Chapter 3

Effect of Dexamethasone Treatment on Metabolic Activities in Rat Liver During Development.

Introduction

The effects of dexamethasone treatment on macromolecules content, cell size and metabolic activity of the postnatal developmental brain as influenced by dexamethasone treatments are detailed in the previous Chapter of the thesis (Chapter 2)

Since liver is a major metabolic tissue and also a target tissue for glucocorticoid action (1),. parallel studies were carried out to examine effects of chronic dexamethasone treatment on liver metabolism and function The results of the investigations are summarized in the present Chapter

Materials and Methods

The experimental design and plan of work as essentially the same as described in Chapter 2 of the thesis

Details of chemicals used are also as described (Chapter 2)

Assay of BChE

The assay procedure as described by Ellman et al (2) and Swegert et al (3) Briefly, the assay medium (total volume 1ml) contained, 50 mM Tris-HCl buffer, pH 8 0, 0 32M DTNB, pH 7 0 and the postnuclear fraction (150-250 µg protein) was used as the source of the enzyme. The reaction was started by adding 5mM BCTI and the increase in absorbance at 412nm was measured at 37 ^{0}C at 5 sec intervals The enzyme activitv is expressed as µmoles of BCTI hydrolyzed/min/g tissue The millimolar extinction coefficient for chromophore 13 6 lit^{-1} cm^{-1} is used for calculation

Butyrylcholinesterase (BChE) rather than acetylcholinesterase (AChE) activity was monitored with butyryl thiocholine iodide (BCTI) as the substrate are from Sigma

Results

The effects of dexamethasone treatment on body weight are given in Fig 1 of the previous Chapter 2 of the thesis Briefly, the weight gain decreased by 8- 28 % in all the age groups after dexamethasone treatment The data of liver weight and relative liver weight are given in Table 1, from which it can be seen that dexamethasone treatment resulted in about 26% liver weight gain in 2 week group However due to decrease in the body weight (Fig 1 of previous Chapter) the relative liver weight seems to be (11-66% increase) in the 2, 3 and 4 week groups after dexamethasone treatment By contrast, in the 5 week and adult animals the liver weight decreased significantly (15-24 % decrease)

The protein, RNA and DNA contents in the liver are shown in Fig 1-3 As can be seen, the protein (mg/g tissue) in the control groups was highest in the 2 week animals, decreased by 3rd week, increased transiently by 4th week and finally reached a steady state value Dexamethasone treatment caused about 24- 49% decrease in protein content during 2- 4 week period, the 5 week group and adults were unaffected by dexamethasone treatment (Fig 1)

The RNA content was the highest in the 2 week control group, declined by about 35% by the 3rd week and remained at this level to 5 week of age, in adults a further decrease was seen (Fig 2) Dexamethasone treatment resulted in 11- 44% decrease in the RNA content during the 2-4 week period, adults showed about 29% increase (Fig 2)

Table 1 The effect of dexamethasone treatment on liver weight and relative liver weight

Group	Treatment	Liver weight (g)	Relative Liver weight. (g/g body weight)
2 week	Control (22)	0 52 ± 0 008	2 43 ± 0 042
	Dex (16)	0 66 ± 0 017 [b]	4.05 ± 0 077 [b]
3 week	Control (18)	1 04 ± 0 015	3 59 ± 0 091
	Dex (22)	1 08 ± 0 019	4 00 ± 0 070 [a]
4 week	Control (16)	1 80 ± 0 056	3 96 ± 0 076
	Dex (22)	1 84 ± 0 044	5 13 ± 0 098 [b]
5 week	Control (22)	2 91 ± 0 153	4 38 ± 0 165
	Dex (28)	2 21 ± 0 084 [b]	4.55 ± 0 137
Adult	Control (24)	8 12 ± 0 184	3 07 ± 0 075
	Dex (16)	6 90 ± 0 279 [b]	2 88 ± 0 089

The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis
[a]$p < 0\ 002$ and [b]$p < 0\ 001$ compared with the corresponding control

