CHAPTER - III

RESULTS AND DISCUSSION

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3:1 STUDIES ON THE ENZYMES OF THE GABA METABOLISM

GABA's role although acknowledged to be one of importance in the neuroendocrine system in mammals, specifically in the hypothalamo-hypophyseal-adrenal axis (DeFeudis, 1984), not many systematic studies are available with elevated corticosterone levels in the blood. These studies were also done to understand the role of corticosterone and the GABA system from a neuroendocrine point of view. Therefore, the classical approach of ablating the source of corticosterone secretion, adrenals, followed by exogenous administration of corticosterone or dexamethasone and monitoring of the GABA system in the CNS were followed.

Studies reported so far pertained mainly to GAD and have shown no change in the enzyme activity in different brain regions such as the hippocampus, striatum and midbrain (Meyer et al,1979; Kendall et al, 1982), and in certain hypothalamic nuclei and amygdala (Acs et al, 1980). GABA levels associated with GAD activity did not change in the midbrain and in the striatum (Kendall et al, 1982). In contrast, a significant decrease in the GABA levels accompanied by a decreased GAD activity has been reported for cerebral cortex (David and Kalyankar, 1986).

Studies on the uptake and binding of GABA have also shown regional variations, e.g., an increased GABA uptake in the hippocampal synaptosomes and not in the cerebellar or frontal cortex (Miller et al, 1978a). An elevation in the GABA receptor binding in the midbrain, and a similar but delayed response in the striatum with adrenalectomy reversed by corticosterone replacement has been reported (Kendall et al, 1982). A selective modulation of benzodiazepine receptors in brain regions has also been reported (De Souza et al, 1986). Adrenalectomy, in contrast to the contradictory alterations of GABA levels, produced a consistent decrease in the levels of glutamic acid and aspartic acid (Woodbury and Vernadakis, 1958; Rindi and Ventura, 1961). All these studies, thus, were made to relate the altered endogenous corticosterone level with the adrenal steroid receptor functions in the brain regions and indicate a possible role of basal corticosteroid levels in the GABA metabolism in specific regions.

The increased blood corticosteroid level in mammals, that ensues as a response to various stressors could have different effects on the GABA metabolism. Several reports to this effect are available in the literature. Animals subjected to various kinds of stressors such as immobilization, swim stress, ether inhalation or electric foot-shock etc., which elevate the blood level of corticosterone have been monitored for various parameters of the GABA/glutamate system. The effect of various stressors on GABA binding and it's functional aspect is summarised in Table-2.

From the Table-2 it is evident that most of the studies pertain mainly to the acute stress treatment resulting in varied region specific effect.

TABLE-2 : Effect of various stressors on GABA-binding and it's functional activity.

	Treatment	Results and Parameter	Region	Reference
a)	Handling	↑ GABA binding	Frontal cortex Caudate nucleus Cerebellum	
b)	Foodshock	↓ GABA binding	Cortex only	Biggo *et al.* (1981)
c)	Footshock to previously handled animal	↑ binding	Frontal cortex Caudate nucleus Cerebellum	
d)	Acute swim stress	↑ GABA binding	Forebrain but not in Cerebellum	Skerritt *et al.* (1981)
e)	Chronic	"	"	
f)	Acute swim stress	↑ Cl^- uptake by muscimol	Cortex and Hippocampus	Schwartz *et al.* (1987)
g)	Acute swim stress	↑ in chloride ionophore/benzodiazapine Complex	Cortex	Havoudjian *et al.* (1986)

Cont...

TABLE-2 (Contd...) Effects on the GABA-related enzymes under stress conditions.

Drug	Mode/dose	Objective	Region/parameters	Results
1)	Immobilization stress repeated for 42 days	To study the short term and long term stress effect on the behaviour of GABA-ergic and cholinergic neurons in individual nuclei of brain. (Adult male rats).	<u>Limbic system</u> N. interstitiales stria termi. puriform cortex. <u>Basal ganglia</u> N. caudatus, G.Pallidus, sub. nigra. <u>Hypothalamic nuclei</u> PVN, ventromedialls/ GABA, GAD and Ch AT.	Demonstrate differential changes in GABA & Ch AT in specific nuclei at 42 days after immobilization stress. GAD ↑ in substantia nigra and n. ventromediales and Ch AT in N. caudatus. - May be involved in inhibition of ACTH secretion. (Gottesfeld <u>et al</u>, 1978).
2)	Ether stress (2 x 2 min within 15 min), to male Wisar rats (140-200g)	To study the interaction between hypothalamo hypophyseal axis (HHA) and GABA system under stress conditions.	Hypothalamus GAD, GABA-T, GABA levels, corticosterone levels.	Ether stress ↑ plasma corticosterone levels. No change in GABA level in Hypo., but ↑ GAD activity. No change in GABA-T activity. ↓ rate of accumulation of GABA with stress. Ether stress activate GABA system which then may prevent a further response of HHA stress. (Manev <u>et al</u>, 1983).
3)	Immobilization stress for 3 h. to male Wistar rats. (180-200g)	To study the effect of environmental stress on cerebral GABA metabolism and content.	CBL, Pons-medulla, STR, HYP, HIP, Cerebral cortex, Mid brain GABA content, turnover, GAD activity.	Stress significantly ↑ GABA contents and GAD activity in HYP and STR which lasted for 12 h after application of stress for 3 h. Glutamic acid ↓ but GABA turnover is not affected. (Yoneda <u>et al</u>, 1983).
4)	Dexamethasone 1 mg IP for 1, 3 or 12 hrs before death. Ether stress (2 x 2 min for 15 min). Male Wistar rats (160-250g)	To study the effect of ether stress and dexamethasone (which are known to increase GAD activity in hypothalamus and on the GABA content in hypophysis.	HYP, Cingulate cortex, Hypophysis/ GABA accumulation.	Neither ether nor dexamethasone altered GABA content in hypophysis. Stress induced ↑ in synthesis of GABA in HYP, need not necessarily lead to an ↑ in Hypophyseal GABA content. (Manev and Pericic, 1985)

Very few studies that are available on changes in the GABA level have been inconsistant, e.g., pain-induced stress caused an elevation in the level of GABA in the cortex but not in the basal ganglia, thalamus or hypothalamus (Sherman and Gebhart, 1974) whereas, a painful stimulus, i.e., subcutaneous injection of formalin did not change the level of GABA in the cortical areas but significantly reduced those in the hypothalamus and lower brain stem nuclei (Elekes et al, 1986).

Of the GABA system enzymes, GAD seems to be the only enzyme studied under various stress conditions. The GAD activity increased selectively in the striatum and the hypothalamus of rats put to acute immobilization stress with a concomittant decrease in glutamic acid content (Yoneda et al, 1983). Acute ether stress also increased hypothalamic GAD activity but not GABA-T activity (Manev and Pericic, 1983). Similar to acute stress, chronic immobilization stress also increased GAD activity in globus pallidus and ventromedial nucleus (Gottesfeld et al, 1978) suggesting an activation of GABAergic system under various stress conditions. These observations suggest that such approach of putting the animal to various stressors have yielded wide ranging alterations in GABAergic system depending on the type and duration of stress. This perhaps may not be unreasonable, as the type of stressors vary the sensory information to the CNS differ, culminating in the activation of different regions and neurotransmitter system/pathways and hence the varied response. Apparently no other enzymes of GABA system have been studied under various stress conditions in detail.

Experiments with exogenous administration of corticosteroids have shown differential effects, unlike a general trend of increased GABAergic activity with stressors, e.g., GAD activity was inhibited in amygdala but increased in certain hypothalamic nuclei (Acs et al, 1980) while no change occurred in the anterior or posterior hypothalamus (Dunn et al, 1978). Studies on other enzymes such as GDH (Sadasivudu et al, 1977) and GS (Patel et al, 1983b) showed increased enzyme activities in the cerebral cortex and cerebellum respectively with corticosteroid administration(Table-3).

Several general tendencies arise out of these studies - (a) irrespective of the approach, GABA/glutamatergic system responds to alteration in the corticosteroid levels in adults; (b) these effects appear to be region specific; (c)the alterations in these systems appear to be different in adrenalectomized, in stressed and in exogenously corticosterone administered intact animals.

Most of the above mentioned studies have been carried out in adult animals wherein the neuroendocrine pathways are well developed. There seem to be very little information about the young animals whose neuroendocrine pathways have not developed properly that respond to various stressors or elevated corticosterone level in circulation with respect to GABA/glutamate system. Patel et al.(1983b) showed that corticosterone administration to young rats induce GS activity in the rat brain regions in vivo; induction being region specific and also dependent on the maturational state of the brain region during

TABLE-3 : Studies reported on the exogenous administration of glucocorticoids on GABA related enzymes in the brain.

Drug	Mode/dose	Objective	Region/parameter		Result
1) Dexamethasone sod.phosphate.	1 mg/kg. i.p. and ADX	To elucidate the effect of DEX on the role of GABA in feedback control of ACTH secretion.	Supreoptic n.(S.n.) Paraventricular n. (P.n.), Arcuate n. (A.n), Nucleus amygdala, Hippocampus	GAD activity after 3 h. of injection of DEX. in adult male rats.	No change in GAD activity in P.n. and A.n. and hippocampus increase in S.n. and ↓ in amygdala suggests a possible role for GABA mediated CRF secretion. (Acs et al, 1980).
2) Corticosterone.	15 mg/kg twice daily (repeated for 4 days) Sacrificed after 24 hr of the last injection.	To find out whether the changes in enzyme activities of TH and GAD.	HIP, STR, Septum, Thalamus, Ant. hypothal. and Post. hypothal. and Cerebellum Tyrosine hydroxylase and GAD. Male adult mice.		No change in GAD activity with corticosterone treatment in any of the regions and GABA changes with C may not be GAD mediated. (Dunn et al, 1978)
3) Hydrocortisone	10 mg/animal of 30 g b.w.	To study the effect of hydrocortisone on neuronal function of the brain.	Cerebral cortex CBL, Brain stem Mg^{++} ATPase, amino acid, Glu, GABA, Gln., Asp, Alanine levels.	GS, Asp. aminotransferase al. aminotransferase, Tyrosine transferase, ODC, protein oxidase, GDH, Na^+,K^+ATPase.	↑ by 65% in C. Cortex, GABA, Glu, Asp & alanine in all regions. Increase Na^+,K^+ ATPase in all brain regions studies indicate ↑ utilization of glutamate in brain as an alternative to glucose by shifting nitrogen metabolism towards catabolism. (Sadasivudu et al, 1977)
4) Cortisone	ADX - 80-120mg lump implanatation S.C. and 5 mg single injection 24 hrs prior to death.	To study the mechanism of action of behaviourally active hormones such as ACTH, Corticosteroneø on postsynaptic receptors and enzyme activities.	Cerebral cortex, HIP, STR, HYP, mid-brain, CBL, and Brain Stem/	GABA levels, GABA binding, GAD	ADX ↑ GABA binding in STR and mid brain only. This ↑ in binding was reverted with cortisone treatment. Time taken for increased binding with ADX was 4 hrs. after surgery. Corticosterone alone did not affect the GABA binding and GAD activity. HYP showed the same changes as ADX, and ACTH also showed these changes. Result suggest that adrenal and pituitary hormones influence GABA receptors in selected brain regions. (Kendall et al, 1982)
5) Hydrocortisone	50 mg/kg 1 hr. and 4 hr after injection.	To study the effect of hydrocortisone on GAD & GABA content in different rat brain regions.	Hip, Cortex, Med.oblongate(M.O) CBL, Diencephalon (Male adult rats)	GAD & GABA	1 hr. after treatment ↓ GABA content in HIP and cortex, whereas ↑ in med.oblongata. Decrease GAD in HIP and Medulla. 4 hr. after treatment ↑ in GABA in HIP, M.O., diencephalon, and CBL but decrease in middle brain. ↑ GAD in HIP but ↓ in M.O. (Kononenko and Mishunina, 1982)
7. Corticosterone	40 mg/kg for 3 days at	To study the *in vivo* enzyme induction of GS by corticosterone in different rat brain regions during development.	CBL, Olfactory bulb (o.b.) and forebrain. (Rats)	GS at 11,20 & 90 days	↑ GS activity in CBL, forebrain and o.b. depending on the maturation and state of the animal. (Patel et al, 1983b).
8. Dexamethasone.	0.077 mg/kg. I.P. for 1-5 days.	To see the effect of steroid on GABA enzymes.	Whole brain (Adult rats)	GAD, GABA-T Tyrosine, aminotransferase.	No changes in the enzyme activities. (Edwards and Rousseau, 1980)
Corticosterone	0.1 mg/kg/dose	-do-			

development. Several neural enzymes have been shown to be regulated by glucocorticoids during development, e.g., tyrosine-hydroxylase (TH), tryptophan hydroxylase (TPH) (Sze et al, 1980) and glycerophosphate dehydrogenase (GPDH) (De Vellis and Inglish, 1973) are responsive to corticosterone mainly during second postnatal week suggesting that developing brain regions may be more responsive to elevated corticosterone level in the blood. Furthermore, during stress non-responsive period - first two postnatal weeks, circulating corticosterone level goes down drastically and does not increase in response to stressors. Besides, the feedback inhibition of elevated corticosterone on the secretion of adrenal is also shown to be delayed during SNRP (Sapolsky and Meaney, 1986). It was therefore of interest to study the effect of exogenously administered corticosterone on the enzymes of GABA and glutamate enzymes during development in different regions of the rat brain. This study would be of importance to find out the susceptibility of GABA system during development to elevated circulating corticosterone levels resulting from certain pathological conditions or from exogenous administration for therapeutic purposes.

3:1:1 Developmental Pattern of The Enzymes of Glutamate And GABA Metabolism In Different Regions of The Rat Brain

With the above mentioned objectives in mind, as a prelude, it became necessary to know about the developmental status of GABA and glutamate related enzymes in different regions of the rat brain. On reviewing the literature, especially on rats, a paucity of information regarding the development of the enzymes, viz., GAD, GABA-T, GS and GDH in brain regions, the hypothalamus, the hippocampus, the striatum and the cerebellum was noticed. The reported enzyme activities during development were either in whole brain (Oja, 1960) or gross regions such as the forebrain and the brain stem (Telang,1975) or on specific regions such as cortex, hippocampus etc.,in species other than rat (Sisken <u>et al</u>, 1960; Kuriyama <u>et al</u>,1968). Most of these studies pertain mainly to GAD (used as a marker for GABAergic neurons) and GDH (as marker for mitochondria). In spite of extensive studies on GABAergic system, the ontogenic pattern of GABA-T and GS activity for the hippocampus and the straiatum is not available. Berl (1966) reported GS activity in different regions of cat brain and Patel <u>et al</u>. (1983a) reported developmental pattern of GS activity in gross regions like the forebrain, the cerebellum and the olfactory bulb in the rat. Since maturational pattern of enzyme is an important determinant in response to corticosteroids, the present experiment of studying the developmental pattern of GABA and glutamate metabolizing enzymes in different brain regions of the rat was undertaken.

The development of enzyme activities related to GABA and glutamate metabolism at 5, 10, 20, 40 days of age and in adult (>90 days of age) are shown in Figs.8 & 9.

The pattern of maturation of GAD in the cerebellum, hypothalamus, hippocampus and straiatum are comparable to several earlier reports (Telang,1975; Coyle and Enna, 1976).

The highest activity of GAD has been observed in adult hypothalamus followed by the hippocampus, striatum and cerebellum. In all the brain regions studied, the GAD activity developed rapidly right from postnatal day 5 with a maximal increase between 10 and 20 days. In the striatum, however, GAD activity continued to increase predominantly after 20 days of age (Table- 4). The adult level of activity was attained at about 40 days in all the regions.

