SYNOPSIS

Animal growth and development are among the most exciting processes in biology. If they are to proceed correctly, they must be regulated precisely by the neuro-endocrine orchestra. Glucocorticoid hormones play an important role in growth and development as well as maintenance of variety of physiological processes.Besides, these hormones have multitude of effects on several tissues including the brain.

The effects on the nervous system range from the regulation of cellular growth and differentiation to alterations in electrophysiological activity. Glucocorticoids can also influence the mood, motivation, learning and behavioral patterns. Administration of glucocorticoids to young animals can exert negative effect on brain devlopment. These include: long lasting decrease in cerebral and cerebellar weight, decreased DNA content and decreased thymidine incorporation into DNA, indicating a general suppression of cell proliferation. Besides, it also retards development of dendritic spines and reduces ganglioside levels and thus can interfere with synapse formation.

Biochemical actions of glucocorticoids are primarily through the regulation of gene expression. However, there is now increasing evidence that glucocorticoids also have non-genomic effects on different membrane systems. Alterations in neuronal function and behavior had been correlated with specific high affinity binding of corticosterone with synaptic

membranes; high affinity binding sites for corticosterone in mitochondria have also been reported.

Development is an energy requiring process, which involves biosynthesis of variety of biological macromolecules. Mitochondria provide 95% of the cellular energy needs in the form of ATP. Hence any alterations in mitochondrial ATP synthesis could have serious consequences for development. The reported negative effects of glucocorticoids overload could be due to the effects of glucocorticoids on mitochondrial ATP synthesis itself. Glucocorticoids are known to cause uncoupling of oxidative phosphorylation and decrease in state 3 respiration rates in mitochondria from tissues such as liver, skeletal muscles and heart under <u>in vitro</u> conditions. Although the deleterious effects of glucocorticoid overload on brain development are well documented, the reports on effects on mitochondrial energy metabolism are scarce and the results of these studies were contradictory. These studies employed not the young but only the adult animals and in most of the cases the glucocorticoids other than corticosterone were used. The physiologic relevence of these studies become debatable in view of the fact that corticosterone is the major glucocorticoid of the rat. Hence, studies employing the the rat hormone i.e. corticosterone only can provide insights in the regulatory role of glucocorticoid in brain development in

relation to energy requirements.

In view of the foregoing, studies were carried out to examine the in vivo effects of corticosterone tretments (acute and chronic) on mitochondrial oxidative energy metabolism in rat brain during development. Male albino rats belonging to different age groups i.e. 14, 21, 35-day-old and adults were used and were given either acute (2 h) or chronic (3 consecutive days) treatment with corticosterone (40 mg/Kg body wt.). Both the tretments - acute and chronic- significantly affected the mitochondrial respiration and oxidative phosphorylation. The effects were age-dependent and substrate specific. The acute treatment resulted in 25 - 70% reduction in state 3 respiration rates with maximum effect being seen in the 35 -day-old animals with glutamate as the substrate. ADP/O ratios also decreased by 10-75% and maximum uncoupling was seen in 21-day-old pups with succinate as the substrate. In the chronic treatment group, overall effects were of lesser magnitude compared to the acute treatment. Nevertheless, the net outcome was a drastic reduction in the rate of mitochondrial ATP synthesis especially in the 21- and 35-day-old animals. Clearly, during this period the mitochondria could not fulfil the high energy requirement of the delveloping brain. The results thus suggest that impairment of mitochondrial oxidative phosphorylation following corticosterone overload may be one of the mechanisms by which

glucocorticoid treatment affects the brain development.

