver and brain (11) As is well recognized, the developmental process is energy dependent (12) Since dexamethasone is recommended in all age groups (9), and since adverse affects have also been noted (10) it is of interest to find out if dexamethasone when used for therapeutic purposes in different developmental age groups would affect the developmental process by modulating organelle functions Since mitochondria are the major site of ATP synthesis we evaluated the effects of dexamethasone treatment on mitochondrial oxidative energy metabolism in rats belonging to different age groups Indeed, our studies have shown that chronic dexamethasone treatment resulted in an age-

dependent and substrate and site-specific impairment of oxidative energy metabolism in the liver mitochondria The results of the these studies are summarized here.

Materials and methods

Chemicals

The details are as given in Chapter 4 of the thesis

Animals and treatment with dexamethasone

The detail plan is as given in the Chapter 2 of the thesis

Isolation of mitochondria

The animals were killed by decapitation and their livers were quickly removed and placed in a beakers containing chilled (0-4°C) isolation medium The isolation medium contained 0 25M sucrose, 10mM tris-HCl buffer, pH 7 4, 1mM EDTA and 250 µg BSA/ml The tissue was minced and 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 centrifugation at 650 x g for 10 min and discarded The supernatant was subjected to a further centrifugation at 7,500 x g for 10 min The resulting mitochondrial pellet was washed by suspending them gently in the isolation medium and by resedimenting at 7,500 x g for 10 min Finally the mitochondria were suspended in the isolation medium to give a protein concentration of about 30-50 mg/ml(13)

Oxidative phosphorylation

Measurement of oxidative phosphorylation was carried out using a Clark type oxygen electrode as described previously(13,14) 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(15, 16)

The assay of dehydrogenases and measurement of cytochrome content are as described in the previous Chapter 4 of the thesis

ATPase activity

ATPase activities were measured at 37^{O}C in the assay medium (total volume of 0 4 ml) containing 50mM tris-HCl buffer , pH 7 4, 75mM KCl, 0 4mM EDTA, pH 7 4 6mM $MgCl_2$ and / or 100 μM DNP Mitochondrial protein (Ca 100-150μg protein) was used as the source of the enzyme After preincubation at 37 ^{O}C for 1min the reaction was initiated by adding 5mM ATP The reaction was terminated after 10 min by the addition of 0 1 ml of 5%(w/v) sodium dodecylsulphate (SDS) as described (13) and the amount

of liberated inorganic phosphorus was estimated by the method of Fiske and Subba Row(17)

Protein estimation was according to the method of Lowry et al (18) using bovine serum albumin as the standard

Results

General

In the control group, the ADP/O ratios and state 3 respiration rates were low in 2 week old animals with glutamate as the substrate but reached the steady state value by the 3rd week of life Similar trend was seen for ADP/O ratio with pyruvate+malate as the substrate pair However, the state 3 respiration rates for pyruvate + malate were very low initially ie in 2 week old animals but increased by 10 fold in 3 week group, remained at this level up to week 5 and then the rates almost doubled in the adult animals With succinate as the substrate also the ADP/O ratio was low in 2 week animals but attained the theoretical value at the later stages The state 3 respiration rates were low and remained so up to 4th week. increased by about 25% in the 5th week and further 75% increase was seen in the adults For ascorbate and TMPD the ADP/O ratio was in the expected range in all the age groups. The State 3 respiration rates were low in 2 week animals but doubled by 3rd week and remained so up to 5 week In the adults. the increase in the state 3 respiration rate was 5 fold Typical maturation patterns were also noted for glutamate dehydrogenase, malate dehydrogenase and succinate cytochrome c reductase activities as well as for the cytochrome profiles and ATPase activity (Tables 1-6, Fig 1 and 2)

Effect of dexamethasone treatment

Dexamethasone treatment resulted in significant reduction in state 3 respiration rates with glutamate as the respiratory substrate in practically all the age groups (48-80% decrease) except for the 4 week old animals The magnitude of inhibition was substantially high in younger ie 2 week and 3 week animals The ADP/O ratio decreased by from 50%-65% in 3 week , 5 week and adult animals(Table 1)

When pyruvate+malate was used as the respiratory substrate, a significant reduction in state 3 respiration was seen only in the 3 week old and the adult animals (45 to 80% decrease) By contrast, in the 4 week group the rate increased by 138% With respect to ADP/O ratio, as in the case of glutamate, the 3 week, 5 week and adult groups seemed to be more susceptible (Table 2)

When succinate was used as the respiratory substrate, the state 3 respiration rates decreased in all age groups (23%– 70% decrease) The ADP/O ratio decreased in the 3 week and 5 week group and in the adults (Table 3)