A similar pattern of GAD maturation is also seen in terms of specific activity in all these regions (Table- 4). The highest specific activity in the hippocampus at 10 day (50% of adult value) increasing till 20 days is in agreement with reported data on cat (Berl and Purpura, 1966).

The development and distribution of GABA-T activity varied among the brain regions studied (Fig.8 and Table- 4). The highest adult activity was found in the hypothalamus followed by the cerebellum, striatum and hippocampus. Although as in the case of GAD, GABA-T activity increased profoundly between 10 and 20 postnatal days in all the regions studied, a continuous but significant increase has been noticed even after the postnatal day 40. In terms of percentage adult activity the striatum seems to attain near adult value by 40 days of age whereas the cerebellum, hypothalamus and hippocampus could only attain around 50-60% at this age (Table-5). It is of interest to note that the striatum possesses only about 8% of adult value by postnatal day 10 compared to 16-26% in other regions, but reaches near

TABLE- 4 : Development of specific activities of GABA enzymes (units/mg protein ± S.E.; no. of observations in parenthesis) in different regions of the rat brain.

Enzyme	Brain Regions	Age (days) 5	10	20	40	Adult (90)
GAD	Cerebellum	0.044 ± 0.011 (3)	0.05 ± 0.010 (4)	0.16 ± 0.010 (6)	0.19 ± 0.05 (6)	0.17 ± 0.007 (4)
	Hypothalamus	0.13 ± 0.015 (4)	0.16 ± 0.00 (4)	0.32 ± 0.01 (6)	0.33 ± 0.013 (6)	0.38 ± 0.029 (3)
	Hippocampus	0.05 ± 0.009 (4)	0.096 ± 0.008 (7)	0.31 ± 0.018 (6)	0.25 ± 0.029 (6)	0.265 ± 0.015 (3)
	Striatum	0.07 ± 0.01 (3)	0.078 ± 0.014 (9)	0.136 ± 0.018 (7)	0.236 ± 0.033 (5)	0.186 ± 0.009 (8)
GABA-T	Cerebellum	-	0.29 ± 0.029 (4)	0.53 ± 0.019 (6)	0.61 ± 0.017 (6)	0.93 ± 0.040 (8)
	Hypothalamus	-	0.32 ± 0.014 (4)	0.60 ± 0.022 (6)	0.70 ± 0.010 (6)	1.04 ± 0.049 (8)
	Hippocampus	-	0.18 ± 0.023 (4)	0.36 ± 0.039	0.47 ± 0.078 (6)	0.58 ± 0.027 (8)
	Striatum	-	0.076 ± 0.004 (8)	0.47 ± 0.043 (8)	0.601 ± 0.027 (5)	1.04 ± 0.049 (8)
GS	Cerebellum	0.78 ± 0.023 (3)	1.22 ± 0.150 (7)	1.43 ± 0.078 (8)	-	2.18 ± 0.17 (4)
	Hypothalamus	0.79 ± 0.150 (5)	1.30 ± 0.031 (5)	1.72 ± 0.095 (7)	-	2.46 ± 0.39 (3)
	Hippocampus	0.81 ± 0.082 (3)	0.97 ± 0.057 (9)	1.90 ± 0.190 (8)	-	1.94 ± 0.430 (3)
	Striatum	0.85 ± 0.12 (3)	1.02 ± 0.030 (6)	2.33 ± 0.317 (7)	-	1.31 ± 0.115 (4)
GDH	Cerebellum	-	0.16 ± 0.016 (8)	0.14 ± 0.008 (8)	0.12 ± 0.017 (8)	0.12 ± 0.015 (8)
	Hypothalamus	-	0.18 ± 0.002 (8)	0.17 ± 0.016 (8)	0.23 ± 0.012 (8)	0.22 ± 0.011 (8)
	Hippocampus	-	0.18 ± 0.011 (8)	0.148 ± 0.013 (8)	0.17 ± 0.013 (8)	0.14 ± 0.009 (8)
	Striatum	-	0.20 ± 0.013 (8)	0.16 ± 0.023 (8)	0.11 ± 0.009 (8)	0.16 ± 0.006 (8)

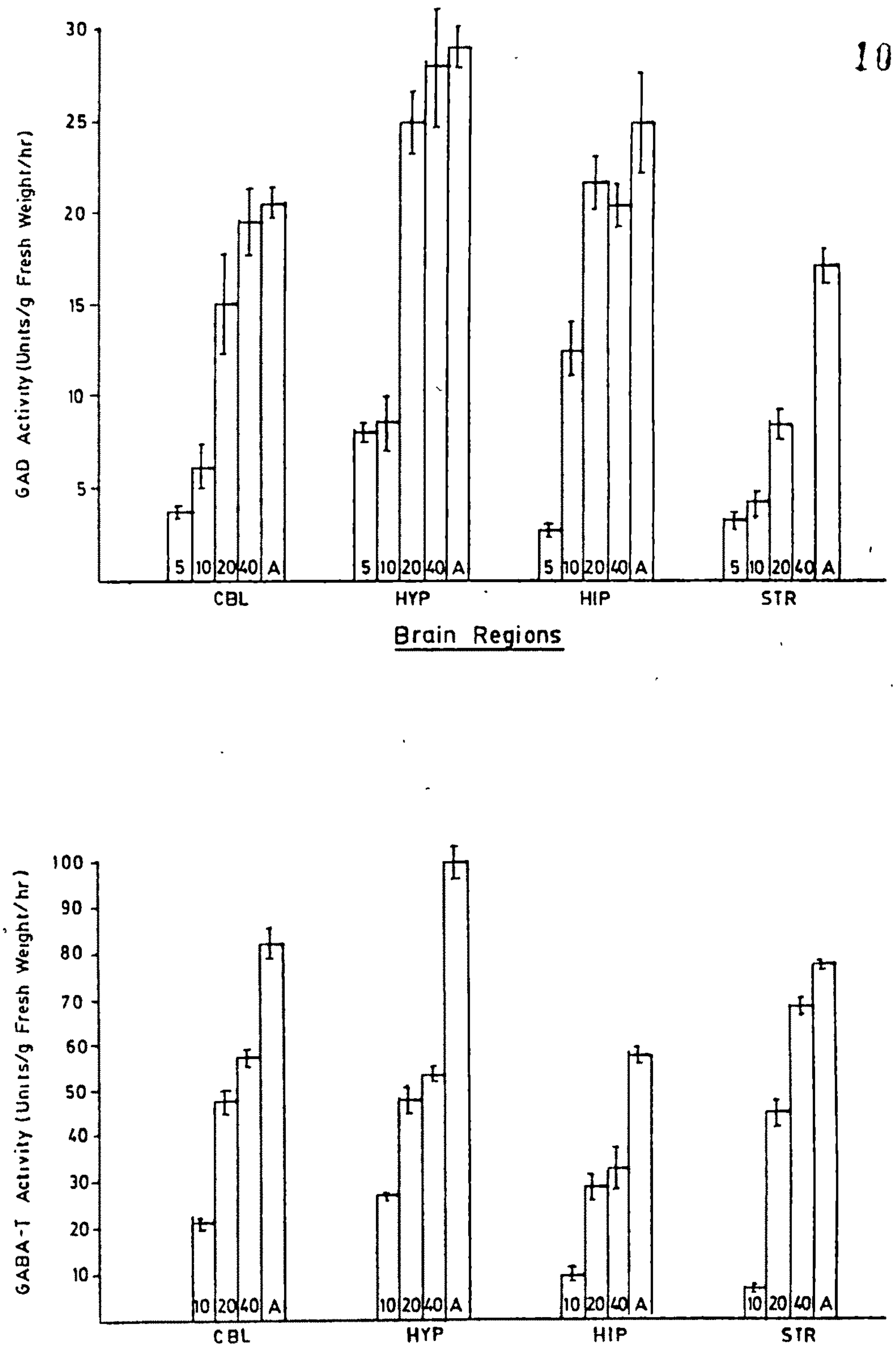


Fig. 8 *Development of GAD and GABA-T Activity In Different Regions of the Rat Brain*

Number inside the bars refer to age of the animal in days; A-Adult (>90 days). Values are Mean of 5-10 observations.

TABLE- 5 : Enzyme activities as percentage of adult value in different regions of the rat brain during development.

Enzyme	Brain regions	Age in days			
		5	10	20	40
GAD	CBL	18	29	73	95
	HYP	27	29	86	97
	HIP	11	50	87	82
	STR	17	24	56	113
GABA-T	CBL	-	26	57	70
	HYP	-	16	47	52
	HIP	-	16	50	57
	STR	-	8	57	88
GS	CBL	21	31	62	-
	HYP	14	28	54	-
	HIP	28	42	124	-
	STR	30	112	147	-
GDH	CBL	-	85	134	119
	HYP	-	52	78	95
	HIP	-	68	93	106
	STR	-	108	115	102

adult activity by about 40 days indicating a rapid rate of GABA-T development in this region.

Specific activity of GABA-T also showed a similar trend of development suggesting an increase in the amount of the specific enzyme protein (Table. 4). Such continued increase in GABA-T is in agreement with an earlier report on the rat whole brain (Vandenberg et al, 1965).

Glutamine synthetase (GS) activity also showed a differential distribution and development in these brain regions (Fig.9). The hypothalamus and cerebellum showed high activity followed by the hippocampus and striatum. The development of GS activity showed different trends in most of the regions studied (Fig. 9). The cerebellum and hypothalamus having 21 and 14% of adult activity at the age of 5 days attained 50% of adult activity by postnatal day 20. GS activity reached adult level by day 10 in striatum and between day 10 and 20 in hippocampus. In both the regions the maximal activity was observed at day 20 which declined to adult level at later stages. A similar decline in the GS activity with development in the striatum has been reported recently (Hansson, 1986) while no reports are available for the hippocampus. The enzyme activity obtained in the present study is in agreement with that reported by Patel et al.(1983a). The developmental pattern of GS activity in the hypothalamus is consistent with that of Vaccoro et al.(1979), although they have measured enzyme activity by the estimation of γ-glutamyl transferase activity which has been shown to give higher specific activity in crude homogenates (Patel et al, 1983a). The continued

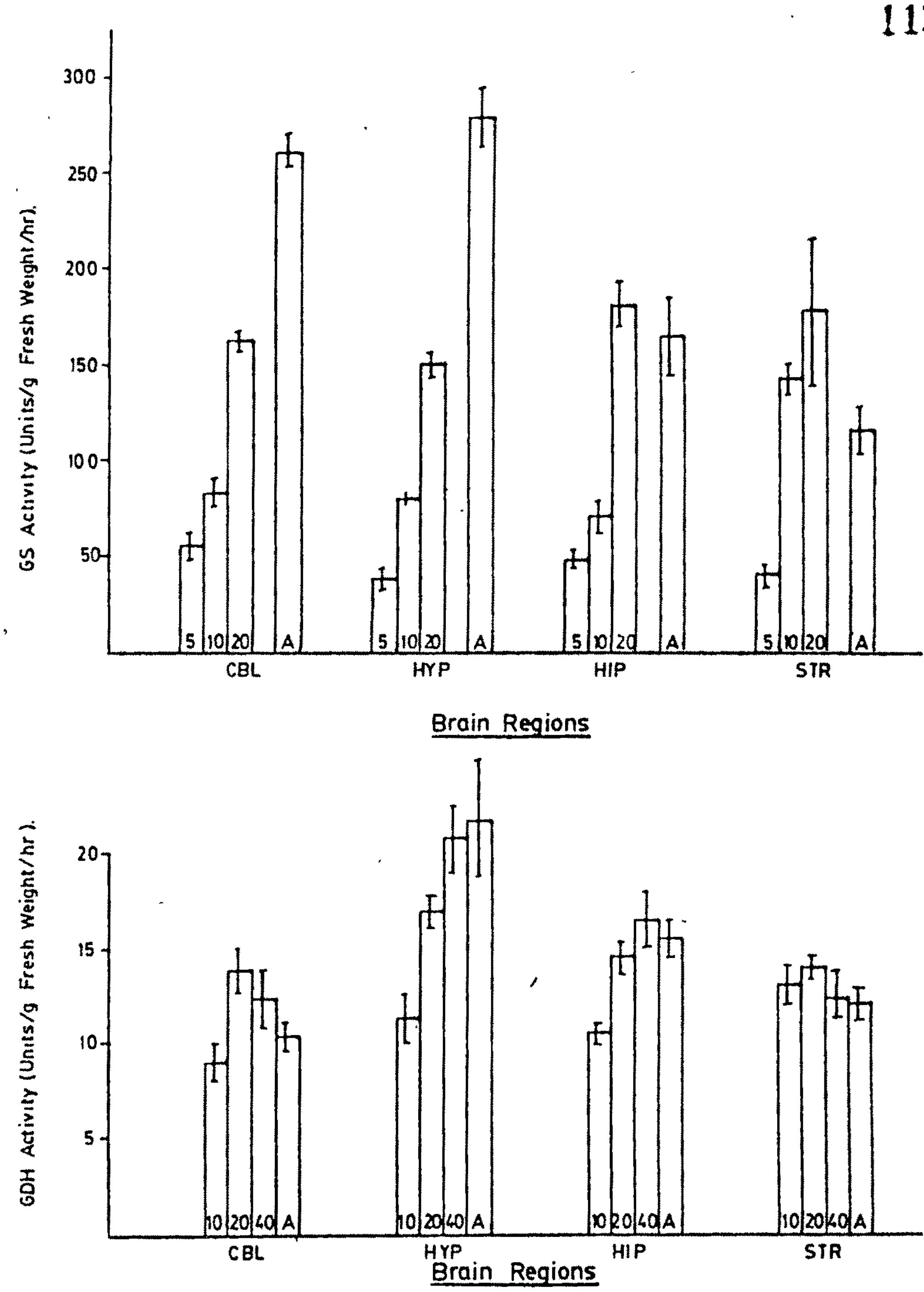


Fig. 9 ***Development of GS and GDH Activity In Different Regions of the Rat Brain***

Number inside the bars refer to age of the animal in days; A-Adult (>90 days). Values are Mean of 5-10 observations.

increase in GS activity in the cerebellum even after postnatal day 20 is in agreement with that reported by Patel et al.(1983a).

The specific activity of GS also showed a trend similar to that observed in unit activity in all these brain regions (Table-4).

The pattern of development and distribution of GDH activity was very unlike those observed for GAD, GABA-T and GS (Fig.8,9 and Table-4). The highest GDH activity was observed in the hypothalamus followed by the hippocampus, striatum and cerebellum. The GDH activity obtained in this study was lower than that of the reported values (Rothe et al,1983; Leong and Clark, 1984a). All the four regions showed the maximal increase in the enzyme activity between 10 and 20 postnatal day with a continued increase only in the hypothalamus and hippocampus beyond 20 days. Similar to the observations made by Leong and Clark (1984a), a slight decrease in the enzyme activity between day 40 and adult stage in the cerebellum was also noticed, although the decrease was not significant. Such a trend in development is in agreement with the earlier reported studies (Leong and Clark, 1984a; Rothe et al, 1983) with respect to the hypothalamus and the hippocampus. The striatum and the cerebellum attained the adult level of activity by around postnatal day 10.

In short, the rat brain regions - the hypothalamus, hippocampus, striatum and cerebellum showed varied levels of GABA enzyme activities. The maturation of GAD and GDH completed by postnatal day 20 in all brain regions except for GAD in striatum, which completed around day 40. GABA-T activity developed till or beyond 40 days in all the four regions. GS activity reached adult value before day 20 in the hippocampus and the striatum and extend beyond 20 days for the cerebellum and the hypothalamus. The striatum exhibited a unique pattern of development of the GABAergic enzymes amongst all the regions studied.