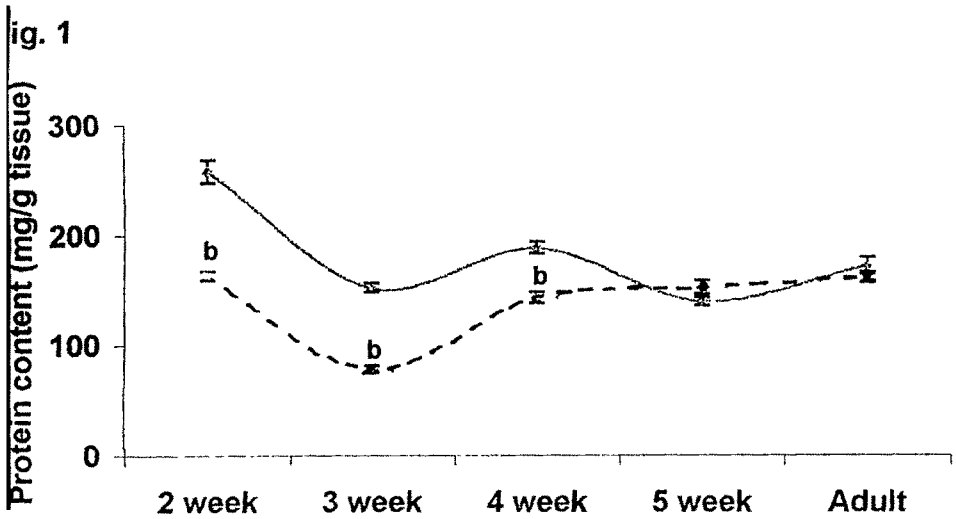
Fig. 1 Effects of dexamethasone treatment on tissue protein content in liver of rats belonging to different age groups The straight line represents the control group whereas the dashed line represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

Fig. 2 Effects of dexamethasone treatment on tissue RNA content in liver of rats belonging to different age groups The straight line represents the control group whereas the dashed line represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

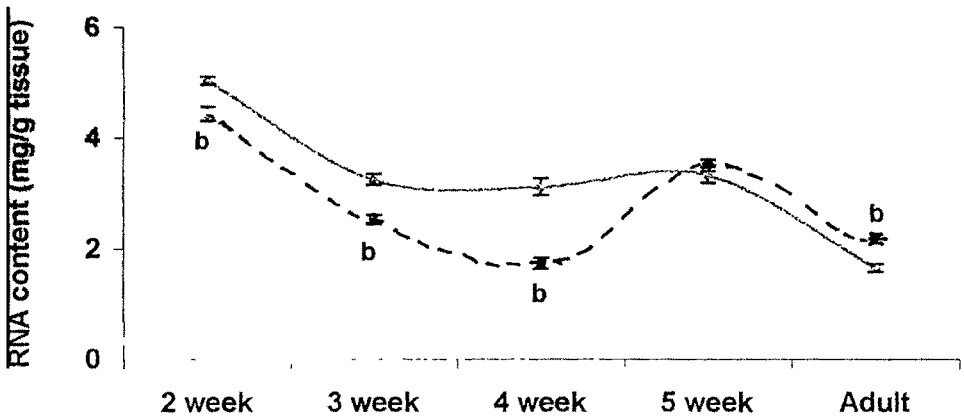
Fig. 3 Effects of dexamethasone treatment on tissue DNA content in liver of rats belonging to different age groups The straight line represents the control group whereas the dashed line represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

[a] p. 0 005 and [b] p.0 001 as compared to controls Fig. 1-3 and Fig 7-8

ig. 1



ig. 2



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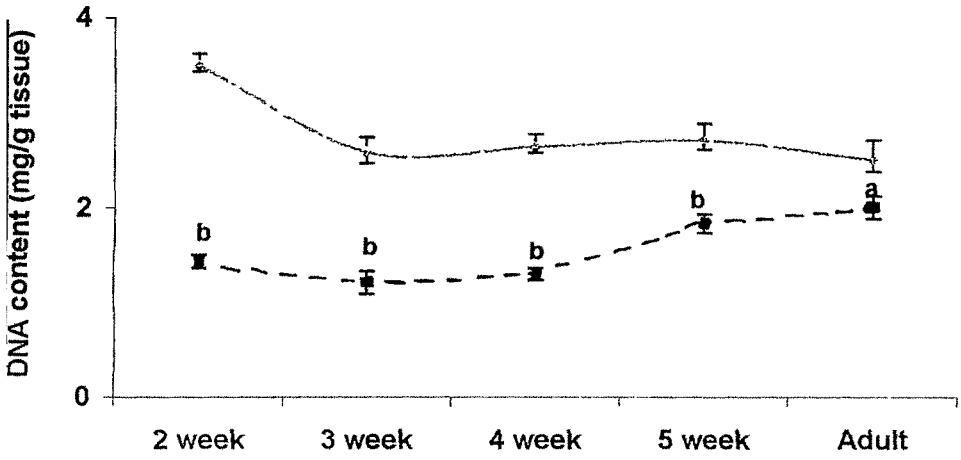


