CHAPTER-IV

S U M M A R Y

SUMMARY

The elevated level of glucocorticoid hormones, as a response to stress, has been known to cause a wide variety of changes in the organism. These include, antiinflammation, immunosuppression, maintenance of elevated blood glucose level and redistribution of the prime energy source - glucose to much needed tissues for obligatory bodily processes. Such effects were attributed to glucocorticoids mainly to facilitate the processes to cope with the stress. The continuous elevation of blood glucocorticoid levels, as under chronic stress and several neuropathological conditions could, however, cause deleterious effects to the organism by uncontrolled activation of the above mentioned processes.

Under normal conditions, the stress related increase in glucocorticoid levels in the circulation has been known to be regulated by a complex interaction of several organs such as pituitary, adrenal, hypothalamus and certain limbic regions of the brain. These organs utilize their specific well established chemical signals, hormones and neurotransmitters. Thus studies reported so far mainly concern with the alteration of these signal systems in the brain and pitutary with elevated glucocorticoid levels. With the advent of sophisticated techniques for neurochemical analysis, it has been very well established that corticosterone receptors are present in various brain regions and that its distribution is very heterogenous

very high in the hippocampal subregions and in hypothalamus, striatum, amygdala and even in the cerebellum at very early postnatal life. These receptors develop during postnatal life in these brain areas in parallel with the blood levels of corticosterone. An interesting feature of this development of corticosterone levels and the stress response is that between day one after birth till the end of second week animal will not respond to stressful simuli by increasing the blood level of corticosterone. In addition, during this period, the elevated corticosterone levels, whatever is the level, will not be brought back to normal condition easily. This particular phase of stress response is referred to as stress non-responsive period (SNRP). These observations reported earlier, indicate the importance of age as a factor for the stress response in the animals.

Studies so far reported in regard to glucocorticoid effects on the brain relate mostly to adult animals where the stress responding system is fully developed. The parameters looked were mainly the bioamine enzymes and levels. Studies other than this, have concerned with the brain weight and cell proliferation during development, that too in the whole brain or gross regions, thus leaving a wide gap in the information available for developing animals which possess an underdeveloped stress responding (in terms of corticosterone levels) machinery. Even in the case of adult whole brain or gross regions, glucocorticoid effects reported so far have been wide ranging and rather confusing owing to the varied experimental designes, glucocorticoid manipulation, be it adrenalectomy, or exogenous administration of different glucocorticoid analogues.

With these points in mind, in addition to the fact that the response of various neurotransmitter system during development would differ, the present study was aimed to investigate the effect of chronic corticosterone treatment on three different aspects of developing rat brain regions - the cerebellum, the striatum, the hypothalamus and the hippocampus possessing varied amount of corticosterone receptors in increasing order. The first aspect studied pertains to the development of GABA and glutamate metabolism enzymes - glutamate decarboxylase (GAD), GABA-transaminase (GABA-T), glutamine synthetase (GS) and glutamate dehydrogenase (GDH). The second aspect related to the levels of precursors of bioamines - tyrosine (Tyr), tryptophan (Try) and the neurotransmitters, Dopamine (DA), Norepinephrine (NE) and 5-hydroxytryptamine (5-HT) and some of their metabolites dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA); trace amines, p- and m-TA - tryptamine (Trp) were also studied. The third aspect involved the cholesterol and galactolipid concentrations during development followed by the ^{14}C-glucose incorporation study into lipid fractions and the enzyme activity of β-hydroxybutyrate dehydrogenase (BDH), a ketone body utilizing enzyme in the rat brain regions.

As a prelude for the study of effect of corticosterone treatment on the GABA enzymes during development, it became necessary to know about the developmental changes in the hippocampus, the cerebellum, the hypothalamus and the striatum with respect to GABA enzymes viz., GAD, GABA-T, GS and GDH.

The results obtained during this study revealed varied levels of GABA enzyme activities in these regions. The maturation of GAD and GDH is completed by postnatal day 20 in all brain regions except for striatal GAD which was observed around day 40. GABA-T activity developed till or beyond day 40 in all the four regions. GS activity reached adult value by day 20 in the hippocampus and the striatum and extended beyond 20 days in the cerebellum and the hypothalamus. Thus the striatum exhibited a unique pattern of GAD development amongst all the regions studied.