With ascorbate + TMPD as the substrate, dexamethasone treatment stimulated state 3 respiration in 5 week old animals but exerted an inhibitory effect in adults The ADP/O ratio decreased in all the groups except for the 4 week animals (Table 4)

The inhibition of respiration and lowering of ADP/O ratio was also reflected in general in the reduction in ADP phosphorylation rates (Tables 1 to 4)

Table 1

Effect of Dexamethasone treatment on oxidative phosphorylation with glutamate as the substrate in rat liver mitochondria

Age	Treatment	ADP/O ratio	Rate of respiration (n moles O_2 /min /mg protein) +ADP	- ADP	ADP phosphorylation rate (n mole/min/mg/protein)
2 week	Control (10)	2 11 ± 0 19	15 13 ± 0 24	8 79 ± 0 89	70 52 ± 15 15
	Dex (10)	2 52 ± 0 36	3 96 ± 0 71 [c]	1 88 ± 0 38 [c]	19 50 ± 5 15 [a]
3 week	Control (27)	2 92 ± 0 11	22.59 ± 1 38	5 41 ± 0 54	128 93 ± 7 82
	Dex (25)	1 01 ±0 08 [c]	4 63 ± 0 31 [c]	2 91 ± 0 25 [c]	9 56 ± 1 17 [c]
4 week	Control (25)	3 34 ± 0 19	23 90 ± 1 36	4 87 ± 0 75	156 84 ± 10 52
	Dex (11)	3 16 ± 0 24	22 30 ± 1 14	8 05 ± 0 41 [c]	140 51 ±12 13
5 week	Control (6)	3 05 ± 0 15	20 72±1 46	7 83 ± 0 66	123 81 ± 8 39
	Dex (28)	1 53 ± 0 15 [c]	14 99 ± 1 25	9 04 ± 0 78	51 07 ± 7 44 [b]
Adult	Control (8)	3 18 ± 0 22	22 10 ± 1.38	4 93 ± 0 63	141 53 ± 13 34
	Dex (10)	1 25 ± 0 13 [c]	11 56 ±1 34 [c]	6 30 ± 1 10	27 66 ± 2 83 [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] $p< 0\ 01$, [b] $p<0\ 002$ and [c] $p<0\ 001$ as compared with the corresponding control

Table 2

Effect of Dexamethasone treatment on oxidative phosphorylation with pyruvate and malate as the substrates in rat liver mitochondria

Age	Treatment	ADP/O ratio	Rate of respiration (n moles O_2/min /mg protein) +ADP	- ADP	ADP phosphorylation rate (n mole/min/mg/protein)
2 week	Control (13)	2 12 ± 0 37	1 57 ± 0 14	1 08 ± 0 21	6 50 ± 1 11
	Dex (13)	2 04 ± 0 26	1 33 ± 0 23 [c]	0 73 ± 0 23 [c]	4 99 ± 0 64 [a]
3 week	Control (18)	2 72 ± 0 14	14 84 ± 1 03	5 69 ± 0 73	80 92 ± 7 03
	Dex (25)	1 01 ±0 08 [c]	4 63 ± 0 31 [c]	2 91 ± 0 25 [c]	9 56 ± 1 17 [c]
4 week	Control (24)	2 71 ± 0 17	12 93 ± 0 46	4 28 ± 0 47	69 53 ± 4 16
	Dex (11)	3 16 ± 0 24	22 30 ± 1 14	8 05 ± 0 41 [c]	140 51 ±12 13
5 week	Control (14)	3 23 ± 0 17	12 07 ± 1 35	5 36 ± 0 70	77 17 ± 8 96
	Dex (28)	1 53 ± 0 15 [c]	14 99 ± 1 25	9 04 ± 0 78	51 07 ± 7 44 [b]
Adult	Control (8)	3 18 ± 0 22	22 10 ± 1 38	4 93 ± 0 63	141 53 ± 13 34
	Dex (10)	1 25 ± 0 13 [c]	11 56 ±1 34 [c]	6 30 ± 1 10	27 66 ± 2 83 [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] $p< 0\ 01$, [b] $p<0\ 002$ and [c] $p<0\ 001$ as compared with the corresponding control

Table 3

Effect of Dexamethasone treatment on oxidative phosphorylation with succinate as the substrate in rat liver mitochondria