control ; Dexamethasone treated.

The DNA content was also highest in 2 week old control animals, decreased slightly by the 3rd week to reach an almost steady state value up to 5 weeks In the adults there was a further small decrease (Fig 3) As can be seen from Fig 3 after dexamethasone treatment, the DNA content decreased in all the age groups by 20- 60% except in the 3 week animals

The total tissue content of the macromolecules were calculated by multiplying the liver weight with the individual specific content of each macromolecules These values for protein, RNA and DNA are shown in Fig 4-6 Thus in the controls the total protein, RNA and DNA content showed developmental increasing pattern reaching the highest value in the adults Dexamethasone treatment caused reduction in the protein content by 24- 49 % in 3, 5 week groups and in the adults whereas in the 4 week animals showed slight increase in protein content (Fig 4) The total RNA content decreased by 19- 51% during 3 to 5 week period, whereas 2 week animal showed 26% increase after dexamethasone treatment (Fig 5) The overall decreasing trend (29- 68 % decrease) was observed in total DNA content in all the age groups after dexamethasone treatment (Fig 6) The significant decrease was about in individual age groups, it is clear that dexamethasone treatment resulted in decreased cell number

The data on protein/ DNA and RNA / DNA ratios which served as an index of cell size and cellular metabolic activity (4,5) are shown in Fig 7- 8

The protein/ DNA ratio in the controls was fairly constant except in the 3 and 5 week groups where the values were somewhat low (Fig,7) Dexamethasone treatment resulted in generalized increase in the protein/DNA ratio except in the case of the 3 week animals where the ratio decreased

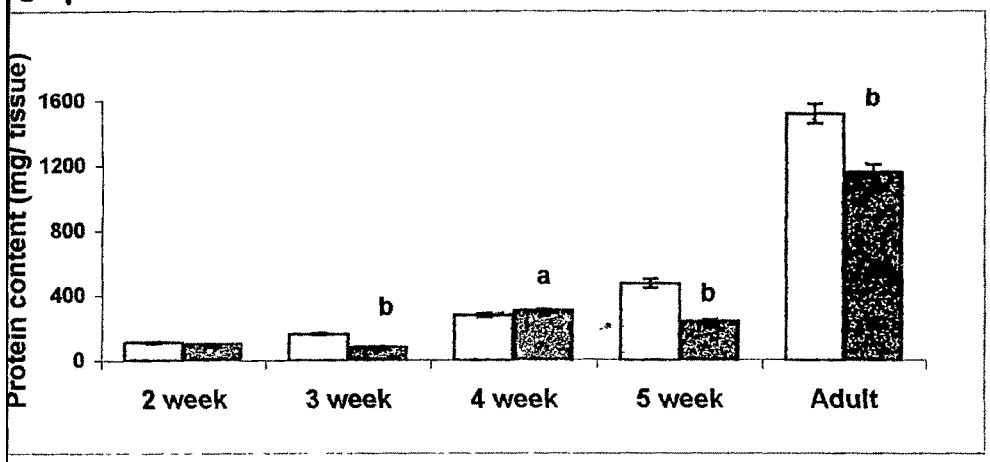
Fig. 4 Effects of dexamethasone treatment on total DNA content in liver of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

Fig. 5 Effects of dexamethasone treatment on protein/ DNA ratio in liver of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