3.1.2 Dose-Response and Specificity of Corticosterone Effect on GAD and GS Activity In The Cerebellum And The Hypothalamus of Rat Brain.

With the endocrine status of the animal being an important factor while looking for any effect of the corticosterone administered exogenously, two parameters that acquire paramount significance are - (a) age of the animal, since the endogenous corticosterone level varies with age, and (b) the physiological state of the target tissue in question. In other words, the effect of corticosterone on any specific parameter may vary with the exogenously administered dose and the age of the animal. The present experiment was undertaken to find out the dose dependency of the effect of corticosterone, if any, on activities of GAD and GS and also specificity of such effects with respect to the steroid hormones used.

Enzymes GS and GAD were chosen because GAD activity, located mainly in neuronal cells and GS in astroglial cells, may represent the corticosterone effect on the neuronal and the glial cells with respect to GABA metabolism. Since the previous experiment clearly indicated a differential developmental pattern of these enzyme activities in different regions of the rat brain, the cerebellum and the hypothalamus were used for assaying the enzyme activities. Besides, these two regions also possess a low (cerebellum) and high (hypothalamus) receptor levels for testosterone and estrogen as well (Stumpf and Sar, 1976). Although this experiment was carried out mainly in adult animals (since the effect of sex steroids may be more pronounced at adult stage), additionally, the specificity of corticosterone effect was also tested on 10 day old rat pups using the highest dose of respective hormones.

The dosages of corticosterone, estrogen and testosterone were selected on the basis of the lowest and the highest amounts used for such experiments as reported in the literature.

The effect of various doses of steroid hormones are as presented in the Figure- 10 indicating dose and specificity to some extent, of the effect of corticosterone on GS and GAD activities. In the adult hypothalamus increasing doses of corticosterone increased GAD activity, but significantly only at 5 and 20 mg kg^{-1} body weight. The magnitude of increase in GAD activity, however, remained same at both these doses. The cerebellar GAD activity was not altered at any of the doses of corticosterone injected.

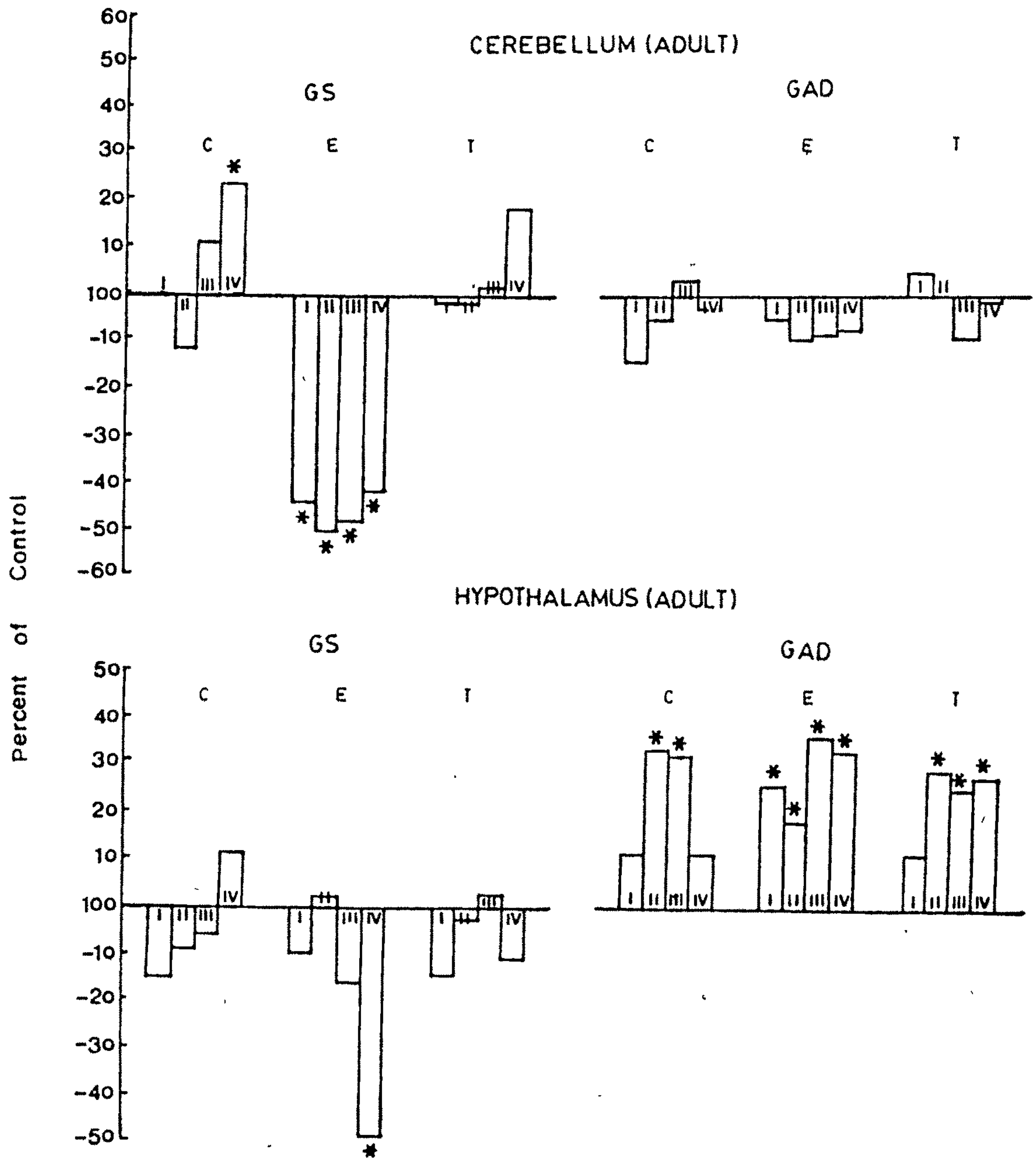


Fig. 10 ***Dose Response Effect of Steroid Hormones on GS and GAD Activity In the Cerebellum and Hypothalamus of the Adult Rat Brain***

I, II, III, IV given inside the bars refer respectively to the doses 0.1, 5, 20, 40 mg/kg b.w. of Corticosterone (C); 0.005, 0.025, 0.5, 4 mg/kg b.w. of Estrogen (E) and 0.05, 0.1, 2, 4 mg/kg b.w. of Testosterone (T).

* *Refers to significantly different from controls. Control values (units/g fr. tissue ± S.E.) of GS was 268.51±29.0 and 16.32 ± 0.97 for GAD in the Cerebellum where as 254.8 ± 10.5 for GS and 32.8 ± 1.77 for GAD in the hypothalamus. Values are mean of 5-7 number of independent observations.*

GS activity was increased with corticosterone treatment only in the cerebellum at 40 mg kg^{-1} body weight, although a slight increase in activity occurred at a lower dose (20 mg kg^{-1}b.w.). The adult hypothalamic GS activity was not altered at any of the doses of corticosterone suggesting a region specific effect. In both the cases of increased enzyme activities (i.e, GS in the cerebellum and GAD in the hypothalamus), dose dependency of corticosterone effect was not observed. A study by Patel et al. (1983b), using a similar corticosterone treatment schedule in 8-day old animals have demonstrated a dose dependent increase in cerebellar GS activity. Taken together, these studies suggest that the responsivity of the cerebellum may decrease with age as smaller doses of corticosterone did not increase GS activity significantly. These results, thus indicate the importance of dose and region specificity of corticosterone effect with respect to GS and GAD activities.

With respect to the specificity of the steroid hormones, only corticosterone increased cerebellar GS activity significantly while estrogen decreased it both in the cerebellum and the hypothalamus. Testosterone did not affect GS activity significantly in these regions, suggesting a corticosterone specific effect of increase in GS activity. Corticosterone effect on GAD activity, however, do not seem to be specific in the adult hypothalamus, as even testosterone and estrogen increased GAD activity atleast at some of the doses injected. This could possibly be due to the heterogenous nature of the hypothalamus involved in various neuroendocrine pathways. The effect of decreased GS activity by estrogen was observed in both the hypothalamus and the cerebellum, and thus could be an uniform effect on GS containing cells. Such an effect of decreased enzyme activity has been attributed to the synthesis of certain inhibitory factors (Seifert and Gelehrter, 1978; Blackwell et al, 1980) for phospholipase A_2 by glucocorticoid.

In young animals (Table- 6), estrogen and testosterone seem to be ineffective in both the cerebellar and hypothalamic GS and GAD activities. Only corticosterone increased GS activity in both the cerebellum and the hypothalamus but had no effect on GAD. These results suggest that the effect of corticosterone on GS activity is specific atleast in the young rats and age dependent as the hypothalamic GS activity was not altered in adult rats but increased in young ones.

In conclusion, this experiment suggest that -

(a) Corticosterone effects can be observed at younger ages (effect on GS) whereas testosterone and estrogen are ineffective and thus become specific at younger age.

(b) of the two regions studied, adult hypothalamus responds to all the three hormonal treatments with respect to GAD activity while GS in cerebellum showed response to corticosterone only.

(c) as the GS activity in young hypothalamus and the cerebellum was altered in addition to the adult cerebellum with 40 mg/kg body weight corticosterone treatment, this dose could be used for further studies.

TABLE- 6 : Effect of various steroid hormones on GS and GAD activity in 10 day old rat brain cerebellum and hypothalamus.

Region	Treatment	GS		GAD	
		Control	Experimental	Control	Experimental
CEREBELLUM	Estrogen	[a] 73.0 ± 8.0 [b] 0.98 ± 0.07	60.0 ± 4.0 0.73 ± 0.08	5.4 ± 0.03 0.07 ± 0.006	5.2 ± 0.03 0.06 ± 0.01
	Corticosterone	117.0 ± 7.0 1.43 ± 0.09	134.0 ± 4.0** 1.73 ± 0.13	4.5 ± 0.66 0.07 ± 0.006	5.4 ± 0.44 0.07 ± 0.004
	Testosterone	86.0 ± 4.4 1.20 ± 0.10	90.0 ± 8.0 1.20 ± 0.14	5.1 ± 0.43 0.10 ± 0.009	4.9 ± 0.46 0.1 ± 0.008
HYPOTHALAMUS	Estrogen	76.0 ± 6.0 1.34 ± 0.16	66.0 ± 14.0 1.19 ± 0.11	9.5 ± 0.4 0.15 ± 0.01	8.2 ± 0.3 0.15 ± 0.01
	Corticosterone	76.0 ± 6.0 1.34 ± 0.16	150.0 ± 14.0** 2.44 ± 0.16	9.5 ± 0.4 0.15 ± 0.01	10.6 ± 0.9 0.17 ± 0.01
	Testosterone	76.0 ± 6.0 1.34 ± 0.16	88.0 ± 8.0 1.48 ± 0.06	9.5 ± 0.4 0.156 ± 0.01	9.7 ± 0.6 0.17 ± 0.01

GS and GDH activities are expressed as - a. units/g fr. tissue;
b. units/mg protein (Mean ± S.E.)

** Significantly different from controls at $P < 0.01$ (n = 6 - 9).

3.1.3. Effect of Chronic Corticosterone Administration on the Enzymes Related to Glutamate and GABA in Different Regions during Development.

From the previous experiment it became evident that corticosterone administration altered the GS activity in 10 day old hypothalamus which seems to obliviate in the adult stage indicating that hypothalamus at young age is sensitive to corticosterone which may become progressively diminished. Since the effect of corticosterone was found to be age and region specific as observed from earlier experiment, studies were carried out in different brain regions viz., cerebellum, hippocampus, hypothalamus and striatum during development at 10, 20, 40 and >90 days of age. The dose of corticosterone used in the present experiment was determined, in addition to the previous experimental result, due to earlier reports that such dosage in intact rats would produce the circulating levels of corticosterone equivalent to the animals under certain type of stress (Dickinson et al, 1985).

Effect on brain regional weights

Inhibitory effects of corticosteroids on body and brain growth have been very well known. Studies based largely on either whole brain or gross regions such as forebrain, hindbrain, cerebellum etc., have reported a clearcut decrease in tissue weights (Cotterellet al, 1972; Kovacs, 1973; Howard, 1965; Bohn and Lauder, 1978) and suggest glucocorticoid inhibition of cell proliferation depending on the time and dose of treatment. Concerned mainly with the longterm effects on brain growth, most of these studies have treated the animals during neonatal period (first four days) and monitored tissue weights at various time points during development. Presumably, owing to the definitive deficits demonstrated by these reports, later studies on corticosteroids' effect on various biochemical parameters in discrete regions such as hippocampus and striatum have not reported about tissue weight changes. Similarly, no reference has been made about these regions in certain exhaustive reviews published recently concerning the effect of corticosteroid on brain growth and cell proliferation (Meyer, 1985). In the present study, body weight and brain regional weights were routinely monitored with corticosterone treatment. Three days of corticosterone treatment resulted in average deficits in the body weight as well as in different regions (Table- 7). The deficits achieved with hormone treatment was comparatively smaller than the batch to batch variation in the growth of the litters. Therefore a comparison of tissue weights from littermates showed a consistent decrease (weights from randomly selected litters are presented in the Table- 7) with steroid treatment. Interestingly, tissue weights from adult rats were also found to be decreased. In this regard, Devenport and Devenport (1985) have demonstrated that the sensitivity of brain growth persists even as the juveniles matured to adults. Furthermore, hindbrain was more sensitive to steroid manipulation than forebrain between 27 to 46 days. In contrast, forebrain was sensitive between 46 to 65 days following a 19 day treatment of corticosterone and deoxycorticosterone. The present data confirms the effectiveness of steroid treatment schedule followed in the ensuing experiments, although it may not reflect on the regional sensitivity to the steroid.

TABLE- 7 : Effect of corticosterone administration on weights of the rat brain regions during development.