An experiment to find out the dose and steroid specific effect of the corticosterone in the adult and 10 day old hypothalamus and cerebellum with respect to GS and GAD activity showed that the corticosterone effect was observed at younger ages (effect on GS) whereas testosterone and estrogen did not show any effect, thus showing a steroid specificity. Adult hypothalamus responded to all the three hormonal treatment with respect to GAD, and since both the young and adult cerebellum exhibited a response in GS activity at 40 mg/kg body weight corticosterone treatment, this particular dose was used for further experiments.

The experiment on corticosterone treatment to rats at various ages (days 10, 20, 40 and adult) showed a region specific as well as age specific effects with respect to GAD, GABA-T, GS and GDH. Amongst the different regions, hippocampus seem to be affected most followed by striatum. The GAD activity

increased mainly at around day 20 in the hippocampus and striatum while GABA-T was altered at around day 40, the time at which a rapid increase in the activity was noticed in the earlier experiment. The cerebellum, showed increased GS activity at all the age points studied while only at 10 and 20 days, the hypothalamus and the hippocampus showed an increase respectively. The GDH activity was inhibited at day 10 in the cerebellum and hippocampus and increased in the striatum at adult stage only. Thus the effect of chronic corticosterone treatment was observed mainly at around day 20 in the hippocampus and striatum and suggest an increased GABA metabolism at this age.

The experiment on effects of chronic corticosterone treatment on the biogenic amine systems showed an uniform decrease mainly in the precursor p-tyrosine and tryptophan levels and the neurotransmitter metabolites DOPAC and 5HIAA at 10 days in all the regions studied. The regional specificity with respect to these responses developed only at adult stage i.e., a decreased dopaminergic activity in the adult hypothalamus and 5HTergic activity in the hippocampus.

Acute corticosterone treatment showed an increased 5HTergic activity in the adult hippocampus and dopaminergic activity in the hypothalamus; the only change in young animals was an increased tryptophan in the hippocampus and suggest a differential effect of corticosterone treatment in terms of region, age and dosage regimen. In view of the earlier reports implicating these neurotransmitter systems in several psychic disorders and also on steroid psychosis in adults, the present

results on bioamines and also on GABAergic enzymes emphasize their importance in childhood psychiatric disorders.

p- and m-tyramines, implicated as neuromodulators by several investigators, has been studied with corticosterone treatment. An increased p-tyramine concentration with a near significant decrease in DAergic activity in terms of decreased DOPAC, only at adult stage but no such relationship at embryonic day 15 and day 10 suggested a possible role of these trace amines in modulation of DAergic activity with the maturation of the latter system.

The experiment on effect of corticosterone treatment on the lipid changes in the hippocampus, the striatum and the cerebellum during development has also been incorporated in the thesis. The developmental changes of cholesterol and galactolipid in these regions suggested a differential pattern of accretion of these membrane components. The adult cholesterol concentration, however, remained same in all these regions while in the hippocampus galactolipid concentration was lower than that of cerebellum and the striatum suggesting differences in the amount of myelinated axons in these regions.

The chronic corticosterone treatment on the above mentioned parameters showed a region and age specific effect. Cholesterol concentration was increased with treatment only in hippocampus and striatum at 10 and 20 days of age in the former and only at 20 days in the latter. The experiment on corticosterone effect on the rate of cholesterol and galactolipid

synthesis from ^{14}C-glucose at 20 days showed a decreased incorporation into various lipid fractions in spite of increased blood glucose level, which could be due to the glucose uptake inhibition in the hippocampus and striatum with treatment.

Experiment to find out the other possible source of carbon for lipid was also performed. Increase in the activity of BDH, an enzyme involved in the utilization of ketone bodies, associated with a significant decrease in the levels of blood ketone bodies with treatment in both the hippocampus and the striatum suggested a possible compensatory mechanism for the inhibited glucose uptake with elevated corticosteroid levels during the rapid phase of brain development.

In view of the above mentioned observations, this thesis embodies the information which emphasizes the possibility of both the hippocampus and striatum as the prime targets for elevated corticosterone, be it exogenously administered or during prolonged stress occurring at the early stages of brain development.