Mitochondrial primary dehydrogenases serve as the ports for entry of electrons from substrates into the electron transport chain. Since the respiration rates were impaired, the activities of primary dehydrogenases i.e. glutamate dehydrogenase, malate dehydrogenase and succinate-DCIP reductase were monitored. The brain mitochondria from 35-day-old and adult animals showed significant decrease in glutamate dehydrogenase activity on acute treatement. Upon chronic treatment 14- and 21-day-old animals showed significant increase in glutamate dehydrognase activity whereas in the 35-day-old and adult animals the activity had decreased. Acute corticosterone treatment significantly lowered the malate dehydrogenase avtivity in all the age groups but chronic treatement had no effect. Acute treatment had no effect on succinate-DCIP reductase activity except for 21-day age group where there was increase in enzyme activity. Chronic treatment led to significant increase in enzyme activity in all the age groups except adults. Increased primary dehydrogenase activities may be a compensatory mechanism to counterbalance the low state 3 respiration rates in the brain mitochondria from corticosterone treated animals.

Effects of corticosterone treatements on the ATPase activity in brain mitochondria were also examined. Both acute and chornic treatments significantly lowered the basal ATPase

activity in almost all the age groups. Mg^{2+} -stimulated ATPase activity was also decreased in 14-day age group but in other age groups it either remained unaltered or increased beyond the control values. DNP -ATPase activity decreased in 14-day-old amimals following both acute and chornic treatments. Chronic treatment led to increase in DNP- and DNP + Mg^{2+} - ATPase activity in 35-day-old and adult animals.

Substrate kinetics studies revealed that the brain SMP (sonic mitochondrial particle) ATPase has two different catalytic sites: high affinity site and low affinity site. In age-dependent and treatment-specific manner the corticosterone treatments affected the kinetic properties of the ATPase. The major effects of corticosterone treatments were on Km rather than Vmax. In young animals it decreased the Km of both the sites but in adults the Km was increased. The 35-day group was least affected by the two corticosterone treatments.

Arrhenius kinetics studies of brain SMP ATPase showed that in 14-day group upon chronic treatment and in 35-day-old and adult animals control as well as corticosterone treatment groups, the break in the Arrhenius plots was completely abolished. These changes correlated well with the higher cholesterol content of brain mitochondria in these groups. The energy of activation E_1 and E_2 of brain SMP ATPase also showed significant alterations upon corticosterone administration in

age-dependent manner.

Many of the mitochondrial enzymes are membrane-bound and require lipid environment for their function. Therefore it was of interest to find out whether corticosterone treatment also influences the lipid content and composition of the brain mitochondria.

It was observed that the acute treatment resulted in significant increase in total phospholipid content in 14- and 21-day age groups. In case of adults the chronic treatment led to significant reduction in total phospholipid content. Two-fold increase in cholesterol content in mitochondria from chronically treated 14-day-old animals was also noted.

Both the treatments -acute and chronic- induced a host of changes in phospholipid composition and content of individual phospholipids in the brain mitochondria. Thus, in 14- and 21-day-old animals the relative proportion (% of total phospholipids) of lysophospholipids and diphosphatidylglycerol had decreased while that of the phosphatidylserine increased in both acute and chronic treatment groups. Corticosterone treatments had no much effect on phospholipid composition of brain mitochondria from adult rats. It was also found that the actual content of the individual phospholipids (µg/mg protein) also changed significantly following corticosterone treatments. Thus in general both acute and chronic treatments

brought about significant increase in the contents of sphingomyelin, phosphatidylinositol and phosphatidylserine; content of lysophospholipids and diphosphatidylglycerol had decreased. The magnitude of the changes were higher in 14- and 21-day-old animals than in the 35-day-old and adult animals. Effects of acute treatment were more drastic than those of the chronic treatment. In chronically treated adults, content of almost all the phospholipids decreased which is consistent with the greater reduction in the total phospholipid content of brain mitochondria from this group referred to above.

In view of these changes brought about by corticosterone treatments, it was of interest to examine the effects of corticosterone treatments on phospholipid metabolism. Hence experiments were performed to study incorporation of ^{3}H lebelled acetate into phospholipid components. In the untreated controls, maximum incorporation of ^{3}H acetate was seen in practically all the phospholipid components of the mitochondria in the 14-day-old animals. As the age advanced, the incoporation decreased gradually. The phospholipid components like sphingomyelin, phosphatidylserine, phosphatidylinositol and diphosphatidylglycerol showed higher rates of incorporation compared to the major phospholipid components e.g. phosphatidylcholine and phosphatidyl-ethanolamine. The effects of corticosterone treatments on ^{3}H acetate incorporation into various phospholipid subclasses

were age-dependent and treatment-specific. In 14-day-old animals both acute and chronic treatment resulted in significant decrease in label incorporation in all the phospholipid components.