Region	Age (days)	Mean weight (mg) of brain regions from littermates						Average weight (mg) ± S.E. (n = 50)	
		I		II		III			
		Control	Experi-mental	Control	Experi-mental	Control	Experi-mental	Control	Experi-ment
CBL	10	93	71	92	68	84	78	87.5 ± 2.0	78.0 ± 2.2
	20	155	144	169	148	168	158	165.4 ± 3.0	148.0 ± 4.0
	40	206	193	206	194	204	197	203.0 ± 4.0	196.0 ± 5.0
	90	232	226	225	213	229	211	230.0 ± 2.0	220.0 ± 4.0
HIP	10	41	38	41	38	58	46	55.0 ± 2.1	48.0 ± 4.0
	20	84	73	77	71	73	71	76.5 ± 1.2	72.0 ± 1.0
	40	92	84	94	88	95	83	91.1 ± 4.3	90.0 ± 3.5
	90	120	103	116	167	118	111	117.6 ± 3.1	110.0 ± 3.6
STR	10	40	36	42	40	45	42	45.0 ± 2.0	41.0 ± 3.6
	20	50	42	49	46	59	57	56.0 ± 1.6	55.0 ± 2.3
	40	60	49	72	56	59	50	60.3 ± 2.0	51.6 ± 2.6
	90	97	71	81	65	163	66	89.6 ± 6.0	74.3 ± 2.6

3.1.3.1 <u>GAD</u> :

As can be seen from Fig.11, the effect of corticosterone treatment on GAD activity seems to depend on both the region and age groups studied. A general trend of increased GAD activity was observed with corticosterone but significant only in the hippocampus and the striatum. In hippocampus, the enzyme activity was increased only at postnatal day 20 whereas in adult the increase was not significant. In the striatum, the increase was observed at postnatal day 20 and 40, but significantly only at day 20. At this point, it would be interesting to note from the earlier experiment that the rapid phase of development of GAD activity occurred between 20 to 40 postnatal day in the striatum and 10 to 20 postnatal day in the hippocampus , the corresponding period at which alteration in the GAD activity occurred with steroid treatment. This change in enzyme activity was accompanied by increased specific activity, albeit not significant owing to a large variation in the values (Table-8). As mentioned earlier, except for a study on the 10 day old rat whole brain which reported no change in GAD activity with corticosterone treatment, there is no report in this regard in any brain regions during development. This study, thus for the first time, showed that the hippocampal and striatal GAD could be altered by exogenous corticosterone treatment during development. The present finding of no significant change in the adult hippocampal GAD activity is consistent with the report of Dunn <u>et al</u>. (1978) administering

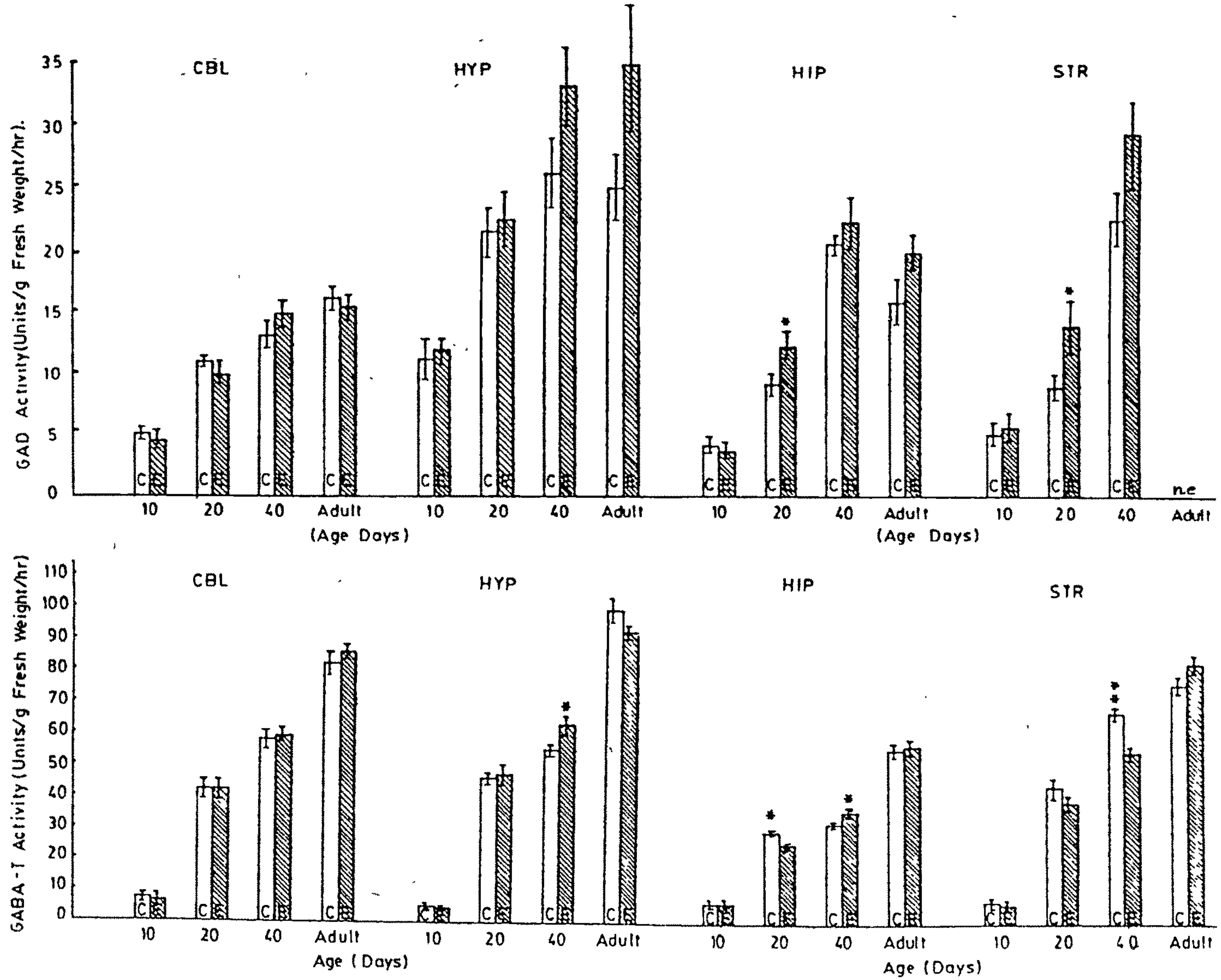


Fig. 11 *Effect of Chronic Corticosterone Administration on GAD and GABA-T Activity In The Rat Brain Regions during development*

(▭), *Control*; (▨), *Experimental*

* $P \leq 0.05$, *values significantly different from controls*; $P < 0.01$ **

Values are mean of 7-10 number of independent observations

Adult refers to >90 day old animals

TABLE- 8 : Effect of chronic corticosterone administration on specific activity of enzymes of GABA metabolism in rat brain regions during development.

Region	Enzyme[a]	Age (days)							
		10		20		40		Adult (90)	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
CEREBELLUM	GAD	0.10 ± 0.009 (6)	0.09±0.005 (6)	0.09±0.009 (5)	0.12±0.010 (5)	0.153±0.013 (6)	0.14±0.013 (6)	0.16±0.012 (7)	0.15±0.018 (7)
	GABA-T	0.097±0.007 (9)	0.092±0.011 (9)	0.46±0.036 (7)	0.48±0.035 (9)	0.592±0.026 (8)	0.574±0.013 (7)	0.802±0.043 (10)	0.93±0.040 (7)
	GS	1.22±0.150 (6)	1.70±0.29 (6)	1.43±0.078 (6)	2.70±0.029 (6)	-	-	1.15±0.071 (5)	1.40±0.125 (5)
	GDH	0.16±0.076 (8)	0.2±0.016 (7)	0.14±0.008 (6)	0.14±0.007 (7)	0.12±0.017 (8)	0.11±0.011 (7)	0.12±0.015 (5)	0.13±0.078 (8)
HYPOTHALAMUS	GAD	0.154±0.011 (6)	0.172±0.008 (6)	0.29±0.029 (5)	0.29±0.025 (5)	0.448±0.029 (6)	0.522±0.050 (6)	0.35±0.041 (7)	0.40±0.041 (7)
	GABA-T	0.076±0.004 (9)	0.070±0.003 (9)	0.471±0.043 (7)	0.49±0.017 (7)	0.60±0.027 (7)	0.643±0.045 (8)	1.04±0.049 (7)	0.96±0.060 (7)
	GS	1.30 ±0.031 (6)	1.46±0.220 (6)	1.72±0.095 (6)	2.09±0.13 (6)	-	-	3.65±0.259 (6)	3.62±0.176 (6)
	GDH	0.18±0.002 (8)	0.20±0.016 (7)	0.17±0.016 (4)	0.19±0.011 (7)	0.23±0.012 (6)	0.21±0.008 (5)	0.22±0.035 (6)	0.22±0.011 (7)
HIPPOCAMPUS	GAD	0.05±0.008 (8)	0.05±0.007 (8)	0.11±0.016 (9)	0.15±0.026 (8)	0.212±0.014 (5)	0.239±0.025 (5)	0.20±0.020 (8)	0.21±0.01 (9)
	GABA-T	0.082±0.004 (8)	0.068±0.008 (8)	0.3 ±0.013 (6)	0.27±0.011 (9)	0.297±0.017 (6)	0.364±0.014 (9)	6.58±0.018 (9)	0.58±0.027 (10)
	GS	0.97±0.057 (5)	1.30±0.160 (6)	1.90±0.190 (6)	2.32±0.12 (7)	-	-	1.82±0.068 (6)	2.00±0.293 (7)
	GDH	0.18±0.011 (9)	0.14±0.008 (8)	0.148±0.013 (5)	0.14±0.004 (7)	0.17±0.013 (5)	0.13±0.011 (7)	0.17±0.011 (7)	0.14±0.009 (7)
STRIATUM	GAD	0.08±0.014 (8)	0.07±0.011 (8)	0.13±0.018 (9)	0.16±0.028 (9)	0.236±0.033 (5)	0.274±0.02 (5)	-	-
	GABA-T	0.098±0.007 (8)	0.075±0.004 (7)	0.515±0.047 (7)	0.930±0.028 (9)	0.676±0.040 (8)	0.578±0.038 (7)	0.75±0.030 (7)	0.89±0.019 (8)
	GS	0.059±0.005 (5)	0.065±0.01 (6)	2.33±0.317 (6)	2.60±0.360 (7)	-	-	-	-
	GDH	0.20±0.013 (9)	0.21±0.11 (7)	0.16±0.023 (4)	0.14±0.012 (9)	0.12±0.015 (7)	0.11±0.009 (7)	0.124±0.060 (6)	0.16±0.006 (8)

a-Values are units mg^{-1} protein; no. of observations in parenthesis an equivalent to 15 mg $kg^{-1}_{b.w.}$ of corticosterone twice daily to adult mice. A similar no change in GAD activity has also been reported in the hippocampus of adrenalectomized and corticosterone implanted adult rats (Meyer et al, 1979) and suggested that endogenous corticosterone may not have a major role in the GABA synthesis in the hippocampus under normal conditions. On the other hand, David and Kalyankar (1986) observed a decreased GAD activity in the cerebral cortex of adrenalectomized adult rat which was reversed by either corticosterone or pyridoxal phosphate (PLP) supplementation and suggested that the GAD activity may be under the influence of adrenal steroids by way of altered brain PLP concentration with adrenalectomy.

Study with respect to corticosterone administration on striatal GAD has come mainly from Dunn et al.(1978), demonstrating no change in the activity in adult mice. Several studies, however, have suggested increased GAD activity in striatum (Yoneda et al, 1983) or certain nuclei of striatum (Gottesfeld et al, 1978) under stress conditions. With the elevated striatal GABA content during immobilised stress as also of GAD activity, an increased GAD enzyme protein rather than the altered availability of its cofactor (PLP) has been suggested (Yoneda et al, 1983). Taken together, these observations would suggest that the striatal GABA system, mainly GAD responds to the elevated corticosterone levels in the blood and may play an important role in the functions of extrapyramidal system during behaviourally significant period at which motor activity transiently increase (15-20 days of age) as proposed by Yoneda et al. (1983).

In the present study, GAD activity in both the cerebellum and the hypothalamus was not affected, although a trend towards increase was observed only in the hypothalamus. GAD activity was, however, found to increase at lower doses (5 mg and 20 mg kg^{-1} b.w.) of corticosterone in the previous experiment. The hypothalamus being involved in various neuroendocrine functions, including the release of corticotropin releasing factor which is suggested to be mediated by the GAD activity (Acs and Stark, 1978), has been a major target of research in this area. Acute dexamethasone treatment to mice has been shown to increase GAD activity in the supraoptic area and decrease in nucleus amygdala while without any effect in paraventricular nucleus and arcuate nucleus (Acs et al, 1980). Chronic corticosterone (15 mg kg^{-1} b.w. twice daily) treatment for four days in mice did not alter GAD activity of anterior and posterior hypothalamus (Dunn et al, 1978). But the same report has mentioned about an increased GAD activity in whole hypothalamus in a preliminary study which may indicate the complexity of this very heterogenous region for the variation and interpretation of enzyme data. Similarly, in the present study also no definite change could be discerned from the hypothalamic GAD activity. Several earlier reports concerned mainly with hypothalamic GAD activity under various stress conditions have demonstrated an uniformly increased specific activity in both whole hypothalamus or specific nuclei (Gottesfeld et al, 1978; Manev and Pericic, 1983; Yoneda et al, 1983) and suggest an increase of enzyme protein. Data, however, has not been reported so far on the effect of corticosterone on either hypothalamus or cerebellum GAD activity during development. Thus the overall picture that emerged from this study is a general trend of increased GAD activity in specific brain regions - hippocampus and striatum at early stages of development (3rd postnatal week specifically).

3.1.3.2 GABA-T :

GABA-T, an enzyme involved in the breakdown of GABA has been studied least with regard to steroid effects; a possibility of this being affected has been suggested by Lajtha and his associates (Banay-Schwartz et al, 1979). The present study indicates that exogenous corticosterone administration affects GABA-T activity in region and age specific manner (Fig.11 and Table-8). Of the four regions studied, except for cerebellum, GABA-T activity was altered in the hypothalamus, hippocampus and striatum. The effect was mainly found at day 40 - a significant increase in hippocampus and hypothalamus but a decrease in the striatum. At day 20, hippocampal GABA-T was decreased as opposed to its increase at day 40. Concommitant changes in specific activities were also observed with corticosterone treatment except in hypothalamus where the change was not significant. Here again, the time of change in enzyme activity pertained mainly with the rapid phase of GABA-T development in these regions as observed in earlier experiment. In adult rats none of these regions showed any effect with the treatment. The change in opposite direction observed in the hippocampus at day 20 and 40 may perhaps be due to altered synthesis or breakdown of the enzyme protein. As there are no report on the developing brain regions with respect to GABA-T response to corticosterone, comparison with the present study was not possible. In adrenalectomized but corticosterone replaced adult rat no change in GABA-T activity in the hippocampus was shown (Meyer et al, 1979). Studies thus reported showed no change in GABA-T activity in the rat whole brain (Edward and Rousseau, 1980) as also in the rat hypothalamus under ether stress (Manev and Pericic, 1983).

3.1.3.4 GS :

At younger ages, with corticosterone treatment, GS activity was increased mainly in the cerebellum and hippocampus (Fig.12). The cerebellum being exception to this showed significant increase even at adult stage whereas the hypothalamus and the hippocampus did not. In the hippocampus GS activity was increased only at postnatal day 20. These changes in the enzyme activity were associated with corresponding changes in the specific activity suggest an increase in the enzyme protein. In the striatum experiments were done only at postnatal day 10 and 20 and the increased enzyme activity was not statistically significant. Hypothalamic GS was increased only at day 10.