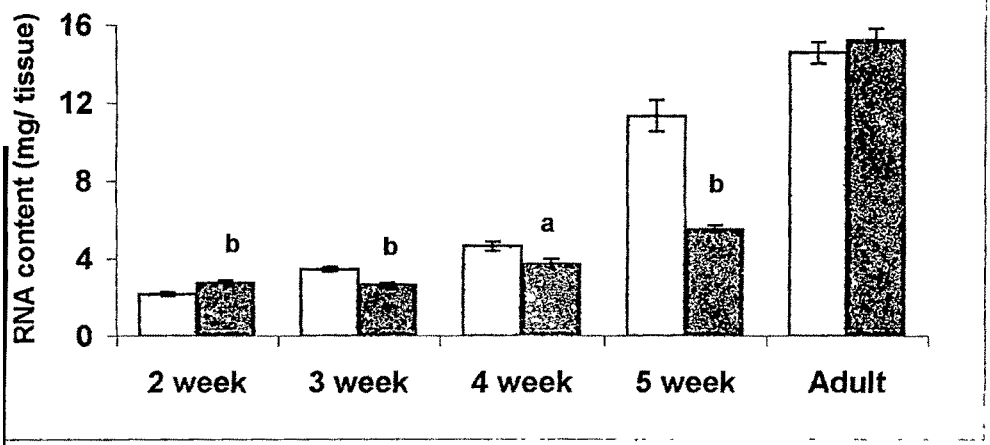
Fig. 6 Effects of dexamethasone treatment on total RNA/DNA ratio in liver of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

[a] p, 0.05 and [b] p,0 001 as compared to controls Fig 4-6

ig. 4


Protein content (mg/ tissue)
1600
1200
800
400
0
b
a
b
b
2 week
3 week
4 week
5 week
Adult

ig. 5


RNA content (mg/ tissue)
16
12
8
4
0
b
b
a
b
2 week
3 week
4 week
5 week
Adult

ig. 6

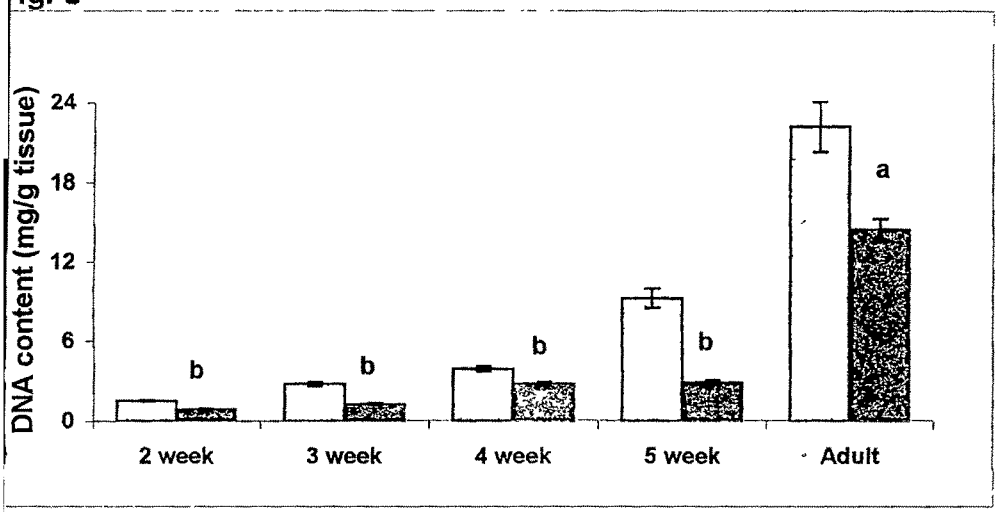

DNA content (mg/g tissue)
24
18
12
6
0
b
b
b
b
a
2 week
3 week
4 week
5 week
Adult

Fig. 7 Effects of dexamethasone treatment on total protein content in liver of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

Fig. 8 Effects of dexamethasone treatment on total RNA content in liver of rats belonging to different age groups The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated The error bar gives the S E M of 20 independent observation

