SYNOPSIS

The adrenal glucocorticoids are important for adaptations to stress Glucocorticoids also have anti-inflammatory property Dexamethasone (Dex), a synthetic glucocorticoid which is one of the most potent anti-inflammatory agent has been used for therapeutic and diagnostic purposes in practically all age groups The use of Dex is recommended by the American National Institute of Health The pharmacological applications of synthetic steroids include the treatment of several diseases eg skin diseases, allergic reactions, eye diseases, bronchial asthma, rheumatoid arthritis and meningitis in children etc A recent survey of British Obstetric Departments showed that 98% are prescribing repeated courses of corticosteroids

The synthetic glucocorticoids are believed to have lesser or no side-effects or toxicity However the pharmacological doses can give rise to adverse side-effect with the incidence being as high as in 50% of the cases

It has been reported that long-term use of adrenal steroids in children shows growth retardation and exerts multitude of effects on nervous systems The development of human brain continues postnatally upto the age of two years Exposure of steroids in this period or during prenatal development of fetus can have adverse effect on the ongoing process of brain development

The "Introduction" **Chapter 1** of the thesis gives a brief review on processes of brain development A detailed literature survey on glucocorticoid history, structure, biosynthesis and its regulation, mechanism of action, metabolism as well as physiological and pharmacological effects are also described in the "Introduction" **Chapter 1** of the thesis An account of the therapeutic indications for use of synthetic steroid, their toxicity

as well as side-effects after prolonged or excessive use are also included Previous studies have reported that the glucocorticoids exert a negative effect on brain growth and cell proliferation and energy metabolism The detailed literature survey on effects of corticosteroids on brain development, cellular metabolism and energy transduction processes are also included in the "Introduction" **Chapter 1.** A brief account of mitochondrial energy conservation mechanisms and membrane structure-function relationships is also included

In the studies reported in the present thesis, experiments were carried out to examine the effects of Dex treatment on biochemical parameters in male albino rats of different age groups eg 2 week to adult

The effects of Dex treatment on brain function and development were monitored in terms of cell number, cell proliferation and metabolic activity by measuring DNA, RNA and protein content of the tissue The ratios of RNA/DNA, protein/DNA were taken as indexes of cell proliferation, size and metabolic activity These studies are included in **Chapter 2** of the thesis

Dex treatment resulted in significant growth retardation during 2 week and 3 week period, the body weight decreased (8-28%) The brain weight decreased by 10% in 3 week animals The total DNA content of brain decreased in all age groups with maximum decrease (41%) observed Total RNA content had no change in general except for 5 week animals where content was higher by 36% The total protein content showed age-specific marginal changes The RNA/DNA ratio increased (24-55%) in all groups except in the 4 week group The protein/DNA ratio increased in 2 week and adult animals The activity

of acetylcholinesterase (AChE)- a neuronal cells marker- showed about 80% to 90% increase during 2 to 4 week following Dex treatment Activity of glycerol-3-phosphate dehydrogenase (G3PDH)- a marker for oligodendroglia- was undetectable up to 2 week period but was stimulated maximally (10 fold) in 4 week age group after Dex treatment The activity of the astrocyte marker, glutamine synthetase (GS) increased in a 2 to 4 week group and in adults by 2 to 2 5 fold

Parallel studies were carried out with liver,- the major metabolic tissue- which served as the internal control for developmental studies These results are summarized in **Chapter 3** of the thesis Thus the liver weight decreased by 8-28% in all the age groups The total DNA content decreased significantly (29-68%) in all the age groups with maximum effect (42% decrease) being seen in the 2 week group The total RNA content showed age-dependent difference in the pattern in the controls Following Dex treatment 2 week group showed 26% increase in total RNA content The total protein content decreased by 24-49% in the 3,5 week group and in adults Thus, the maximum effects were manifested in 2 week to 4 week groups The RNA/DNA ratio in all the groups with maximum increase (118%) being observed in the 2 week group The Protein/DNA ratio resulted in generalized increase in all age group The GS activity increased significantly in all the groups The maximum effect (5 5 fold increase) was seen for 2 week group The butyrylcholinesterase (BChE) activity increased only marginally (11% to 17%) in all the developmental groups The G3PDH activity was undetectable in 2 week and 3 week groups Dex treatment brought about 8 fold increase in the adults

Thus. the results given in the **chapter 2 and Chapter 3**, taken together, showed that Dex treatment caused significant tissue- specific changes in the developmental pattern of the macromolecules and activities of the selected marker enzymes