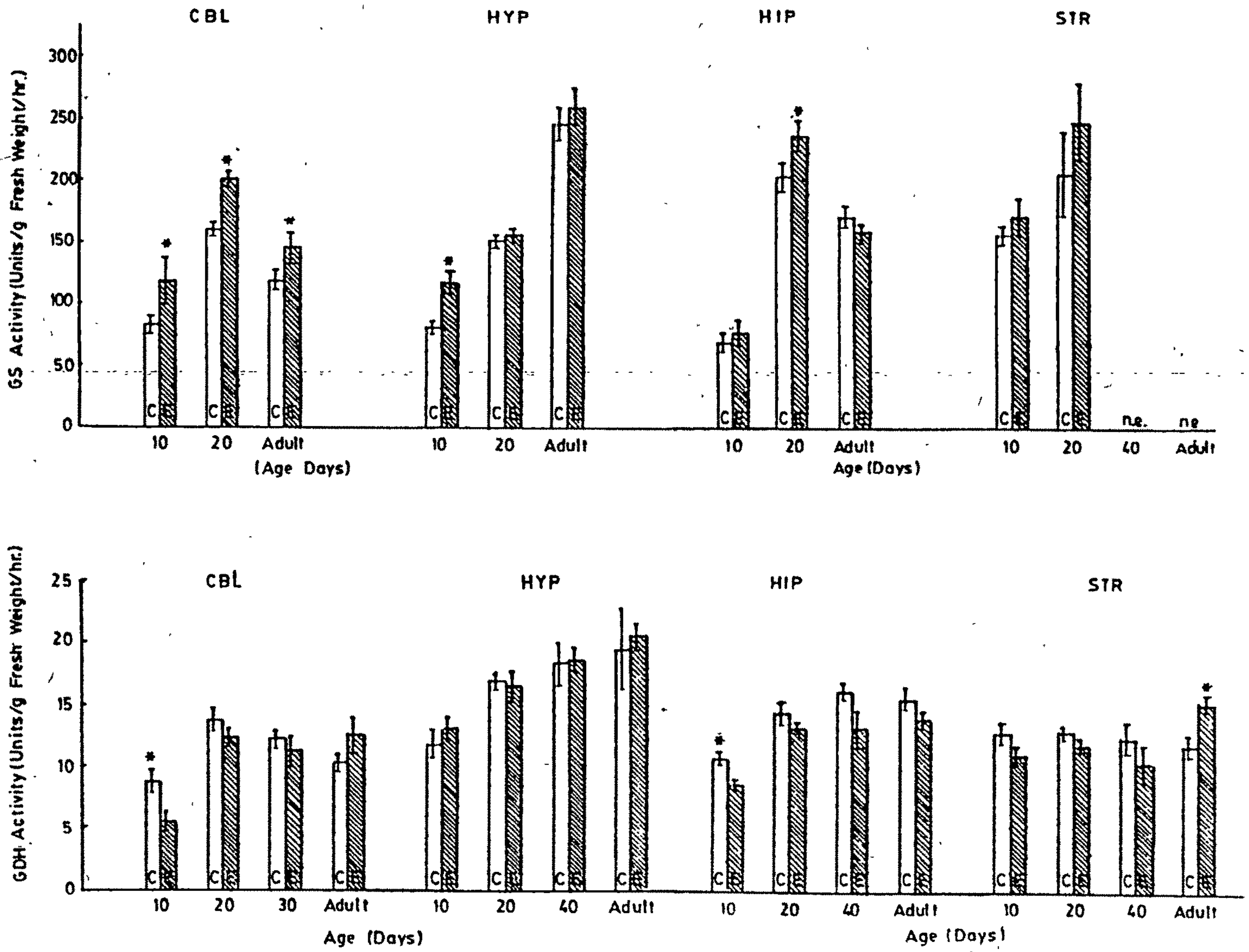


Fig. 12 *Effect of Chronic Corticosterone Administration on GS and GDH Activity In the Rat Brain Regions during development*

(□), Control; (▨), Experimental

* *P <0.05, values significantly different from controls*

Values are mean of 7-10 number of independent observations

Adult refers to >90 day old animals

Because of it's exclusive presence in astrocytes, GS has been used as a marker of astrocyte differentiation. Glucocorticoid induction of GS during development both *in vivo* and *in vitro* has been demonstrated by several investigators (Hallermayer *et al*, 1981; Patel *et al*, 1983b; Chatterjee and Sarkar, 1984). The present study extends these results to developing brain regions such as hippocampus, hypothalamus and cerebellum. Patel *et al*. (1983b) have obtained a similar result and showed age dependent GS increase in the forebrain and olfactory bulb only at day 10 and 20 while in the cerebellum increased GS activity was noticed even at adult stage. The differences in responsiveness with age and regions were attributed to the maturational stage of the tissue and also to the differences in biochemical make up of the astrocytes in these regions. Furthermore, the increased GS activity has been shown to be due to the increased enzyme protein (Patel *et al*, 1983b). Other than glucocorticoids, several hormones also induce GS activity in astrocyte culture derived from cerebral cortex. Thyroid hormone and insulin induced synergistically GS activity with hydrocortisone in astrocyte culture (Aizenman and De Vellis, 1987) suggesting a complex interplay of various hormones during brain development. In addition to the development of GS activity in the rat brain, glucocorticoids have also been shown to be essential for maintaining high levels of GS in the adult (Chatterjee and Sarkar, 1984). The implications of age dependent increase of GS by corticosterone administration in hippocampus and hypothalamus, however, are not yet clear. Several earlier reports cited above in this regard seem to suggest that hormonal imbalance during development could affect the processing of amino acid transmitters as glutamine synthesized in glial cells may serve as precursors for the synthesis of glutamate, GABA, in the synthesis of proteins, nucleotides and glucosamine-6-phosphate.

3.1.3.4 GDH :

Glutamate dehydrogenase which catalyses interconversion of glutamate and 2-oxoglutarate is a key enzyme linking the tricarboxylic acid cycle with amino acid metabolism and also closely involved in the formation of ammonia. It's activity was significantly decreased in 10 day old hippocampus and cerebellum (Fig.12 and Table- 8). Except an increased activity in the adult striatum none of the regions showed any effect at days 20, 40 and adult stage. As mentioned earlier, reports on corticosterone effect on GDH activity in brain regions are very meagre. Sadasivudu et al. (1977) have shown an increased GDH activity only in cerebral cortex with chronic hydrocortisone administration to adult mice. No change in adult cerebellar GDH activity observed in the present study compares well with a report by Sadasivudu et al. (1977). The increased GDH activity in the cerebral cortex has been attributed to increased utilization of amino acids as an alternative source of energy in the event of blockage of glucose oxidation by hydrocortisone. Decreased GDH activity in the cerebellum and hippocampus observed only at day 10 may reflect a specific pool/compartment susceptible during development. It may be relevant to note here that in liver cells,GDH activity was reduced by certain steroid hormones derived from pregnenolone by the alteration of GDH structure so as to favour disaggregation into monomers (Tomkins and Yielding, 1965). Interestingly, both GDH and GABA-T localised in the mitochondria were inhibited with corticosterone treatment, although at different ages.

To summarize, it is evident that corticosterone has an age dependent effect with younger animals more responsive than the adults. The effect seems to be region specific as hippocampus showed maximal response followed by striatum by around postnatal day 20. A general increased GAD and GABA-T activities in these regions, atleast, during the rapid phase of development of these enzymes, suggest a possible increased turnover of GABA with an elevated corticosterone level in the blood at younger ages. Adult brain regions were not responsive to hormone treatment inspite of having large number of corticosterone receptors.

3.2 STUDIES ON BIOAMINES

Association between corticosteroids and bioamines has been very well known. Most of the studies in this regard pertained to adrenal - pituitary - hypophysis relationship which is thought to be central to human adaptation to changing internal and external environment. Glucocorticoids have been implicated in several behavioural alterations, psychiatric disorders in humans and associated with alteration in bioamines in the limbic system (Schatzberg *et al*, 1985). These studies were mainly concerned with adults and not much attention was paid to its importance in young animals.

It was evident from the studies on enzymes of GABA metabolism that the effects of corticosterone administration in young and adult animals have been quite different in different regions depending upon the maturational stage of the enzyme. A greater responsiveness of animals at 20 days of age was observed in general. In contrast to the enzymes of GABA metabolism, considerable information regarding the ontogenic pattern of key enzymes involved in bioamine metabolism (TH, DBH, and TPH) have been reported. Studies on these enzymes with respect to steroid treatment indicate that there is an age specific induction of TH and TPH activities between 2nd and 3rd postnatal weeks (Sze *et al*, 1980). Considering the fact that adult hippocampus and striatum are known to be responsive to corticosteroids, there is no information available with regard to sensitivity/response of immature hippocampus and striatum.

Glucocorticoids, as mentioned earlier, affect hepatic tryptophan and overall amino acid metabolism by the induction of several enzymes such as tryptophan oxygenase, tyrosine transaminase etc., and lead to decreased blood levels of these amino acids. It has been established that the blood levels of tryptophan and tyrosine influence their availability in brain for synthesis of catecholamines and 5-HT and lead to altered levels of these neurotransmitters. Several factors that influence the blood levels of tyrosine and tryptophan and consequently their supply to the brain include age (Sarna et al, 1982), certain pathological conditions (Mans et al, 1979) involving altered kinetic characteristics of the tryptophan uptake by the brain, physiological changes associated with stress (Curzon et al, 1972) and increased blood corticosteroid level by exogenous administration in adult animals (Table- 9). Recently Kennett et al. (1986) have suggested that the increased brain tryptophan and 5-HT synthesis under immobilization could also be due to altered amino acid metabolism, efflux or altered influx kinetics. Furthermore, during development, the unidirectional transport of tryptophan across the blood-brain barrier due to saturable mechanism is higher than in adult brain (Sarna et al, 1982). In the context that glucocorticoid has been shown to alter tryptophan uptake by

TABLE-9 : Reported studies on the effect of exogenous administration of glucocorticoids on changes in bioamines of the adult brain.

Drug	Dose and mode	Objective	Regions/parameters	Result
1) Hydrocortisone (saline)	Single dose of 15 mg kg^{-1}.	To confirm their earlier finding of a decreased brain 5-HT metabolism with hydrocortisone treatment.	Whole brain/free and total tryptophan, brain 5-HT, 5HIAA.	Showed a ↓ 5-HT, 5HIAA and tryptophan both in whole brain and liver 6 h after I.P. injection of HC. (Green et al, 1975a).
2) Corticosterone (Brij.35 0.005g)	50 mg kg^{-1} x 2 daily for 4 days and sacrificed after 20 h.	To find out the association on relation of C with the changes in behavioural and 5-HT associated mechanism under immobilization stress.	Striatum and Hind. brain/ Try, 5HT, 5HIAA, DA, DOPAC & HVC.	No changes in 5HT & DA metabolism except that HVA ↑ with treatment in striatum. However, behavioral changes in response to 5-HT releaser (PAC (p-chloroamphetamine) and agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) were reduced indicating postsynaptic changes in response. Rats response to open field activity such as hyperactivity and ↓ defaecation suggest the stress-induced C release may oppose some of the behavioral consequences of stress. (Dickerson et al, 1985).
3) Methyl prednisolone sod. succinate. (Adult cats)	15, 30, 90 mg kg^{-1} I.V. Single dose - sacrificed after 1 and 24 hr.	To see the effect of increased GC activity on central 5HT and catecholaminergic system as a function of dose and time.	Cat lumbar spinal cord/ DA, NE & 5HT.	Acute 90 mg kg^{-1} of GC produces a biphasic effect. ↑ DA, NE & 5HT after 1 h but ↓ in DA, NE & 5HT after 24 h. shows dose related in DA & 5HT levels but not in NE. (Hall and McGinley, 1982)
4) Hydrocortisone (Mice)	20 mg/kg	Action of GC on uptake of Tryptophan in the brain and the mechanism of hormone regulation of 5HT metabolism.	5HT, whole brain/Tryptophan, TH, Tyrosine in synaptosome.	5HT ↓ in whole brain. No change in TH activity. ↑ tryptophan uptake into brain and synaptosomal fraction. (Neckers and Sze, 1975)

TABLE-9 (Contd..)

Drug	Dose/model	Objective	Regions/parameters	Result
9) Dexa-methasone (saline)	20 ug I.P.; sacrificed either 1 or 4 hr after injection**	To examine C effect on DA, 5HT levels and turnover in discrete brain areas.	Cortex, n. accumbens, STR and HYP/NE, 5HT, DA, DOPAC, HVA & 5HIAA.	Region specific effect on DA system seen. No effect on behavior. ↓ in DA in hyp., n.accumbens Cortex and STR. ↓ DA turnover in n. accumbens. ↑ 5HT in hypothalamus and ↓ in 5HT in cortex. (Rothschild et al,1985)
10) Cortico-sterone (rats) in 10% ethane.	1.0 or 10.0 mg kg^{-1} I.P. 30 or 120 min prior to the decapita-tion.	To clarify the action of C admini-stration in differ-ent doses and at different time inter-vals on cerebral 5HT metabolism.	Hypo., Hippo-mesencephalon, Frontal cortex, parietal cortex/5HT, 5HIAA & 5HT turnover by pargyline (75 mg/kg i.p.) at 10 or 90 min interval.	1.0 mg kg^{-1} C ↑ 5-HT in hypo. and mesencephalon whereas 10 mg kg^{-1} ↓ 5HT after 30'. No effect after 120' either with 1 or 10 mg mg^{-1} C. ↑ 5-HIAA in Hypo. but ↑ in Hip. at 1 mg kg^{-1}. At 10 mg kg^{-1} ↓ 5HIAA Hypo. No changes in 5HIAA at 120 min after injection. 5HT turnover ↑ after 1.0 mg kg^{-1} administration in hyp. and mesence-phalon. No change in Hip. or P-, F.cortex. but ↓ at 10 mg kg^{-1} in hyp. (Kovacs et al, 1977).
11) Hydrocor-tisone Gerbil & rats.	50 mg/kg 4 h before sacrifice.	To find out the rela-tionship between peripheral tryptophan metabolism with 5HT metabolism in brain.	Whole brain/5HT & 5HIAA liver pyrrolase activity.	Liver pyrrolase activity ↑ and brain 5HT and 5HIAA ↓ suggesting a ↓ in 5HT synthesis as a result of ↓ in brain tryptophan concentration. (Green et al, 1975a)

* Dosage chosen because of the level of C after acute immobilization stress equals this level in plasma.

** 4 hr time frame used since a low dose of D suppress C.

TABLE-9 (Contd..) Effect on bioamines in the developing brain.

Drug	Mode/dose	Objective	Region/Parameters	Result
1) Cortisol acetate	1 mg S.C. on day1.	Effect of single, neonatal injection on brain 5HT development.	Pons-medulla, hypothalamus/ Tryptophan pyrrolase and 5HT.	Premature induction of tryptophan pyrrolase and ↓ 5HT in pons-medulla but ↓ in tryptophan pyrrolase from day 15 with concomittant ↑ in 5HT suggest a linkage between tryptophan pyrrolase and brain 5HT production. (Yuwiller et al, 1974)
2) Dexamethasone	1 mg kg^{-1} S.C. for 4 days after birth.	To find out whether the effect of/or alterations in development oe neurotransmission with glucocorticoid reflect a general retardation of growth or on synaptic maturation.	Whole brain, heart, synaptosome, levels, turnover(t.o), uptake of NE & DA in synaptosomes and vesicles -MPT - 300 mg/kg I.P. 5 h before estimations at 2,4,8,11,15,18 & 21 days ODC and polyamine levels.	Development of ODC and polyamine levels are delayed in brain (2 days) and heart (obliterated) - delays central CA synaptic development but seems secondary to cellular development and growth. - uptake of NE into synaptosomes and vesicles were affected. - NE & DA levels were spared. - ↑ in NE t.o. both in cardiac tissue as well as brain seen. (Slotkin et al, 1982).
3) Corticosterone. Rats.	20 ug/kg b.w. I.P. (days 2-4) & (16-18) sacrificed on 16,18 & 21 days	To elucidate the effect of Thyroxine & corticosterone on the development of hypothalamic and mesencephalic NE and DA content.	Hypothalamus and mesencephalon - DA, NE at 16, 18 and 21 days.	NE ↓ both regions at 16,18 & 21 days. DA ↓ at 21 days in hypo. DA ↑ at 18 & 21 days in mesencephalon. C-treatment at 16-18 ↑ hypothalamic. NE on day 21. (Lengvari et al,1980)
4) Hydrocortisone (Sal) Rats.	57 mg/kg/day 12 or 15 day of gestation. Sacrificed at 20-21 day C and 12-13 of neonatal period.	-do-	Whole brain DA & NE, 5HT levels	- Does not affect the proliferation of amine cell bodies or the arrival of axon terminals in the regions where synapses form. (Van Geijn et al, 1979)
5) Hydrocortisone.	1 mg in C.M.C. by S.C. at birth. Single injection.	To determine whether enzyme induction in the neonate might effect the CNS.	- Liver tryptophan oxygenase Tryptophan, 5HT. - Pons-medulla, Thal., Hypothalamus. 5HT turnover at 10 days whole brain TH activity.	-Brain 5HT pathway is minimally affected by Try. oxygenase induction. No change in 5HT turnover in pons-medulla and Thalamus. Induced at 7 & 14 days but not at 21 days. - delays blood brain barrier. (Yuwiller et al, 1978).
6) Hydrocortisone acetate	1 mg S.C. at birth.	With a view to assess the changes associated with alteration in the circadian rhythm neonatal HC treatment.	Basomedial hypothalamus NE, DA & 5HT corticosterone (Plasma).	- ↑ NE & 5HT but no change in DA levels. (Ulrich et al, 1975).

synaptosomes (Neckers and Sze, 1975) and several transport processes both in brain (Cremer et al, 1976) and peripheral tissues (Cf. Munck et al, 1984), the effect of elevated corticosteroid during development in the amino acid precursors tyrosine and tryptophan availability in brain regions was studied. In addition, neurotransmitter metabolites, DOPAC, 5HIAA and HVA were also measured in different brain regions during development as these compounds have been widely measured in the CSF of both adult and child neuropsychiatric populations (Cohen et al, 1980; Shaywitz et al, 1983). Taking these points into consideration, studies were carried out essentially on the effect of corticosteroid treatment at different ages in three regions namely, hypothalamus, hippocampus and striatum with respect to precursors, neurotransmitters and their metabolites discussed above. In order to correlate the change , if any, with the maturation of bioamine systems, developmental pattern of these parameters was obtained by a separate experiment.