Age	Treatment	ADP/O ratio	Rate of respiration (n moles O_2 /min /mg protein) +ADP	- ADP	ADP phosphorylation rate (n mole/min/mg/protein)
2 week	Control (9)	0 33 ± 0 37	43 75 ± 4 17	30 88 ± 4 36	27 14 ± 1 58
	Dex (14)	0 28 ± 0 06	13 19 ± 1 18 [b]	11 53 ± 0 23 [b]	8 44 ± 2 59 [b]
3 week	Control (28)	1 77 ± 0 09	45 16 ± 2 04	20 43 ± 1 85	158 53 ± 9 79
	Dex (14)	0 12 ± 0 01 [b]	19 74 ± 1 32 [b]	17 74 ± 0 87	5 00 ± 0 70 [b]
4 week	Control (28)	2 09 ± 0 11	44 77 ± 3 03	12 97 ± 0 47	186 96 ± 15 88
	Dex (19)	1 76 ± 0 18	26 14 ± 2 26 [b]	10 91 ± 1 24	97 59 ± 12 61 [b]
5 week	Control (14)	1 83 ± 0 17	55 71 ± 5 68	28 25 ± 2 38	188 29 ± 14 91
	Dex (20)	0 65 ± 0 06 [b]	30 84 ± 2 25 [b]	23 78 ± 2 20	40 12 ± 5 07 [b]
Adult	Control (29)	1 49 ± 0 12	77 15 ± 4 63	36 65 ± 0 63	210 78 ± 10 03
	Dex (26)	0 64 ± 0 08 [b]	59 54 ± 3 35 [a]	43 54 ± 3 43 [a]	72 11 ± 10 55 [b]

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

Table 4

Effect of Dexamethasone treatment on oxidative phosphorylation with ascorbate + TMPD as the substrates in rat liver mitochondria

Age	Treatment	ADP/O ratio	Rate of respiration (n moles O_2/min /mg protein) +ADP	- ADP	ADP phosphorylation rate (n mole/min/mg/protein)
2 week	Control (9)	0 26 ± 0 03	14 16 ± 1 63	9 00 ± 1 38	6 70 ± 0 52
	Dex (12)	0 11 ± 0 02 [c]	20 96 ± 4 45	8 62 ± 2 22	3 70 ± 0 47 [c]
3 week	Control (24)	0 28 ± 0 02	28 24 ± 2 25	17 54 ± 1 73	15 06 ± 1 26
	Dex (12)	0 09 ± 0 01 [c]	27 63 ± 2 69	18 42 ± 2 79	4 34 ± 0 28 [c]
4 week	Control (11)	0 27 ± 0 05	39 60 ± 2 53	24 32 ± 2 28	20 60 ± 2 36
	Dex (19)	0 26 ± 0 02	30 12 ± 2 48 [b]	17 04 ± 2 41 [a]	14 63 ± 1 01 [a]
5 week	Control (24)	0 23 ± 0 02	31 65 ± 2 11	19 91 ± 1 46	13 18 ± 0 75
	Dex (20)	0 10 ± 0 01 [c]	45 40 ± 3 77 [c]	27 17 ± 3 35	8 26 ± 0 77 [c]
Adult	Control (42)	0 20 ± 0 01	73 43 ± 5 57	42 90 ± 3 34	27 64 ± 1 93
	Dex (26)	0 09 ± 0 05 [c]	48 36 ± 3 52 [c]	26 63 ± 2 51 [c]	8 67 ± 0 73 [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] $p< 02$ and [c] $p<0\ 001$ as compared with the corresponding control

Fig. 1 Effect of dexamethasone treatment on rat liver 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] $p < 0.001$ as compared with the corresponding control