The initial postnatal period is characterized by neuronal proliferation, connectivity and myelination Because of this it may be anticipated that during this period the energy requirement would be higher Hence, further studies were performed to find out the effect of Dex treatment on mitochondrial oxidative energy metabolism The effects on brain mitochondrial energy metabolism are summarized in **Chapter 4** of the thesis and those on the liver mitochondrial energy metabolism are summarized in **Chapter 5**

Thus, when glutamate was used as a substrate, after Dex treatment in the brain mitochondria the state 3 respiration rate increased significantly from 25% to 245% during 2 to 5 week period However, the adults showed 68% decrease The ADP/O ratio was unchanged but ADP phosphorylation rates showed corresponding changes With pyruvate + malate as the substrates, 4 and 5 week groups showed 103 to 117% increase in the state 3 respiration, adults showed 72% decrease Uncoupling of ATP synthesis was observed in the 3 , 4 and 5 week groups and in adults State 3 respiration with succinate increased by 52% to 105% in 2, 4 and 5 week groups, maximum increase was observed in the 5 week group (105% increase) Adults showed 75% decrease The state 3 respiration rate increased by 65 to 214% in all the developmental groups following Dex treatment GDH activity increased for all the age groups with maximum increase (71%) in 5 week age group, while the adults showed 20% decrease after Dex treatment The malate dehydrogenase (MDH) activity increased by 20-40% in the 2, 3 and 5 week groups, adults showed 14% decrease Cytochrome aa_3 and b contents decreased significantly in

all the groups from 14% to 50%, maximum effect was observed in the 3 week group where the content of both the cytochromes decreased by 32% The cytochrome $c+c_1$ content increased in 2 and 3 week groups marginally (12% and 18%) and decreased in adults (20% decrease) The mitochondrial ATPase activity altered in an age dependent manner in 2 and 5 week groups These results are included in **chapter 4** of the thesis

Parallel studies in liver mitochondria revealed that following Dex treatment the state 3 respiration rates decreased by 48% and 80% in 2 and 3 week groups and in adults with glutamate as the substrate The ADP/O ratio decreased from 50% to 65% in 3 and 5 week groups and in adults, consequently ADP phosphorylation rates decreased by 50% to 92% in all the age groups except for 4 week With pyruvate + malate as the substrates, the state 3 respiration rate showed age specific changes However, the ADP/O ratio decreased (50% to 63% decrease) in all the age groups except for 4 week group When succinate was used as the substrate, the state 3 respiration rate decreased (22% to 56% decrease) Likewise ADP/O ratio also decreased from (15% to 93%) in all age except 2 week The ADP phosphorylation rates decreased by 46% to 96% No changes in state 3 respiration rates were observed for ascorbate + TMPD as the substrates, although the ADP/O ratio decreased by 55% to 68% GDH activity decreased by 36-67% in all the groups with maximum decrease (67%) being observed in the 2 week group MDH activity decreased by 55% to 66% in all the age groups after Dex treatment The SDR activity decreased by 19% to 67% with major decrease being observed for 2 and 3 week groups (about 65% decrease) Contents of cytochromes aa_3, b and $c+c_1$ decreased from 10% to 30% in all the age groups The 2 week old rats showed decrease only in cyt aa_3 content (26% decrease) and increase in cyt b and cyt $c+c_1$ (20%-32% increase) The basal ATPase activity

increased in 3 week group, whereas 2 and 4 week groups resulted decrease in the activity These results are included in **Chapter 5** of the thesis

Taken together the results of **Chapter 4** and **Chapter 5,** suggest that Dex treatment shows tissue-specific age-dependent adverse effects on mitochondrial energy transduction processes

Since the mitochondrial ATPase activity in both the tissues was significantly affected in different age groups following Dex treatment, it was of interest to see if the substrate and temperature kinetics of the enzyme was also altered in the experimental groups