3.2.1 Studies on 5-HT Metabolism

3.2.1.1 Development : Table- 10 gives the developmental profile of tryptophan in the hippocampus, hypothalamus and striatum. Tryptophan, in all the three regions was highest at 10th day and decreased between 10 and 30 days sharply to near adult level. Regional concentration of tryptophan was highest in striatum followed by hippocampus and hypothalamus at 10 days. A maximal fall was observed in hippocampus between 10 and 30 days. Such changes in tryptophan levels* in the whole brain and gross regions have been reported (Sarna et al,1982; Shaywitz et al, 1985).

* refers to concentration throughout.

TABLE- 10 : The developmental pattern of tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindole-acetic acid (5HIAA) in rat brain regions.

Brain region	Age (days)	Tryptophan		5-HT		5HIAA	
Hypothalamus	10	8290 ± 290	(11)	380 ± 10	(11)	510 ± 30	(11)
	30	5100 ± 150	(13)	830 ± 20	(12)	710 ± 20	(12)
	50	5490 ± 140	(6)	840 ± 30	(6)	780 ± 20	(6)
	Adult	5900 ± 180	(10)	1040 ± 10	(10)	660 ± 40	(11)
Striatum	10	12860 ± 580	(10)	160 ± 10	(10)	410 ± 30	(11)
	30	5760 ± 180	(13)	490 ± 20	(12)	840 ± 20	(12)
	50	6320 ± 110	(6)	570 ± 40	(6)	730 ± 40	(6)
	Adult	5220 ± 240	(12)	520 ± 10	(11)	620 ± 20	(11)
Hippocampus	10	11220 ± 390	(11)	170 ± 10	(10)	270 ± 10	(10)
	30	4860 ± 190	(11)	370 ± 10	(13)	430 ± 20	(12)
	50	5960 ± 160	(6)	380 ± 20	(6)	520 ± 20	(6)
	Adult	4460 ± 140	(6)	400 ± 20	(6)	420 ± 20	(6)

The concentrations are in ng g-1 of fresh tissue (± S.E. of mean, number of experiments in parenthesis).

In contrast, 5-HT levels increased in the hypothalamus rapidly after 10 day and rather slowly between day 50 and adult stage (Table-10). In the striatum and hippocampus, adult levels were reached by day 30 only. This is in agreement with the attainment of adult level of activity of TPH by postnatal day 30 in the whole brain (Schmidt and Sanders-Bush, 1971) and the development of neurotransmitter storage capacity in 5HT neurons. (Tissari, 1973).

During development values of 5HIAA were similar to adult values between day 10 and 30 in both the hypothalamus and hippocampus (Table-10). A slight increase in 5-HIAA levels were observed between day 30 and 50 which declined to 30 day levels in both these regions. This pattern of 5HIAA levels in the rat and mouse brain has been reported earlier, with a peak in 5HIAA level at day 20 (Sarna et al, 1982; Baker et al, 1974). In the case of striatum, 5HIAA levels increased maximally by 30 days, well above adult level, and declined thereafter. A higher level of 5HIAA at younger ages has been attributed to the increased breakdown of 5HT formed in 5HT neurons by MAO-A activity (Bourgoin et al, 1974) which may also be due to the lower storage capacity of 5HT at early age. At adult stage, however, the levels of 5HIAA in the three regions paralleled that of 5HT concentration with the hypothalamus having maximal value followed by striatum and hippocampus. These observations, thus, indicate a maximal activity of synthesis, storage and/or transport of 5HT and 5HIAA in these brain regions between day 10 and 30. Therefore, the age groups of day 10, 30 and adult were chosen for the steroid treatment experiment.

3.2.1.2 Effect of Corticosterone Treatment

(a) Chronic treatment : Chronic corticosterone treatment at various ages affected 5HT metabolism in a region specific manner in adult animals but not in the young (Fig. 13 and Table- 11). At 10 days of age, tryptophan levels were decreased in all the three regions while changes were not observed at adult stage. Only 30 day hippocampus showed a decreased tryptophan concentration with the treatment.

Inspite of dramatic decrease in tryptophan levels at 10 days of age, there was no effect on the 5HT concentration in any of the regions studied. On the other hand, even though no change in tryptophan was observed at adult stage, only hippocampus had a significantly decreased 5HT concentration.

Significant decrease in 5HIAA levels were observed at 10 day old hypothalamus, hippocampus and striatum while in adults decrease was observed only in the hippocampus. Interestingly at day 30 no parameter of 5HT metabolism was affected with steroid treatment except the decreased tryptophan level in the hippocampus (Table- 11).

If alteration in the metabolite level is considered as an index of alteration in the activity of serotonergic cells, present results indicate a significant decrease in the serotonergic cell activity in adult hippocampus and perhaps in all

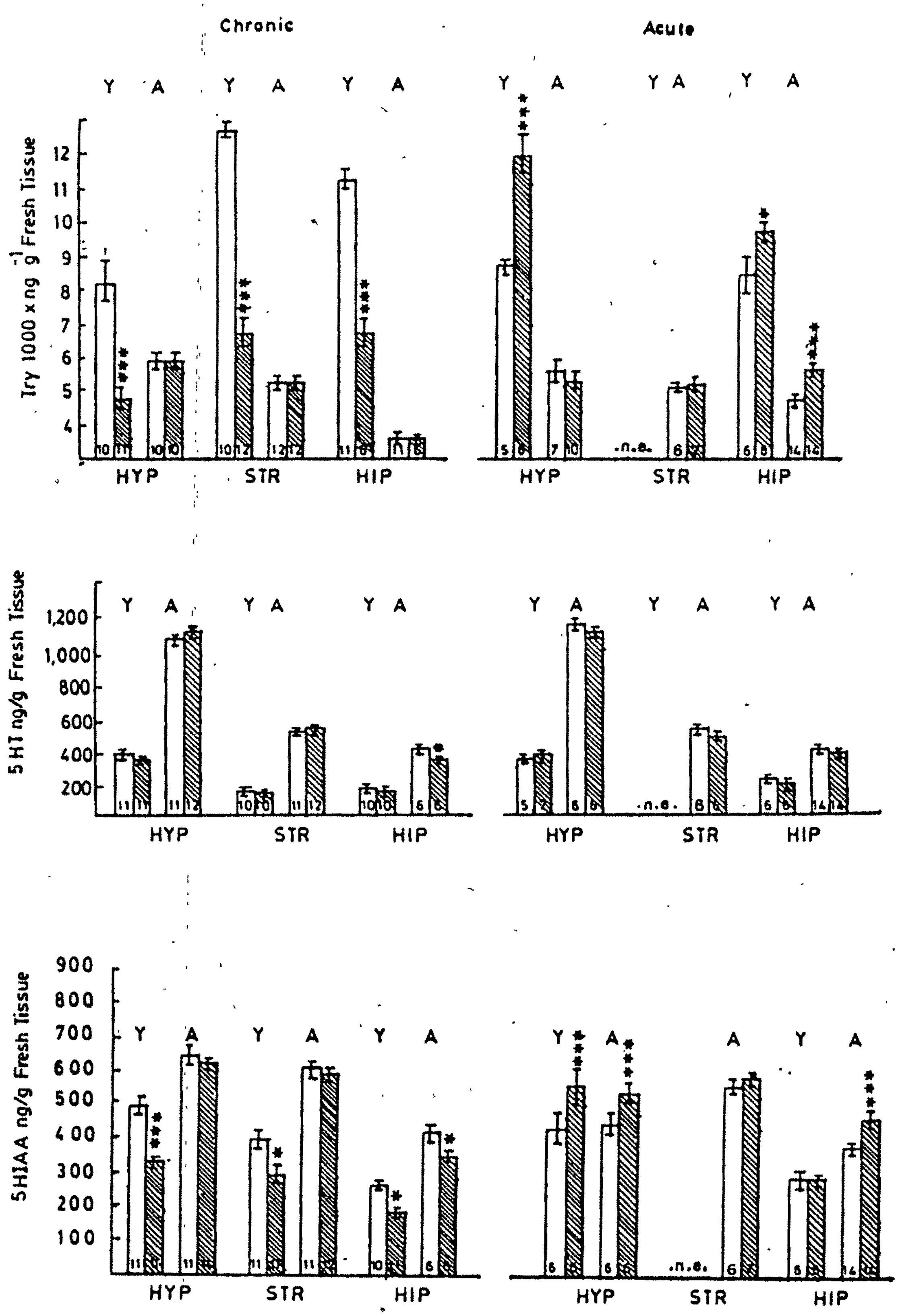


Fig. 13 ***Effect of Chronic and Acute Corticosterone Administration on Try, 5HT and 5HIAA Concentrations In The Rat Brain***

(▭), Control; (▨), Experimental

Values significantly different from Controls * $P<0.05$

** $P<0.01$

*** $P<0.001$

n.d., not detectable; n.e., not estimated; y, 10 day of age and A, >90 day of age. Number inside the bar refers to number of observations

TABLE- 11 : The effect of chronic corticosterone treatment on tryptophan, 5HT and 5HIAA concentration in rat brain regions at different ages.

Regions	Age (days)		Tryptophan		5HT		5HIAA		5HIAA/5HT
HYPOTHALAMUS	10	C	8290 ± 800	(10)	380 ± 10	(11)	510 ± 30	(11)	1.34
		T	4800 ± 260***	(11)	350 ± 10	(11)	340 ± 15***	(11)	0.97
	30	C	5100 ± 150	(13)	830 ± 20	(12)	710 ± 20	(12)	0.855
		T	4590 ± 240	(7)	900 ± 40	(7)	750 ± 40	(7)	0.833
	Adult >90	C	5890 ± 230	(10)	1060 ± 30	(11)	660 ± 40	(11)	0.623
		T	5900 ± 180	(10)	1100 ± 40	(12)	630 ± 20	(11)	0.573
STRIATUM	10	C	12860 ± 580	(10)	160 ± 10	(10)	410 ± 30	(11)	2.56
		T	6730 ± 470***	(11)	140 ± 10	(10)	300 ± 30*	(11)	2.14
	30	C	5760 ± 180	(13)	490 ± 20	(12)	840 ± 20	(12)	1.71
		T	5400 ± 200	(6)	440 ± 20	(7)	840 ± 30	(7)	1.91
	Adult (90)	C	5220 ± 240	(12)	520 ± 10	(11)	620 ± 20	(11)	1.19
		T	5300 ± 230	(12)	530 ± 10	(12)	600 ± 20	(12)	1.14

Contd...

TABLE- 11 (Contd...)

Regions	Age (days)		Tryptophan		5HT		5HIAA		5HIAA/5HT
	10	C	11220 ± 390	(11)	170 ± 10	(10)	270 ± 10	(10)	1.59
		T	6730 ± 470***	(11)	160 ± 10	(10)	190 ± 10*	(11)	1.18
HIPPOCAMPUS	30	C	4860 ± 200	(11)	370 ± 10	(13)	430 ± 20	(12)	1.16
		T	3900 ± 90*	(7)	360 ± 20	(7)	390 ± 20	(8)	1.08
	Adult (90)	C	3600 ± 110	(6)	400 ± 20	(6)	430 ± 20	(6)	1.08
		T	3390 ± 80	(6)	330 ± 20*	(6)	360 ± 10*	(6)	1.09

Values are expressed as ng g^{-1} tissue ± S.E. with number of observations in parenthesis;

* $P < 0.05$; *** $P < 0.001$.

C and T refers to Control and Corticosterone treated animals.

the regions in young animals, as evident from 5HIAA/5HT ratio (Table- 11). Studies available in this regard, as given in the Table- 9 , are very scarce and confine only to adult animals. On comparison, no change in the 5HT metabolism in adult striatum is in agreement with that of Dickinson et al. (1985) who have suggested a possible alteration in the post-synaptic response. In contrast, Walkowitz et al. (1986) have reported an increased 5HIAA level in caudate nucleus but not in other limbic structures. The difference may perhaps be due to the discrete nuclei analysed by these authors, for steroid effect, unlike that of striatum used in the present study. Thus corticosterone treatment alters 5HT metabolism in all the three regions in 10 day old animals and only in hippocampus of adults indicating a differential response of adult and young animals.

Most of the studies with stressors are comparable to acute elevation of steroid levels and have been reported in specific regions of adult animals. Since the chronic corticosterone treatment resulted in age and region specific differential response, it was of interest to check whether similar age and associated region specificity can be observed with acute treatment in 10 day old and adult rat brain regions.

(b) Acute Treatment : As shown in the Figure 13 and Table-12, a single dose of corticosterone treatment in young and adult rats seem to have differential effects. As in the case of chronic treatment, effects were observed mainly in precursor and metabolite levels

TABLE- 12 : The effect of acute corticosterone treatment on tryptophan, 5HT and 5HIAA concentrations in hypothalamus and hippocampus at different ages.

	Age (days)		Tryptophan		5HT		5HIAA	
HYPOTHALAMUS	10	C	8710 ± 1720	(5)	340 ± 30	(5)	440 ± 50	(6)
		T	11940 ± 1260	(7)	360 ± 20	(7)	570 ± 50	(6)
	Adult (90)	C	5620 ± 200	(6)	1130 ± 30	(6)	450 ± 40	(6)
		T	5370 ± 270	(10)	1080 ± 20	(6)	550 ± 30***	(6)
HIPPOCAMPUS	10	C	8330 ± 660	(6)	200 ± 10	(6)	310 ± 20	(6)
		T	9730 ± 300*	(6)	190 ± 10	(6)	310 ± 20	(6)
	Adult (90)	C	4780 ± 160	(14)	390 ± 20	(14)	390 ± 10	(14)
		T	5680 ± 140***	(14)	380 ± 10	(13)	480 ± 20***	(14)

Values are expressed as ng g^{-1} tissue ± S.E. with number of observations in parenthesis;

* $P < 0.05$; *** $P < 0.001$, Significantly different from respective controls. C - Control, T - Corticosterone treated animals.

but not in the concentration of 5HT. Tryptophan concentration was increased in both the hypothalamus and the hippocampus at 10 day, however, at adult stage, only hippocampus showed such an increase. Increased 5HIAA concentrations in the adult hypothalamus and the hippocampus but not in the young suggested a possible increased 5HTergic activity mainly at adult stage with acute corticosterone treatment, unlike a decreased activity observed with chronic treatment.