[a] p, 0.005 and [b] p,0 001 as compared to controls Fig 1-3 and Fig. 7-8

Fig. 7

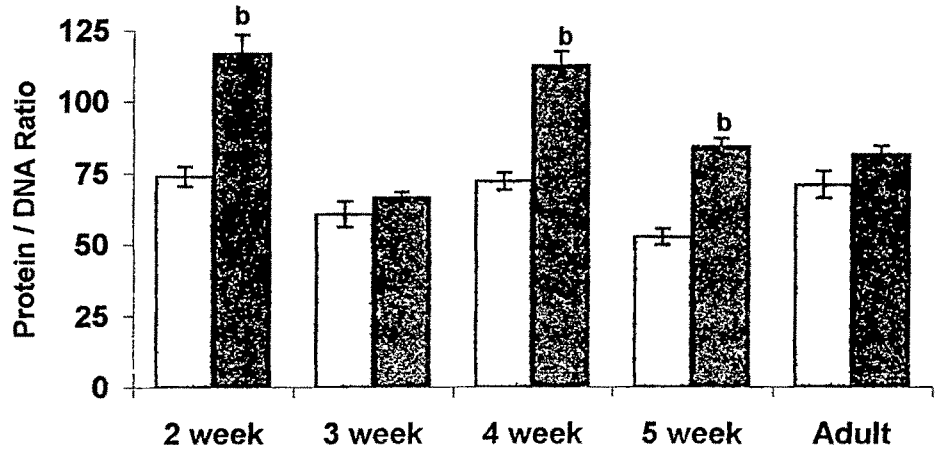
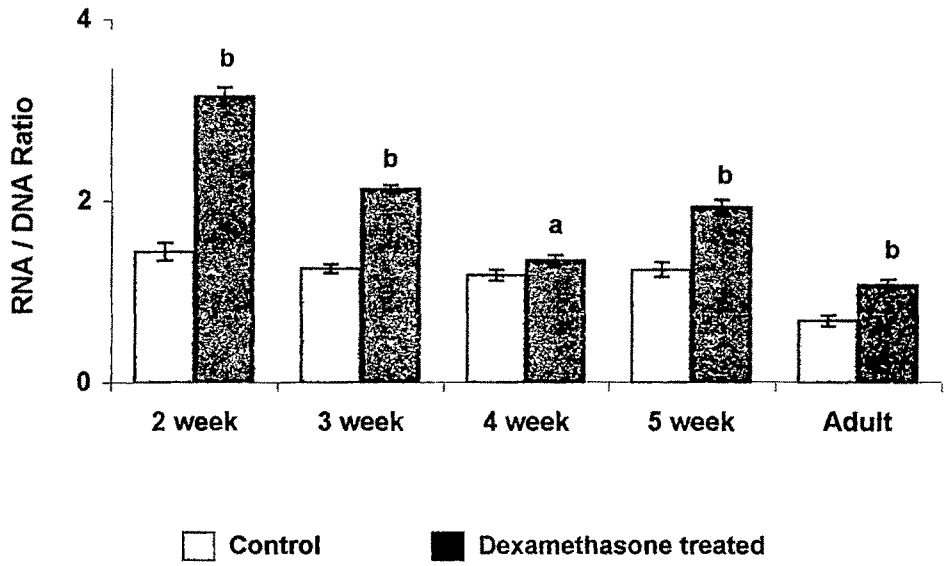

Protein / DNA Ratio
125
100
75
50
25
0
b
b
b
2 week
3 week
4 week
5 week
Adult

Fig. 8


RNA / DNA Ratio
4
2
0
b
b
a
b
b
2 week
3 week
4 week
5 week
Adult
Control
Dexamethasone treated

The RNA / DNA ratio (Fig 8) in the cells decreased with development Dexamethasone treatment caused a significant (118%) increase in the 2 week animals and 40% in crease in 5 week animals In other group the changes were marginal The exception was due to no change in DNA content in 3 week animals compared to the other age groups

As can be seen in Fig 9, the BChE activity in control group was lowest in 2 week, doubled 3 week animals, and was increased in the adults (5 fold increase) Dexamethasone treatment caused early stimulation of BChE activity but the changes are of marginal nature, in the adults effect was opposite and about 96% activity was lost after dexamethasone treatment