The observed changes in lipid/phospholipid content of the mitochondrial membrane could affect the membrane fluidity. Hence, effects of corticosterone treatments on membrane fluidity were examined. In brain mitochondria from 14-and 35-day age groups the corticosterone treatments made membrane more fluidized whereas in case of 21-day-old and adult rats the membrane fluidity had decreased significantly.

Earlier it had been reported from our laboratory that in vivo corticosterone treatment led to impairment of energy metabolism in the liver mitochondria. Acute treatment resulted in generalized decrease in state 3 respiration rates, without having any significant effect on ADP/O ratios. Chronic treatment resulted in uncoupling of oxidative phosphorylation without having significant effects on respiration rates. Thus, both the treatments significantly lowered the rates of ATP synthesis. However, the underlying mechanisms were different for the two treatments regimens. The effects were also age-dependent; older animals showed increased resistance to the corticosterone treatments. These observations on liver

mitochondria were further extended by measuring the effects on activities of ATPase and primary dehydrogenases, kinetic properties of ATPase, lipid/phospholipid profiles and phospholipid biosynthesis and membrane fluidity.

Acute treatment significantly increased glutamate dehydrogenase activity in all the age groups except adults. Chronic treatment resulted in increased glutamate dehydrogenase activities in almost all the age groups except 14-day-old animals, where the activity had decreased by a factor of 4. The activity of malate dehydrogenase had decreased significantly by both acute and chornic treatment with corticosterone in all the age groups. Acute treatment had no much effect on succinate-DCIP reductase activity except that there was slight increase in the 21-day-old rats. In 14-day group and in adults the chornic treatment led to significant decrease in succinate-DCIP reductase activity but in case of 21-day-old animals the activity had increased. The results thus point out that even at the level of primary dehydrogenases, corticosterone effects are age dependent and tissue-specific.

Corticosterone treatments also altered mitochondrial ATPase activity in the liver. 21-day-old animals were most affected by both the corticosterone treatments; basal, Mg^{2+}, DNP-and Mg^{2+} + DNP- ATPase activities decreased significantly.

Besides having effects on the activity of mitochondrial

ATPase, the corticosterone treatments caused significant alterations in phase transition temperature and energies of activation: E_1 and E_2 of liver SMP ATPase. In control animals of 14- and 35-day group, no break in Arrhenius plots was observed. This could be attributed to higher cholesterol content in mitochondria from animals of these age groups.

The effects of corticosterone treatments on lipid content in liver mitochondria were also examined. Total phospholipid content of mitochondria increased significantly by both acute and chronic treatments; the extent of increase was generally higher following acute treatment. The cholesterol content was significantly lowered by both the treatments in 21-and 35-day-old animals; no effect was seen in the 14 -day-old and adult animals.

Corticosterone treatments also altered the phospholipid composition of liver mitochondria. Both acute and chronic treatment significantly decreased lysophosholipids and phosphatidylserine components in all the age groups except 14-day-old group. The phosphatidylethanolaime was also decreased significantly in 14-, 21-day-old and adult animals. Sphingomyelin component increased upon both acute and chronic corticosterone treatments. Diphosphatidylglycerol had increased only in chronically treated animals of age 14- and 21-day. Contents of individual phospholipids remained more-

or-less unaffected by corticosterone treatments in 14-day-old and adult animals. The maximum changes were observed in 21- and 35-day-old animals. In 21-day-old animals both the treatments led to increase in contents of almost all the phospholipid classes. This is attributable to the significant increase in total phospholipid content in mitochondria from this particular age group. In 35-day-old animals there was significant increase in phosphatidylcholine, phosphatidylethanolamine and diphosphatidylglycerol contents. On the other hand, contents of lysophospholipids, sphingomyelin, phosphatidylinositol and phosphatidylserine had decreased.