Fig.1

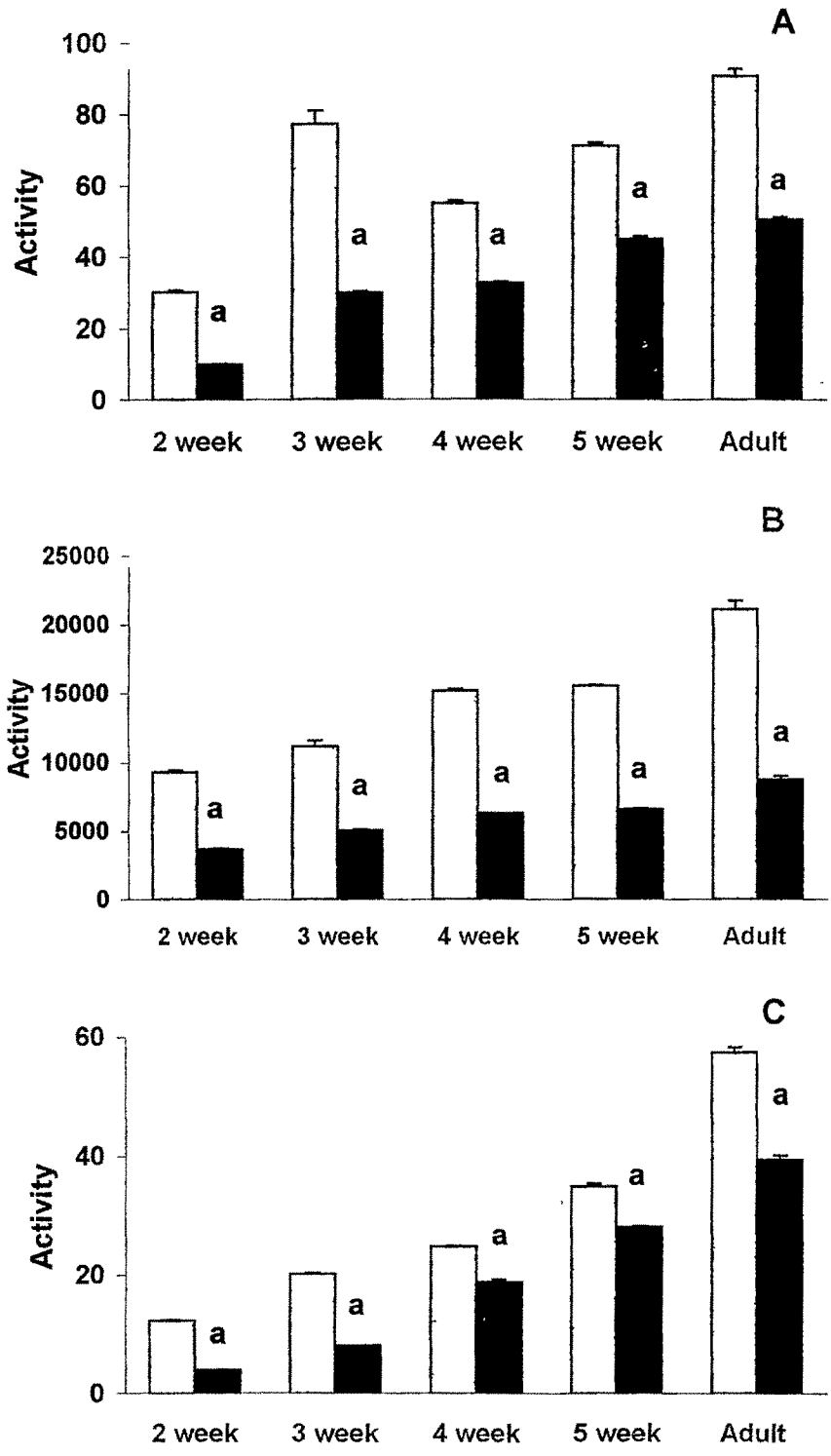
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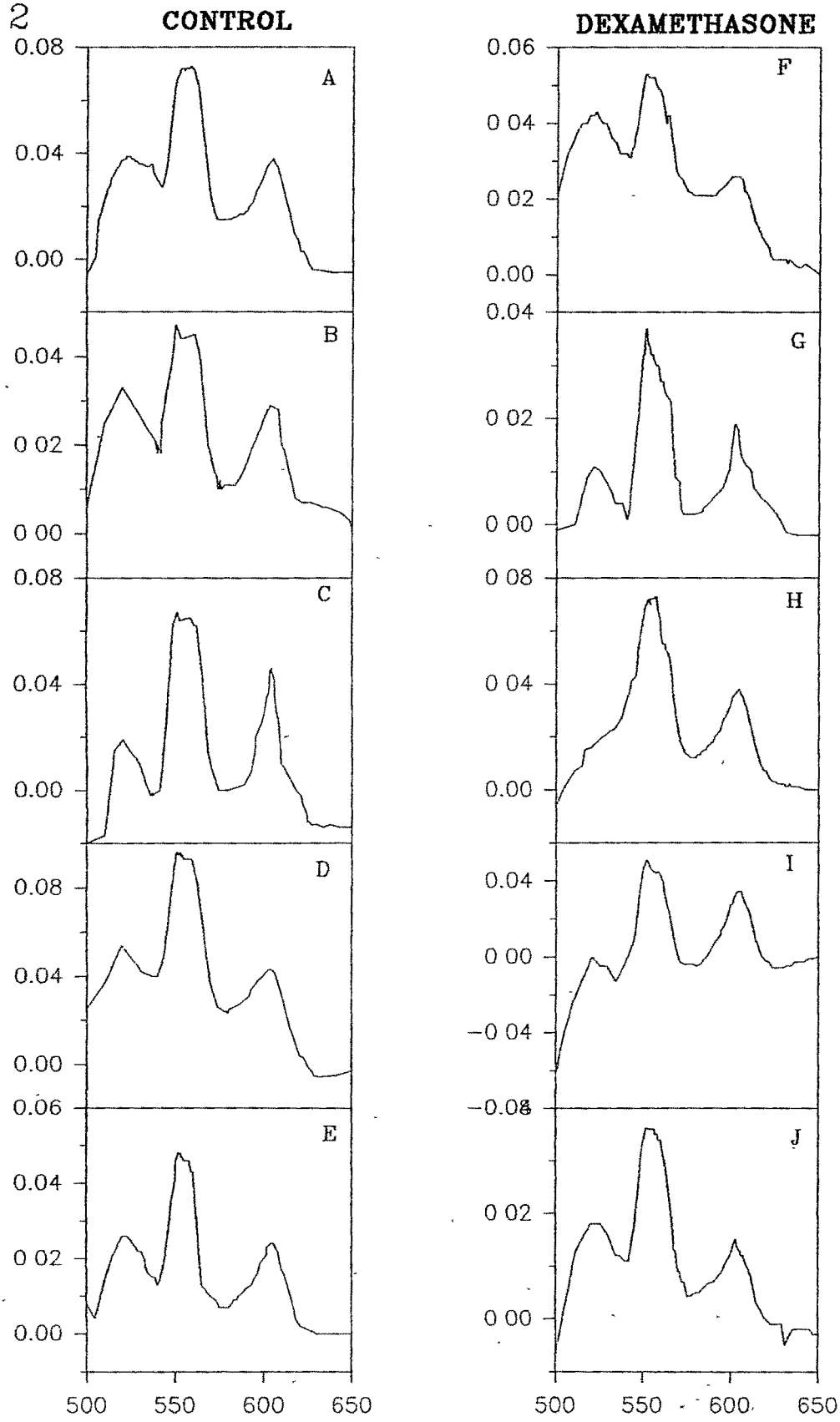
Fig. 2 Typical cytochrome spectra of liver mitochondria form individual age-groups The spectra of control group (A to E for 2,3,4,5, week and adult respectively) are given on the left hand side panel and those for dexamethasone treated group (F to J for corresponding age groups) are given on the right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