In the cells only α G3PDH activity (Fig 10) was non-detectable in 2 week group but increased progressively thereafter up to 5th week. the adults showed significant decline Dexamethasone treatment caused about 2 fold increase in α G3PDH activity (Fig 11) in 4 and 5 week animals and about 7 fold increase in the adults The GS activity, in control groups showed a peak at 4 week period dexamethasone treatment resulted in significant stimulation of GS activity (100 – 450 % increase)without affecting the developmental profiles (Fig 11)

Discussion

From the data presented, it is clear that the effects of dexamethasone treatment on liver metabolism were very different from those noted earlier for brain Thus dexamethasone treatment caused initial increase in the liver weight but have adverse effects on latter stages i e 5 week and adults. The adverse effects of dexamethasone in early age groups are also evident from decrease content of protein, RNA and DNA (Fig 1,2 and 3) The

Fig. 9 Effects of dexamethasone treatment on tissue activity of acetylcholinesterase in liver of rats belonging to different age groups The activity is given as moles/ min/ gm tissue The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

Fig. 10 Effects of dexamethasone treatment on tissue activity of glutamine synthase in liver of rats belonging to different age groups The activity is given as moles/gm tissue The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

Fig. 11 Effects of dexamethasone treatment on tissue activity of GPDH in liver of rats belonging to different age groups The activity is given as moles/ gm tissue The dotted bar represents the control group whereas the filled (black) bar represents the dexamethasone treated groups The error bar gives the S E M of 20 independent observation

a, $p < 0.001$ as compared to initial body weight of controls

Fig. 9

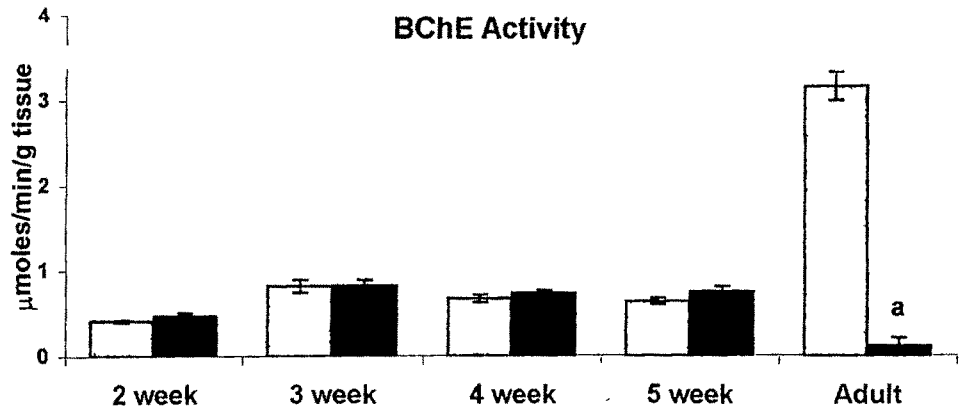


Fig. 10

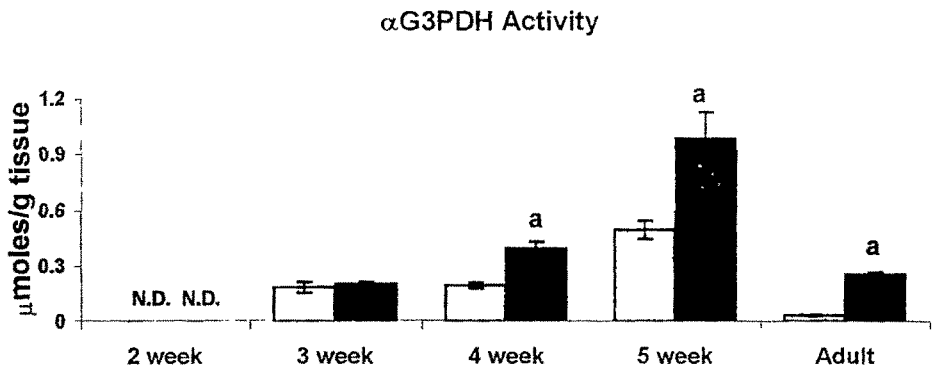
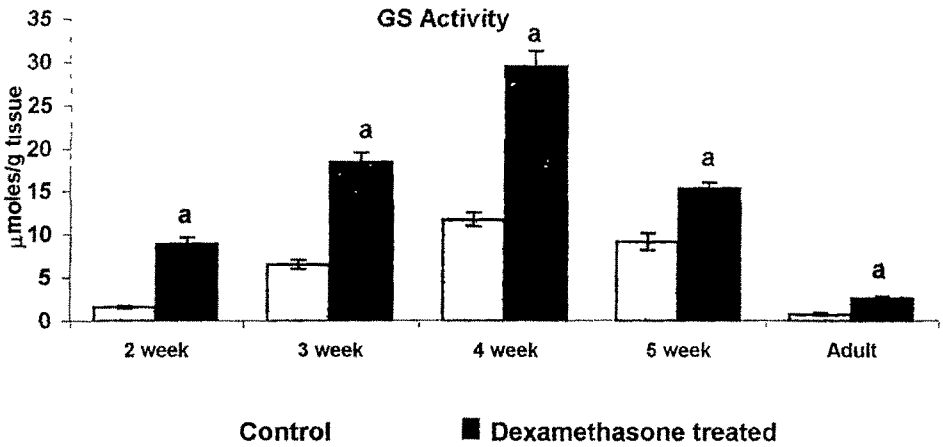