3.2.2.1 Development of Catecholamines :

Catecholamines, NE and DA with their precursors and metabolites were estimated in the hypothalamus whereas in the hippocampus and the striatum only NEergic and DAergic parameters were estimated respectively.

The developmental pattern of the precursor tyrosine is presented in Table- 13 . At adult stage, tyrosine concentration did not differ amongst the three regions. In young animals, a remarkable difference in the tyrosine concentration was evident in all the three regions. During development, the maximum decrease of about 6 fold in the hippocampus and 2-3 fold in hypothalamus and striatum was observed between 10 and 30 days of age. Although on the whole brain basis such decline in brain tyrosine has already been reported (Sarna et al, 1982; Diez et al, 1977), information in this regard is not available with respect to different regions.

TABLE- 13 : The developmental pattern of p-tyrosine, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and noradrenalin (NE) in rat brain regions.

Brain region	Age (days)	p-Tyrosine	DA	DOPAC	HVA	NE
Hypothalamus	10	43680 ± 2750 (10)	130 ± 10 (11)	240 ± 20 (11)	180 ± 10 (11)	510 ± 20 (11)
	30	19700 ± 880 (10)	280 ± 10 (12)	120 ± 10 (13)	n.d.	1630 ± 30 (13)
	50	13590 ± 890 (6)	300 ± 20 (6)	120 ± 10 (6)	n.d.	1805 ± 30 (6)
	Adult (>90)	10050 ± 810 (5)	390 ± 10 (6)	120 ± 5 (6)	n.d.	2430 ± 130 (6)
Striatum	10	59620 ± 3470 (11)	2950 ± 110 (11)	700 ± 20 (11)	410 ± 30 (11)	n.d.
	30	21290 ± 860 (12)	6950 ± 220 (12)	1120 ± 30 (9)	840 ± 20 (12)	n.d.
	50	15650 ± 1120 (6)	9030 ± 410 (6)	1570 ± 60 (6)	930 ± 50 (6)	n.d.
	Adult (>90)	12270 ± 660 (6)	11260 ± 70 (6)	1540 ± 40 (6)	680 ± 40 (5)	n.d.
Hippocampus	10	56940 ± 1220 (9)	n.d.	n.d.	n.d.	60 ± 10 (9)
	30	12790 ± 300 (6)	n.d.	n.d.	n.d.	270 ± 20 (12)
	50	14560 ± 1060 (6)	n.d.	n.d.	n.d.	320 ± 20 (6)
	Adult (>90)	10820 ± 760 (6)	n.d.	n.d.	n.d.	530 ± 40 (5)

The concentrations are in ng g-1 of fresh tissue (±S.E. of mean, number of experiments in parenthesis). Values that were found below the limit of sensitivity of the measurement are indicated by n.d.

Decrease in tyrosine was associated with increase in DA and NE levels in the hypothalamus, DA in the striatum and NE in the hippocampus. The maximum increase in neurotransmitter levels were observed between day 10 and 30 in all the three regions with a small but significantly continued increase beyond 30 days.

With regard to metabolites of DA, both DOPAC and HVA concentrations increased in striatum during development. In contrast, in hypothalamus DOPAC concentration was maximum at day 10 which decreased sharply to adult levels by day 30. The developmental changes in the concentration of neurotransmitters (DA and NE) and metabolite DOPAC are consistent with the pattern reported earlier on whole brain (Cf. Lanier et al, 1976; Shaywitz et al, 1985) and of HVA in the striatum (Nomura et al, 1979).

Thus the maximum changes occur between 10 and 30 days of age with a small but significant change beyond 50 days of age suggesting a very slow development of the catecholaminergic system in comparison with the 5HT system. Notable amongst the three regions is the striatum for such a slow maturational pattern.

3.2.2.2 : Effect of Corticosterone Treatment

(a) Chronic Treatment : Chronic corticosterone treatment resulted in decreased concentration of tyrosine in all the three regions at day 10 and in the hypothalamus only at day 30 (Table-14 and Fig.14). At adult stage, both hypothalamus and striatum showed a decrease while the hippocampal tyrosine concentration was not estimated.

DA concentration was not altered in all the three brain regions at any time period with steroid treatment. NE, however, increased only in the hypothalamus at day 10 and 30 and not in other two regions.

Metabolites DOPAC and HVA were altered with chronic corticosterone treatment. DOPAC concentration was decreased in all the three regions at day 10 whereas in adult, it decreased only in the hypothalamus. On the other hand, HVA concentration was increased only in striatum at day 10 but not in the hypothalamus.

From these results, it is evident that as in the case of 5HT metabolism, the precursor tyrosine and the metabolite DOPAC concentrations were decreased uniformly in all the three regions at day 10 but only in the hypothalamus at adult stage.

TABLE- 14 : The effect of chronic corticosterone treatment on p-Tyr, DA, DOPAC, HVA and NE concentrations regions of the rat brain at various ages.

Region	Age (days)		p-Tyrosine	DA	DOPAC	HVA	NE	DOPAC/DA
HYPOTHALAMUS	10	C	43680 ± 2750 (10)	120 ± 10 (11)	240 ± 10 (11)	180 ± 10 (11)	510 ± 20 (11)	2.0
		T	23560 ± 680*** (10)	140 ± 10 (10)	190 ± 10** (11)	160 ± 10 (11)	570 ± 20* (10)	1.36
	30	C	19700 ± 890 (10)	270 ± 10 (12)	120 ± 10 (13)	n.d. (12)	1630 ± 30 (13)	0.44
		T	13950 ± 720*** (6)	300 ± 20 (6)	130 ± 10 (7)	n.d. (7)	1830 ± 80* (7)	0.43
	Adult (>90)	C	18930 ± 640 (10)	600 ± 20 (11)	110 ± 20 (11)	n.d. (11)	[illegible] (4)	0.16
		T	13460 ± 450*** (10)	630 ± 20 (11)	80 ± 10* (11)	n.d. (11)	2140 ± 80 (11)	0.13
STRIATUM	10	C	59620 ± 3470 (11)	2950 ± 110 (11)	700 ± 20 (11)	410 ± 30 (11)	70 ± 6 (10)	0.24
		T	31980 ± 1510*** (11)	2800 ± 100 (11)	520 ± 20*** (10)	470 ± 30** (10)	70 ± 5 (11)	0.19
	30	C	19300 ± 760 (7)	6940 ± 230 (12)	930 ± 20 (6)	600 ± 20 (6)	60 ± 10 (6)	0.09
		T	18920 ± 690 (6)	7360 ± 150 (6)	980 ± 30 (6)	660 ± 20 (6)	70 ± 10 (6)	0.10
	Adult (>90)	C	15420 ± 510 (11)	9200 ± 250 (12)	1100 ± 10 (11)	750 ± 30 (12)	50 ± 20 (10)	0.12
		T	13,270 ± 550 (11)	9840 ± 420 (12)	1120 ± 50 (12)	790 ± 40 (12)	60 ± 10 (10)	0.12
HIPPOCAMPUS	10	C	56940 ± 4080 (10)	n.d. (10)	170 ± 10 (10)	n.d. (10)	60 ± 5 (9)	-
		T	26860 ± 730*** (11)	n.d. (11)	120 ± 4*** (10)	n.d. (10)	70 ± 3 (10)	-
	30	C	12,700 ± 300 (6)	n.d. (6)	50 ± 2 (12)	n.d. (6)	270 ± 10 (12)	-
		T	11630 ± 450 (8)	n.d. (6)	52 ± 8 (6)	n.d. (6)	260 ± 10 (10)	-
	Adult (>90)	C	n.e.	n.d. (6)	n.d. (6)	n.d. (6)	260 ± 20 (6)	-
		T	n.e.	n.d. (6)	n.d. (6)	n.d. (6)	250 ± 20 (6)	-

Values are expressed as ng g^{-1} tissue ± S.E. with number of observations in parenthesis;
* P ∠0.05; ** P ∠0.01; *** P ∠0.001;
n.d. - non detectable and n.e. refers to not estimated.
C - Control; and T - Corticosterone treated animals.

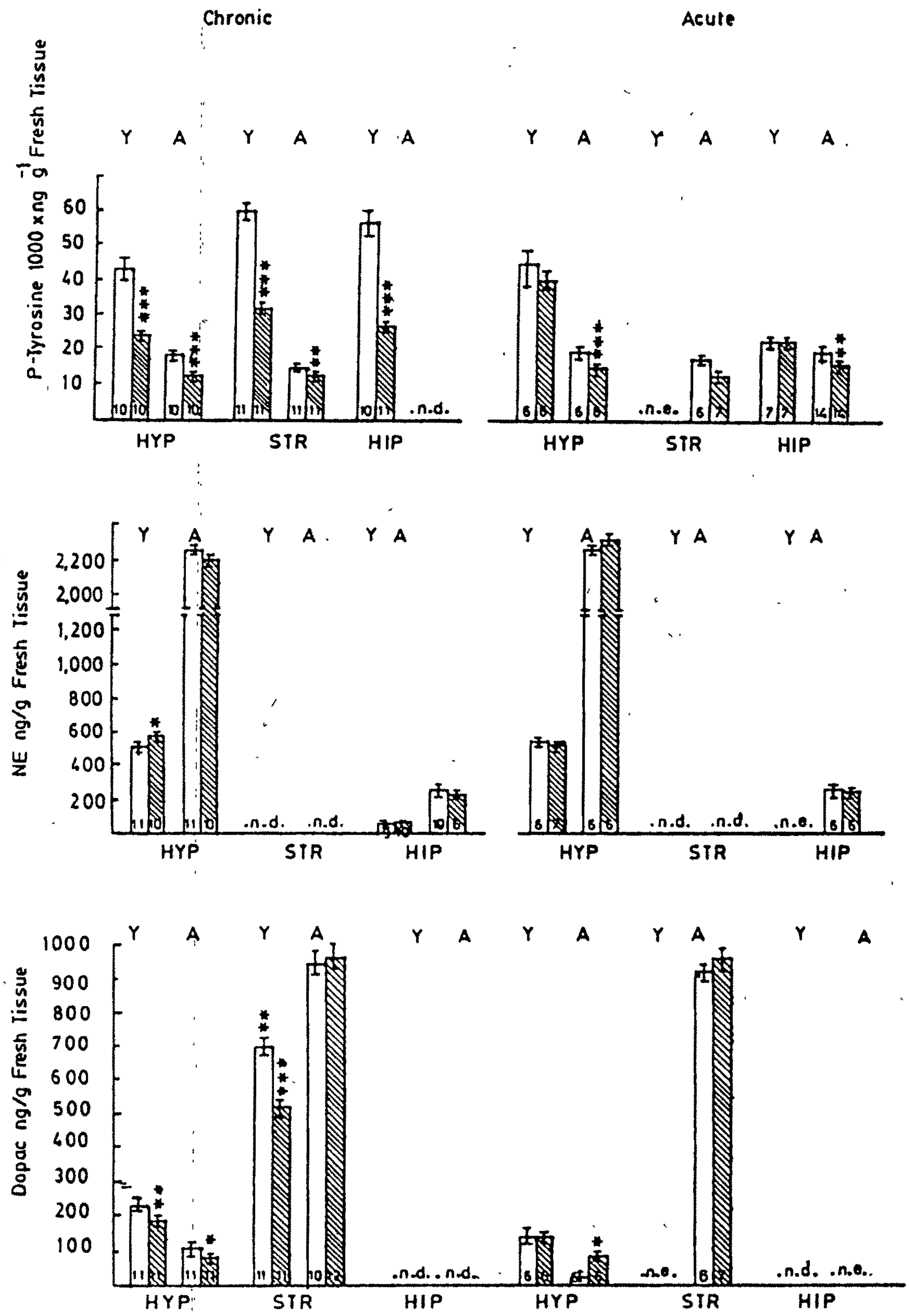


Fig. 14 **Effect of Chronic and Acute Corticosterone Administration on p-Tyrosine, NE, Dopac Concentrations In the Rat Brain Regions**

(☐), Control; (▨), Experimental

Values significantly different from Controls * $P<0.05$

** $P<0.01$

*** $P<0.001$

n.d., not detectable; n.e., not estimated; y, 10 day of age and A, 90 day of age. Number inside the bar refers to number of observations

(b) Acute Treatment : The results of acute corticosterone treatment on the above mentioned parameters in the hypothalamus and the hippocampus are given in the Table- 15 and Fig.14. In contrast to chronic treatment, acute treatment decreased p-tyrosine concentrations only at adult stage both in the hypothalamus and the hippocampus.

The concentrations of DA and NE were not changed in both these regions at day 10 and adult stage. Only DOPAC concentration was decreased significantly in the adult hypothalamus. The above mentioned observations indicate that with acute treatment the hypothalamic dopaminergic system was altered.

Thus, effects of steroid treatment on bioamine metabolism (Table-14,15) in different brain regions at different ages, indicate that the level of precursor amino acids, tyrosine and tryptophan were affected most. Amongst the two, tryptophan seems to be greater responsive. The alteration in brain tryptophan levels associated with elevated blood corticosterone levels reported so far has been controversial. Several studies using different stressors have observed a consistent rise in the brain tryptophan and 5HT turnover (Kennett and Joseph, 1981)(Table- 9 This rise has been attributed to increased corticosteroid level in the plasma. With the exogenous administration of a single dose of corticosteroid both an elevation (Millard et al, 1972; Hiller et al, 1975; Neckers and Sze, 1975) and a consistent

TABLE- 15 : The effect of acute corticosterone treatment on p-Tyr, DA, DOPAC and NE concentration in rat hypothalamus and hippocampus at 10 and 90 days of age.

Brain region	Age (days)		p-Tyrosine	DA	DOPAC	NE
HYPOTHALAMUS	10	C	45,590 ± 3970 (6)	100 ± 10 (6)	150 ± 10 (6)	541 ± 23 (6)
		T	40,290 ± 2050 (6)	100 ± 5 (6)	150 ± 2 (6)	518 ± 26 (7)
	Adult (>90)	C	20,790 ± 970 (6)	450 ± 30 (6)	25 ± 20 (6)	2260 ± 70 (6)
		T	15,020 ± 480*** (6)	520 ± 40 (6)	90 ± 10 (6)	2340 ± 20 (6)
HIPPOCAMPUS	10	C	22,990 ± 1430 (7)	n.d. (7)	n.e.	n.e.
		T	22630 ± 360 (7)	n.d. (7)	n.e.	n.e.
	Adult (>90)	C	20,250 ± 890 (14)	n.d. (14)	n.d. (14)	260 ± 20 (6)
		T	17,120 ± 640** (14)	n.d. (14)	n.d. (14)	250 ± 20 (6)

Values are expressed as ng g^{-1} tissue ± S.E. with number of observations in parenthesis;
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. n.d. - not detectable and n.e. - not estimated.
C - Control; and T - Corticosterone treated animals.

decrease (Green et al, 1975a) in the brain tryptophan has been reported. The difference in the result has been attributed to several factors such as the time, dose and form of the hormone injected apart from time lapse between the hormone treatment and the analysis (Green et al, 1975b); the former two have estimated 1 hr and 3 hr after the treatment whereas the latter after 6 hr. Studies of Diez et al. (1976), however, report a consistent increase in brain tryptophan level from 30 min after treatment till several hours, and attribute it to increased availability of the tryptophan from plasma. All these three studies have been done on whole brain. In the present study, the increase in tryptophan concentration was evident only in the hippocampus but not in the hypothalamus. With regard to young animals, Yuwiller et al.(1978) have shown an increased uptake of tryptophan 12 days after a single dose of steroid given at the time of birth and this effect lasted till 44 days. Such an age dependent effect has also been suggested by Green et al. (1975b). In the present study, only hippocampus of 10 day old animal showed a significant increase but not the hypothalamus. This increase in brain tryptophan could be due to increased availability of free tryptophan in the plasma or due to alteration in the kinetic properties of the uptake system.
In contrast to the single dose of steroid treatment, chronic injection for three days significantly decreased the concentration of tryptophan in all the regions in 10 day and only in the hippocampus at day 30; adult brain regions did not show any effect. This response to chronic treatment, perhaps, can be attributed to increased pyrrolase activity in the peripheral

system or alteration in the transport process as in young animals; the kinetics of transport process indicate a higher influx and less responsiveness to other plasma factors (Sarna et al, 1982). The unidirectional transport is saturation dependent and therefore, plasma levels of tryptophan results in alteration in the regional levels of tryptophan. The regional specificity seems to arise by 30 days of age mainly in the hippocampus which shows maximal change in tryptophan levels between day 10 and 30 and respond to steroid treatment in the adult stage predominantly with respect to 5HT turnover.