The results on effects of Dex treatment on substrate and temperature kinetics of brain mitochondrial ATPase are included in **Chapter 6** of the thesis The substrate saturation curves for the control group in general (3 , 5 week and adults) showed allosteric pattern for substrate saturation, the curves were sigmoidal Chronic Dex treatment resulted in loss of the allosterisity and the enzyme displayed normal substrate saturation curves The 2 week Dex group did not show sigmoidal curve As compared to the 2 week control, after Dex treatment the Km and Vmax values of component I decreased significantly For the groups which displayed allosteric pattern, Hill plot analysis was performed Analysis of temperature-activity pattern in terms of Arrhenius plots showed that in 3 week group, the energies of activation increased after Dex treatment In 5 week and adults, the pattern became reversed as compared to control group after Dex treatment, ie E_1 increased (81-129% increase) while E_2 decreased (55% and 81% decrease) The phase transition temperature (Tt) decreased by 8-9^{O}C in 2 week group and in adults

Substrate saturation kinetics of liver mitochondrial ATPase showed presence of three components in control group, treatment with Dex conferred allostrisity in the 3 week group In the 2 week group, the Km_2 and Km_3 decreased (46% and 99% decrease) and all the Vmax values ie $Vmax_i$ to $Vmax_3$ decreased from 30 and 56% The component I was lost in adults Temperature kinetic analysis by Arrhenius plots revealed that after Dex treatment, the values of E_1 and E_2 increased or decreased marginally in the 3 week group The 5 week group and adults showed significant decrease in both E_1 and E_2 (30-44% decrease) after Dex treatment The Tt was not changed in the 2 and 3 week group whereas in the 5 week group the Tt increased by 20°C, in the adults there was a marginal decrease in Tt after Dex treatment These results are included in **Chapter 7** of the thesis

The foregoing results (**Chapter 6** and **Chapter 7**) of the thesis suggested that Dex treatment possibly was bringing about changes in membrane lipid milieu Many of the mitochondrial enzymes including ATPase are membrane-bound and require lipid environment for their function Therefore, it was of interest to find out the effect of Dex treatment on lipid profiles and membrane fluidity of the brain and liver mitochondria This aspect was examined in the next series of the experiments The results are included in **Chapter 8** of the thesis

It was observed that the brain mitochondrial total phospholipid (TPL) content decreased in all the age groups, maximum decrease (58%) was noted for adults The cholesterol (CHL) content increased in 3 week and 4 week group (69% and 35% increase), while that in 5 week group and adults showed 31% and 41% decrease This was further reflected in the TPL/CHL molar ratio in all the age groups except for 5 week Significant changes were observed also in the phospholipid (PL) composition after Dex treatment which

indicated that the major phospholipids PC and PE were somewhat low in 3, 4 and 5 week groups Lysophopholipid decreased by 8% to 23% in all the age groups except for the 3 week group while PS and PI increased in all the age groups. However, the mitochondrial membrane fluidity was unchanged in 3 and 4 week groups, in the adults the membrane becomes more fluidized

Chapter 9 includes studies on liver mitochondrial lipid profiles and membrane fluidity after Dex treatment Thus, after Dex treatment, the TPL content increased significantly in 2 week and 5 week groups by 69%, the adults showed a significant decrease in both TPL (66%) and CHL (43%) content Dex treatment resulted in increased PE (14%-32% increase) in 4 and 5 week and adult groups PC decreased (29%) in 4 week group and adults(14%) Age specific changes were observed also for minor phopholipid components The liver mitochondrial membrane was more fluidized in 2 and 5 week animals and the adult rats after Dex treatment

Taken together, the results in **Chapter 8** and **Chapter 9** substantiate the notion that Dex brings about significant age-dependent and tissue-specific alterations in membrane lipid profiles

Corticosterone is the glucocorticoid hormone of the rat Fluorimetric and RIA methods for estimation of serum corticosterone levels have been described However applications for estimation of tissue or subcellular ie mitochondrial corticosterone content has not been detailed Hence, efforts were made to develop a fluorimetric method which can be applicable for quantitative determination of corticosterone in plasma, tissue and mitochondria The difficulty of foggy interphase encountered in Silber Method (Silber

R H et al Clin Chem (1958),4 278-285) was overcome by directly extracting the samples with chloroform rather than with dichloromethane which Silber procedure recommends The procedure for development of fluorophore was treatment with 0 1N NaOH followed by treatment with 30N H_2SO_4

Recovery experiments showed that the efficiency of the procedures was in the range of 90% to 100% These results are included in **Chapter 10** of the thesis

The results thus suggest that the caution must be exercised against excessive use of Dex (as medication) in early developmental period Since adverse effects are seen not only in the brain but also the major metabolic tissue such as liver The results embodied in the present thesis can serve as a useful guidelines to exercise this caution while using synthetic steroids especially in the growing children