^{3}H acetate incorporation studies revealed that the pattern of label incorporation in liver mitochondria was quite different compared to the brain mitochondria. Thus the rate of label incorporation did not decrease with advancement of the age. Individual phospholipid components followed a specific developmental pattern. In general, both acute as well as chronic treatment resulted in decreased label incorporation into individual phospholipid components. The counts in lysophospholipids were reduced drastically; in some instances it was undectectable. This is suggestive of decreased phospholipid degradation in corticosterone treated animals. The effects of chronic treatment were more pronounced compared to the acute treatment.

Determination of membrane fluidity of liver mitochandria revealed that in 14-day-old and adult animals the membrane became more fluidized after corticosterone treatments. In case of 21-and 35-day-old animals the corticosterone treaments led to decrease in membrane fluidity. Hence, the corticosterone effects on membrane fluidity of liver mitcohondria were also age-specific as was observed for the brain mitochondria.

It is clear from the foregoing that corticosterone overload affected the mitochondrial membrane function of brain as well as liver. It was therefore of interest to find out how the serum levels, and the tissue and mitochondrial corticosterone contents were affected by the two treatment conditions. It is expected that such quantitative determinations can give proper insights about what concentration of the hormone can affect the mitochondrial membrane fuction.

Although methods for estimation of serum corticosterone levels are described, there are hardly any reports on tissue or mitochondrial corticosterone contents. Also, no method has been described thus far for estimation of tissue/mitochondrial corticosterone contents. Hence, a rapid, sensitive, economical and reproducible fluorimetric micromethod was developed to estimate the corticosterone contents in serum and in the

tissues/mitochondria. By employing this method it was possible to extract corticosterone quantitatively from the samples (50 µl serum, 0.5 mg tissue/mitochondrial protein). The efficiency of the extraction ranged from 80-100%.

In control animals the serum corticosterone level was highest in 7-day-old animals and then gradually decreased as age advanced; compared to the 7-day-old animals the contents decreased about 10 times in the adults. Acute treatment resulted in increased levels in serum of animals belonging to all the age groups with the extent of increase being much more in the young animals than in the adults. Upon chronic treatment, the serum corticosterone levels actually decreased in animals of all the age groups except adults.

The content of corticosterone in the whole brain and brain mitochondria was 2 to 3 times higher compared to the liver, indicating that the brain has greater ability to accumulate corticosterone. Corticosterone content was highest in the brain and liver in 21-day-old animals while the adults showed the lowest content. On acute treatment the corticosterone content in brain increased in the 35-day-old and adult animals. Chronic treatment caused decrease in the 14- and 21-day-old animals. Corticosterone content of brain mitochondria increased in 14- and 21-day-age group upon acute treatment while in case of the adults the content decreased

significantly. On the other hand, chronic treatment led to decreased corticosterone content of brain mitochondria from 21-day-old animals but in case of 14-day-old animals the content had increased significantly.

In livers of 14- and 21-day-old animals the acute treatment resulted in significant increase in the corticosterone content. The chronic treatment led to decreased liver corticosterone content in almost all the age groups except 14-day-old animals. In case of liver mitochondria from 14-day-old and adult animals, both the treatments increased corticosterone content. Increase was of greater magnitude upon chronic treatment than the acute treatment. In 21- and 35-day-old corticosterone treated animals, the content of mitochondrial corticosterone decreased significantly.

The studies reported here thus provide information about corticosterone effects on structure and function of mitochondrial membranes. 21- and 35-day-old animals were more prone to deleterious effects of corticosterone overload. Interestingly, this is the critrical age during which neuronal connectivities are formed and brain development becomes nearly complete. Corticosterone treatment resulted in drastic reduction in ATP synthesis in brain mitochondria from young animals. This could possibly be one of the primary underlying mechanisms by which corticosterone treatment affects the brain development.