Protein concentrations for determination of cytochrome spectrum are 10 42, 5 21, 11 26, 10 14 and 5 07 mg/ml in the control group (A to E) These values for the comparing dexamethasone treated groups are 6 84, 10 95, 10 34, 4 12 and 4 93 mg /ml (F to J)

Fig. 2


CONTROL
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0.08
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0 04
0 02
0 00
−0 04
500
550
600
650
Wavelengths, nm

Table 5.
Effect of antimalarial treatment on liver mitochondrial cytochrome contents

Age	Treatment	Cytochrome		
		aa_3	b	$c+c_1$
2 week	Control (12)	159 93 ± 9 35	234 44 ± 7 30	236 00 ± 10 47
	Dex (11)	118 80 ± 4 38 [c]	282 65 ± 6 88 [c]	311 88 ± 7 60 [c]
3 week	Control (13)	167 26 ± 6 17	243 23 ± 9 12	268 17 ± 9 76
	Dex (14)	113 66 ± 6 30 [c]	166 87 ± 5 24 [c]	219 48 ± 10 38 [b]
4 week	Control (16)	190 08 ± 4 08	291 09 ± 5 69	345 83 ± 4 16
	Dex (9)	165 19 ± 7 64 [b]	204 90 ± 13 96 [c]	292 73 ± 18 57 [a]
5 week	Control (13)	196 72 ± 7 50	300 31 ± 8 94	334 22 ± 12 28
	Dex (9)	187 89 ± 6 29	216 34 ± 11 30 [c]	307 31 ± 8 93
Adult	Control (14)	207 76 ± 7 78	302 87 ± 7 82	336 85 ± 13 43
	Dex (14)	180 86 ± 11 11	274 67 ± 5 55 [b]	277 11 ± 18 94 [a]

The experimental conditions are as described in the text Cytochrome contents are given as pmoles / mg protein The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis
[a] $p< 0\ 02$, [b] $p<0\ 01$, [c] $p<0\ 001$ as compared with the corresponding control

Table 6
Effect of Dexamethasone treatment on liver mitochondrial ATPase

Age	Treatment	Basal (-,-)	+Mg	+DNP	+Mg.+DNP (+,+)
2 week	Control (8)	4 89 ± 0 33	13 81 ± 0 55	14 91 ± 0 98	16 47 ± 0 80
	Dex (4)	1 91 ± 0 09 [d]	5 53 ± 0 32 [d]	6 30 ± 0 18 [d]	7 85 ± 0 40 [d]
3 week	Control (8)	4 64 ± 0 37	16 29 ± 0 74	19 14 ± 0 50	20 58 ± 0 64
	Dex (8)	3 38 ± 0 26 [b]	13 16 ± 0 56 [d]	11 94 ± 0 30 [d]	15 26 ± 0 77 [d]
4 week	Control (8)	6 53 ± 0 31	19 96 ± 0 47	18 10 ± 0 52	27 99 ± 1 16
	Dex (8)	5 28 ± 0 36 [b]	10 36 ± 0 40 [d]	9 66 ± 0 82 [d]	17 79 ± 0 82 [d]
5 week	Control (14)	10 63 ± 0 46	19 28 ± 1 27	18 43 ± 1 40	32 52 ± 1 12
	Dex (16)	5 76 ± 0 36 [d]	14.35 ± 0 69 [c]	15 03 ± 0 76 [a]	22 80 ± 0 65 [d]
Adult	Control (8)	14 27 ± 0 41	29 64 ± 0 60	18 22 ± 0 97	38 83 ± 1 91
	Dex (8)	5 79 ± 0 33 [d]	11 69 ± 0 54 [d]	9 99 ± 0 28 [d]	15 15 ± 0 52 [d]