Fig.11



decrease DNA content would mean that as in case of brain the cell number had decreased significantly However the extent of decrease in the content of protein. DNA and RNA were differential and age dependent This was reflected in terms of a generalized increase in protein / DNA ratio in all the age groups except in the 3 week animals The increased ratio suggests that the cell size had increased in response to dexamethasone treatment However the 3 week group seem to be highly sensitive and displayed opposite pattern The metabolic activity as assessed suggest in terms of RNA / DNA ratio increased substantially in 2 week animals followed by 5 week and adult group, 3 week and 4 week group are insensitive Thus the overall picture one gets is that the early age groups are more prone to dexamethasone treatment whereas the 5 week and adults are less sensitive

Since dexamethasone treatment significantly enhanced the AChE activity in the brain, it was of interest to find out the effect of this glucocorticoid on BChE in the liver BChE is synthesized in the liver and is secreted in to serum (6) The serum BChE is believe to be involve in the catabolism of opiod narcotics (7) Paradoxically it was found that up to the age of 5 week dexamethasone treatment had nil or only marginal effect on the BChE activity whereas in the adult there was a drastic 95% reduction Although the plasma levels were not monitored, it is reasonable to assume that such a drastic decrease in liver enzyme would cause significant lowering the plasma BChE levels This in turn could compromised the ability to catabolise opiods type of xenobiotics The a-G3PDH is believe to be involve in carbohydrate and phospholipid metabolism (8) The enzyme in situ brings about NAD^+ (H) dependent inter conversion of dihydroxy acetone phosphate and glycerol 3 phosphate The early induction of α-G3PDH activity by dexamethasone treatment would suggest that this may result in a switch over of carbohydrate/lipid

metabolism due to dexamethasone treatment Indeed the results that followed in Chapters 6 and 7 shown that the mitochondrial phospholipid profiles in brain and liver were significantly altered by dexamethasone treatment

From the data presented it is also clear that dexamethasone treatment resulted in decrease protein content of the tissue (Fig 1) The catabolic effect of glucocorticoids of protein metabolism are well recognized The observed stimulation of glutamine synthetase activity may thus be considered as a response to increase protein catabolism

In conclusion the results presented in this Chapter shown that dexamethasone treatment in different age groups can adversely affect the cellular metabolism even in a tissue such as liver Therefore caution has to be exercised in indiscriminate use of dexamethasone

Summary

Effects of dexamethasone on metabolic activities of liver in rats belonging to different age groups were examined Dexamethasone treatment in 5 week and adult groups decreased the liver weight Dexamethasone treatment also resulted in general decreased in the in DNA content in all the age groups while protein and RNA content decreased up to 4^{th} week of age The protein/DNA ratios were generally high signifying increased cell size. The RNA/DNA ratios also follow similar pattern However, the 3week animals showed effects in opposite direction Dexamethasone treatment caused significant stimulation of α-G3PDH activity from the 4^{th} week onwards The GS activity was stimulated in all the age groups to a variable extent The results suggest that treatment with dexamethasone during postnatal developmental period adversely affected the metabolic activity of the liver in an age-dependent manner.

References

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