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

The observed decrease in respiration rates was paralleled by decrease in glutamate dehydrogenase, malate dehydrogenase and succinate cytochrome c reductase activities (Fig 1) Nevertheless the respective dehydrogenase activities were sufficiently high to support the corresponding observed respiration rates(Tables 1-4)

We then checked if dexamethasone treatment also affected the intramitochondrial cytochromes content The representative difference spectra of the cytochromes are shown in Fig 2 and the contents of individual cytochromes as affected by dexamethasone treatment are given in Table 5 Thus following dexamethasone treatment the cytochrome aa_3 content decreased up to the age of 4 weeks with maximum decrease(32% decrease) being seen in the 3 week group The content of cytochrome b increased in 2 week old animals, but was significantly low in other age groups and an almost similar trend was seen for cytochrome $c+c_1$ content (Table 5)

The basal. Mg^{2+} stimulated. DNP stimulated and DNP + Mg^{2+} stimulated ATPase activities were generally always low after dexamethasone treatment (Table 6)

Discussion

The present studies were undertaken to evaluate the effect of dexamethasone treatment on mitochondrial energy transduction functions The rationale for these studies was that dexamethasone is used in all age groups prenatal, very young and adults as medication for treatment of various ailments as cited above in the 'Introduction' section (4-7) Use of dexamethasone even during pregnancy for continuous 6 week for alleviating respiratory distress syndrome in fetus has been recommended (6,9,10,19,20) However, it is possible that such a chronic exposure to a potent glucocorticoid such as dexamethasone can affect

development and/or metabolic function in tissues other than the lungs. As is well recognized, the process of development is energy dependent(12) Therefore we decided to evaluate the effects of chronic dexamethasone treatment on liver mitochondrial function in rats belonging to different age groups

From the data presented it is clear that the dexamethasone treatment adversely affected the oxidation of various substrates and the effects were age specific Thus with glutamate as the substrate the age groups affected were 2 week and 3 week animals and the adults For pyruvate and malate, the adverse effects were seen for 3 week group and the adults, whereas succinate oxidation in all the age groups was severely impaired For ascorbate + TMPD the adverse effects were seen in 4 week old animals and in the adults Interestingly dexamethasone treatment stimulated respiration with pyruvate and malate in 4 week group, similar stimulatory effect was seen for TMPD mediated ascorbate oxidation in 5 week old animals (Tables 1- 4)

The other interesting aspect of dexamethasone treatment was uncoupling of oxidative phosphorylation i e lowering of ADP/O ratio which was seen predominantly in 3 week and 5 week animals and in the adults In the case of ascorbate and TMPD, even the 2 week animals were affected (Tables 1- 4)

When the data in Table 1-4 were analyzed to find out if the dexamethasone effects with respect to uncoupling of ADP/O ratio were site specific (data not shown, but can be easily computed from the Tables 1-4), the effects seemed to be generally related to the uncoupling of the 2nd and the 3rd sites of phosphorylation although the 1st site was also susceptible in the 3 week and the adult groups It also became apparent that in the 2 week animals the 2nd site

was not operative even in the controls which is consistent with the observation by us and other researchers (21), In the 2 week group dexamethasone treatment resulted in about 60% uncoupling of the 3rd site In 3 week group dexamethasone treatment caused 100% uncoupling of the 2nd site. the other two sites were affected by 40 to 68% In the 4 week group, only the 2nd site was marginally affected (26% decrease) In the 5 week group the major effect was on the 2nd and the 3rd sites (55-60% decrease) with marginal effects on the 1st site In the adults. the major effect was on the 1st and the 2nd sites (50-55% uncoupling) while the 3rd site was not affected It is thus apparent that the 3 week old animals were highly susceptible to dexamethasone treatment followed by 5 week animals Also the 1st site was susceptible to dexamethasone treatment in 3 week animals and in adults

From the results it was also apparent that the 5 week group was also severely affected by dexamethasone treatment Especially one begins to see the uncoupling effect on the 1st site, the magnitude of the effect is amplified in the adults The overall effects is a severe impairment of ATP synthesis rates ie ADP phosphorylation rates (Tables 1-4) One can anticipate that the results of energy restriction can severely affect the process of development It is interesting to note that both 3 week and 5 week periods are crucial in development At 3 week the weaning rats switch to solid diet whereas the process of neuronal proliferation, arborization and connectivity occurs from the 3 week onwards up to the 5 week (11) It is possible that dexamethasone interferes with these processes which ultimately affects energy metabolism in the liver mitochondria

Commensurate with the impaired respiration rates, the glutamate dehydrogenase activity also decreased significantly A similar trend was seen for malate dehydrogenase and succinate DCIP reductase activity (Fig 1).

The contents of cytochromes were also affected in an age specific manner for eg the content of cytochrome aa_3 decreased significantly up to the age of 4 week. Cytochrome b and $c+c_1$ content was higher in 2 week animals but decreased in general in all other age groups The decrease in cytochrome contents would also add to the impairment of the process of electron transport (eg see Tables 1-4) Besides, the potential of Fo F1 ATPase had decreased in practically all the age groups In conclusion, our results show that the treatment with dexamethasone can severely impair liver mitochondrial energy metabolism and that the effects are age specific

Glucocorticoids are known to exert both genomic and nongenomic membrane effects (22) Thus the observed effects could relate to direct effects of dexamethasone on mitochondrial metabolism or to its genomic effects

Several investigators have examined the in vitro effects of various steroids on oxidative energy metabolism in isolated mitochondria (23-26) These in vitro experiments in general demonstrated that incubation with steroids resulted in an inhibition of respiration as well as uncoupling of mitochondria (23-26) Morin et al (27) demonstrated that under in vitro conditions 1μM dexamethasone inhibited the respiratory control ratio and state 3 oxygen consumption by 10-15% Martens et al (25) reported that under in vitro condition dexamethasone inhibited the pyruvate + malate dependent respiration However only few studies have evaluated the in vivo effects of steroid hormone treatment on mitochondrial energy metabolism

Previously we have shown that acute and chronic treatments with corticosterone differentially affected mitochondrial respiration and coupling efficiency of rat liver and brain mitochondria (21,28)

The first in vivo studies using dexamethasone were carried out by Wakat and Haynes (29) These authors injected the rats with 15μmoles dexamethasone /100 g body weight (Ca 6mg dexamethasone / 100 g body weight) and found that state 3 and uncoupler-stimulated respiration with β-hydroxybutyrate and succinate increased maximally by 3 hours Subsequently, similar observations have been reported by Allan et al (30)

The results of our present studies do not agree with those of Wakat and Haynes (29) cited above This could be attributed to several reasons Firstly, the dose of dexamethasone which Wakat and Haynes (29) used was about 30 times higher than the dose which we have employed Secondly, these authors examined the early effects of acute dexamethasone treatment It is possible that the observed stimulation of respiration in their studies may be related to known uncoupling effect of dexamethasone (29-30) and\or may relate to membrane effects (22)

Our studies, on the other hand, have shown that dexamethasone treatment resulted, in general, in inhibition of respiration and uncoupling of mitochondria In our studies dexamethasone was injected subcutaneously to ensure slow release of the hormone If one makes a simple assumption that dexamethasone gets evenly distributed in the body over a long time span due to subcutaneous route of administration, the effective concentration reached at the mitochondrial level could be in submicromolar or in micromolar range Morin et al (27) have shown that under in vivo studies micromolar

concentration of dexamethasone the effects on respiration are only marginal, no data on coupling efficiency were provided

Dexamethasone is a potent glucocorticoid and binds to the glucocorticoid receptors (GR) with high affinity in the liver (2) It may hence be anticipated that this potent glucocorticoid may mimic the actions of the natural glucocorticoid i e corticosterone As is evident from our data, paradoxically dexamethasone treatment resulted in adverse effects on energy metabolism Muller and Renkawitz (2) proposed that glucocorticoid can act as positive as well as negative regulator of gene expression It is possible that in our present studies the effects we observe may be related to negative regulatory aspects after dexamethasone binds to the GR Alternately, dexamethasone may prevent the binding of the natural glucocorticoid i e for example corticosterone to the GR and thereby bring about the negative regulatory effect However these possibilities need to be verified experimentally

Recently, it has been shown that the mitochondria themselves have dexamethasone binding site on cytochrome oxidase I gene (31-33) Crucial peptides of cytochrome oxidase, cytochrome b and Fo F_1 ATPase are known to be coded by mitochondrial DNA (34) One therefore wonders if dexamethasone also exercises negative regulatory control on the mitochondrial gene expression for eg we are already seeing the decreased content of the cytochrome aa3 and cytochrome b and decreased Fo F_1 ATPase activity

In conclusion, our studies have shown that chronic exposure to synthetic glucocorticoid such as dexamethasone can result in an impairment of oxidative energy metabolism in an age-dependent manner The results also suggest that the well documented side-effects

following prolonged exposure to corticosteroids may result with impaired cellular functions especially the energy metabolism

Thus our results caution against indiscriminate use of dexamethasone and its adverse effects in energy metabolism in critical age groups which can adversely affects the process of development

Summary

Effects of chronic dexamethasone treatment on oxidative energy metabolism in liver mitochondria from rats belonging to different developmental age groups were examined Dexamethasone treatment adversely affected the state 3 respiration rates in 2 and 3 week groups and in the adults with glutamate as the substrates, whereas for pyruvate + malate, the adverse effects were seen for the 3 week and the adult groups Oxidation of succinate was severely impaired in all the age groups For ascorbate + TMPD as the substrate, elevated respiration was noted for the 4 week group and the adults Dexamethasone treatment also resulted in site specific uncoupling with the the effect being seen predominately in the 3 and 5 week animals The activity of dehydrogenases decreased in a manner comparable to the respiration rates The ATPase activity also decreased significantly The mitochondrial cytochromes especially aa_3 and b decreased in an age dependent manner The results thus emphasize the adverse effects of dexamethasone treatment on mitochondrial energy metabolism especially in critical age groups

References

1 Milgrom, E , (1990) Steroid hormones. In Hormones from molecules to disease Baulieu, E.E , Kelly, P A (Eds), Hermann Publishers, and Hall , New York and London, pp 387-438

2 Muller, M , Renkawitz, R , (1991) The glucocorticoid receptor Biochim Biophys Acta 1088, 171-182

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 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 Experimental Brain Res 61, 117-123

5 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

6 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 59, 266-274

7 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

8. Brocklehurst, P , Gates, S., McKenzie-McHarg, K., Alfrevic, Z , Chamberlain, G , (1998) Are we prescribing multiple courses of antenatal corticosteroids? A survey of practice in the UK Br J Obstet Gynaecol 170, 206-214

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

10 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 282, R55-R63

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

12 O'Neill, J (1974) Carbohydrate metabolism in the developing nervous system In . Himwich, W (Eds), Biochemistry of the developing brain, Vol II, Marcel Dekker, New York

13 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

14 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

15 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

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

17. Fiske, C.H , Subba Row, Y , (1925) Colorimetric determination of phosphorus J Biol Chem 66, 375-400

18 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

19 Yeh, T,E , Torre, J A , Rastogi, A , Anyebuno, M , Pildes, R S , (1990) Early postnatal dexamethasone therapy in premature infants with severe respiratory distress syndrome A double blind, controlled study J Pediatr , 117, 273-282

20 Ferguson, S A and Holson, R (1999) Neonatal dexamethasone on day 7 causes mild hyperactivity and cerebellar stunting Neurotoxicol Teratol 21, 71-76

21 Jani, M S , Telang, S D , Katyare, S S , (1991) Effect of corticosterone treatment on energy metabolism in rat liver mitochondria J Steroid Biochem Molec Biol 38, 587-591

22 Duval, D , Durant, S , Homo-Delarchi, F , (1983) Non genomic effects of steroids Interactions of steroid molecules with membrane structures and functions Biochimica et Biophysica Acta 737, 409-442

23 Podvigina, T T , (1980) Corticosteroid hormone effect on oxygen consumption of rat brain and hippocampus mitochondria and homogenates Urk Biokhim Zh 52, 36-39

24 Leshchenko,(1986) Effect of corticosterone on mitochondrial respiration of slices of rat liver Phorbl Endokrinol 32(2), 67-69

25 Martens, M E , Peterson, P L , Lee, C P , (1991) In vitro effects of glucocorticoid on mitochondrial energy metabolism Biochimica et Biophysica Acta 1058, 152-160

26 McIntosh, M K , Pan, J,S , Berdanier,C D , (1993) In vitro studies on the effects of dehydroepiandrosteinedione and corticosterone on hepatic steroid receptor binding and mitochondrial respiration Comp Biochem Physiol 104, 147-153

27 Morin, C , Zini, R , Simon, N , Charbonnier, P , Tillement, J P , Louet, H.L , (2000) Low glucocorticoid concentrations decrease oxidative phosphorylation of isolated rat brain mitochondria an additional effect of dexamethasone Fundam Clinic Pharmacol 14, 493-500

28 Sureshkumar, M B , (1994) Effect of corticosterone treatment on energy metabolism in rat brain mitochondria M Sc Dissertation, M S University of Baroda, India

29 Wakat, D K , Haynes, Jr R C , (1977) Glucocorticoid –stimulated utilization of substrates in hepatic mitochondria Arch Biochem Biopyhs 184, 561-571

30 Allan, E H , Chisholm, A B , Titheradge, M A , (1983) The stimulation of hepatic oxidative phosphorylation following dexamethasone treatment of rats Biochim Biophys Acta 725, 71-76

31 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

32 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

33 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

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