With regard to tyrosine levels not many studies have been reported, partly because of the fact that no clearcut relationship has been established between the plasma levels of tyrosine and the related transmitters in the brain. The effects on regional tyrosine levels were uniform both with acute and chronic treatment. Diez et al. (1977), with a single dose of hydrocortisone in adult mice have reported a biphasic effect on whole brain tyrosine levels. In their study brain tyrosine levels increased transiently immediately after steroid injection and decreased below control levels after 2 hrs which remained low till 8 hrs. In another study by the same group (Neckers and Sze, 1975) tryptophan concentration increased with hydrocortisone suggesting that with acute treatment both tyrosine and tryptophan levels show opposite changes. This is consistent with the present study which monitored these precursor amino acid levels 2 hr after steroid treatment. This pattern remained uniform at 10 days and perhaps even at adult stage. The result that, such changes were not observed in both the striatum and the hippocampus at day 30 with the change at adult stage in the striatum is difficult to explain.

Thus with steroid treatment precursor levels were altered uniformly in all the three regions at day 10 with the region specific changes occurring in adult animals and even at postnatal age of 30 days specificity with respect to steroid effect on precursors seems to be evident even at the postnatal age of 30 days.

Unlike the alterations in precursor amino acid levels with steroid treatment, changes in the neurotransmitter concentrations were very few. With chronic treatment, only hypothalamic NE levels increased at 10 and 30 days whereas 5HT levels decreased in adult hippocampus alone. Acute steroid treatment did not affect the neurotransmitter levels in any of the regions at any age studied.

As mentioned earlier, there are only two studies with chronic corticosteroid administration effect on bioamines in different regions of the rat brain (Dickinson <u>et al</u>, 1985; Walkowitz <u>et al</u>, 1986). Both these studies have reported no change in the concentrations of 5HT and DA in the striatum which is in consonance with the present study. Decreased 5HT and DA concentration, however, has been reported in other region such as cortex with chronic treatment (Walkowitz <u>et al</u>, 1986).

Surprisingly, there seems to be no report with chronic corticosterone treatment on the hippocampal bioamine changes.

In contrast to chronic treatment, several reports have appeared on acute treatment in adult animals which are certainly contradictory. Earlier reports on the whole brain 5HT concentration with acute corticosteroid treatment have shown a decrease (Green and Curzon, 1975; Neckers and Sze, 1975) as also no changes by certain other study (Hiller et al, 1975). In different regions, however, the effect on 5HT concentration seems to be differential. Rothschild et al.(1985) have reported an increased 5HT in the hypothalamus and a decrease in the cortex. Kovacs et al.(1977), experimenting with two different doses of corticosterone at various time points have highlighted the variability of effects with respect to both these factors - at shorter duration (30 min), smaller dose increased 5HT concentration in the hypothalamus and mesencephalon whereas higher dose decreased 5HT in these regions. Both doses did not show any change in 5HT at a longer time duration of 2 hrs. The results of no change with acute treatment in the hypothalamus and the hippocampus agree with this report as the estimations were done from samples sacrificed 2 hrs after the steroid treatment.

As in the case of 5HT, with acute treatment, NE and DA changes varied with respect to the regions studied. However, NE level did not change in the whole brain (Iuvone et al, 1977).

An increased DA concentration in the hypothalamus and nucleus-accumbence in adult animals have been reported (Rothschild _et al_, 1985). No change in these parameters, in adult animals, obtained in the present study contradicts with the hypothalamic changes observed by Rothschild _et al_.(1985). This may perhaps be due to the difference in the form of hormone (dexamethasone) used by this group compared to the present study. In young animals, chronic treatment from 16-18 postnatal days has been shown to increase NE concentration in the hypothalamus at 21 days (Lengvari _et al_, 1980). Interestingly, such an effect of chronic treatment has also been observed in the present study at younger ages - day 10 and 30 but not at adult stage. This age specific effect on NE, perhaps, is consistent with the age related induction of the TH reported by Sze (1980).

These results, on the whole, suggest a relatively unaltered neurotransmitter levels in these three brain regions. The fact that the increased NE levels in young hypothalamus associated with a decreased tyrosine levels, and decreased 5HT level in adult hippocampus with no change in tryptophan seems to indicate that, these changes in precursor amino acid levels with corticosterone treatment may not influence the neurotransmitter levels _per se_. This may not be surprising, because of the fact that the neurotransmitter levels are assiduosly maintained which gets affected only by rather drastic conditions. Generally, the system adjusts to the altered immediate environment responding to it by way of altering its turnover in order to maintain the homeostasis.

From the metabolite levels (DOPAC and 5HIAA) it appears that the corticosterone treatment, be it acute or chronic, affects the metabolism rather than the level of neurotransmitters. In the present study, chronic treatment resulted in significantly low levels of 5HIAA in all the three regions at 10 day while affecting only the hippocampus at adult stages In contrast, acute treatment increased 5-HIAA levels in both the hypothalamus and the hippocampus at 10 day while doing so only in adult hippocampus. Taken together, with alteration in levels of precursors it would indicate a decreased 5HT metabolism due to decreased level of tryptophan in all the regions of young animals with chronic treatment and conversely, increased 5HT metabolism with acute treatment. In adult animals only hippocampus showed such a change although the tryptophan level was not affected by chronic treatment. This study, thus, reaffirms the fact that metabolism of 5HT is regulated by availability of tryptophan; however, the effects are not evident in the 5HT levels but are reflected in the 5HIAA levels.

Such decrease in 5HIAA levels associated with decreased tryptophan levels in young animals and unaltered 5HT levels suggest a possibility of decreased 5HT turnover with chronic corticosterone treatment. However, alteration in the metabolite level as an index of change in the functional activity of the cell has been questioned, at least with respect to 5HT metabolism and discussed in detail by Green and Grahamme Smith (1975) In this connection, they have mentioned that 5HT is normally synthesized in excess of functional needs and only a portion of this is released into synaptic cleft . It is also supposed that two pools of 5HIAA may be present, one derived from synaptically released 5HT and the other derived from the non-released 5HT oxidatively deaminated within the neuron. Therefore, it would not be correct to assume any change in 5HT-ergic activity unless further evidences to that effect are obtained.

With regard to metabolite of DA, DOPAC level decreased significantly in all the three regions at 10 day and only in adult hypothalamus with chronic treatment. Acute treatment increased DOPAC levels only in adult hypothalamus and not in young animals. Taken together, it indicates that in young animals chronic treatment decreased tyrosine levels with no effect on DA in all the three regions while increasing NE level in hypothalamus only. The decrease in tyrosine was also associated with decreased DOPAC in all the three regions which may perhaps be due to decrease in MAO activity or decreased DA turnover. However, it has been postulated that alteration in DOPAC levels can be considered as an index of presynaptic breakdown of DA i.e., nonfunctional breakdown. Thus, it is likely that in young animals this breakdown could reflect the decreased breakdown in the nonfunctional pool. In contrast, acute treatment showed opposite effect with respect to tyrosine which decreased wherever there is an increased breakdown. The possibility of alteration in the functional activity cannot be ruled out under such conditions.

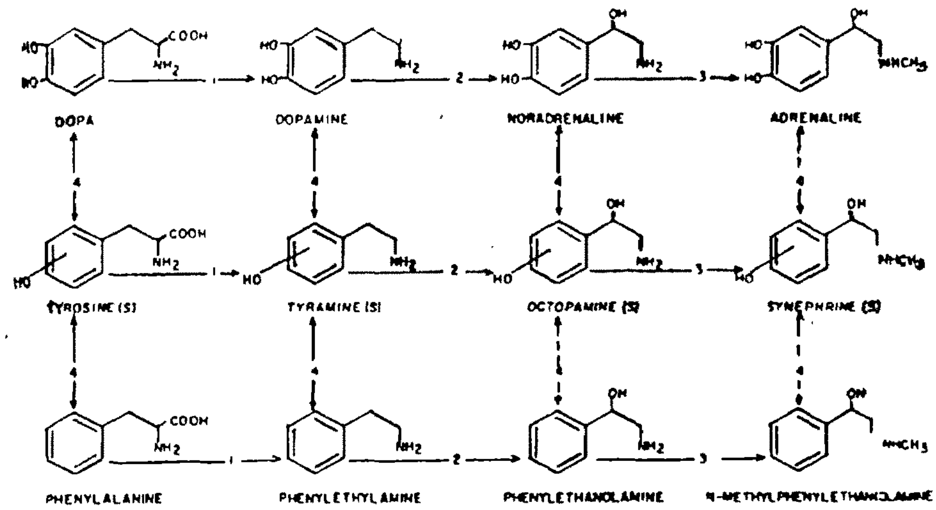
To summarize the effects on 5HT and DA systems, it appears that chronic corticosterone treatment decreased functional turnover of 5HT/ decreased intraneuronal breakdown in all the three regions studied in young animals, whereas at adult stage hippocampus and hypothalamus were affected with respect to 5HT and DA, respectively. In contrast, acute treatment affects only adult hypothalamus for both DA as well as 5HT and only hippocampus for the latter showing increased functional or intraneuronal breakdown.

3:3 STUDIES ON TRACE AMINES

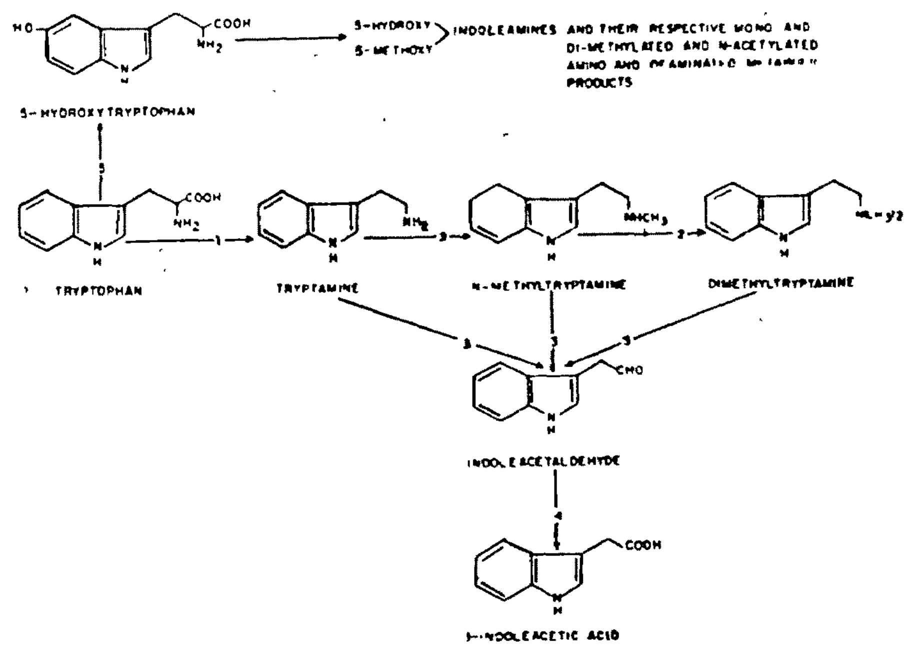
The trace amines are present in discrete areas of the mammalian brain and possess a high metabolic rate as determined by their accumulation after monoamine oxidase inhibition. In the rat brain, highest concentration of p-tyramine (p-TA), m-tyramine (m-TA) and tryptamine (T) is present in the hypothalamus and striatum (Jurio et al, 1985). Although the precise role of these trace amines are not yet known p-, m-tyramine and tryptamine have been suggested to act as neuromodulators (Jurio, 1984). Besides the well known sympathomimetic action of p-tyramine, it's role has been implicated in the release of endogenous dopamine (Kamal et al, 1981) as well as serotonin (Raiteri et al, 1977) in striatum. It's ability to reduce high prolactin levels achieved by pharmacological dopamine depletion and blockade of dopamine receptors indicate a possible interaction of p-tyramine and the dopaminergic system related to neuroendocrine control of prolactin secretion (Becu-Villabos et al, 1985).

Animals subjected to various kinds of physical stress, although did not show any change in the level of dopamine (Bliss and Zwanziger, 1966; Thierry et al, 1968; Saavedra et al, 1979), showed increased dopamine turnover rate (Lavielle et al, 1979). The altered dopamine turnover in mouse striatum subjected to aggregation stress was associated with decreased p-TA and increased m-TA indicated a reciprocal relationship between dopamine turnover, m-TA and p-TA concentration and led to the suggestion of a possible role for striatal p-TA to act as a modulator or transmitter to control the activity of dopaminergic neurons (Juorio, 1980). In humans, subjected to the ~~phy~~ psychological stress of inflight training, p-TA and m-TA in both conjugated and unconjugated forms decreased in urine over basal rates of excretion (Harris et al, 1985). Besides this level of p-octopamine - another trace amine, seems to be very sensitive to the stress of electrical shock in rat brain regions (Ennaceur et al, 1986). The synthesis of p-octopamine has been shown to be by the β-hydroxylation of p-tyramine (Boulton, 1979) (Fig.15). Therefore during stress, apart from being affected directly, these trace amine concentrations might be altered by being the precursor for p-octopamine itself. The trace amines (p-TA, m-TA and T) have been implicated in various neurological and psychiatric disorders (Juorio, 1982) which are invariably associated with altered glucocorticoid levels in plasma. Stressors of various kind are very well known to elevate plasma glucocorticoid level in mammals. Elevated glucocorticoid levels affect the brain concentration of p-tyrosine and L-tryptophan which are known to affect the concentration of brain p-, m-tyramine and tryptophan respectively in the presence of monoamine oxidase inhibitors (Mc Quade and Juorio, 1982; Warsh et al, 1979). In view of the above findings it was of interest to study the effect of acute and chronic administration of corticosterone on the rat striatal concentrations of p-, m-tyramines and tryptamines associated with dopamine (DA) and 5-hydroxytryptamine (5HT), their precursors and metabolites. In addition, studies were

166



Synthesis of the phenolic amines. 1. decarboxylase: 2. β-hydroxylase. 3. N-methyltransferase: 4. ring (de)hydroxylase.



Metabolism of tryptamine 1. decarboxylase. 2. N-methyltransferase; 3. monoamine oxidase: 4. aldehyde oxidase: 5. tryptophan hydroxylase

Fig. 15 *Metabolism of p-TA, m-TA and Tryptamine in The Rat Brain*

Taken from : Boulton, and Juorio (1984) done on the effect of acute corticosterone treatment to pregnant females on embryonic day 15 brain concentrations of p- and m-tyramine because octopamine very sensitive to stress in rat brain is reported to be present in very high concentration during gestation (Saavedra et al. 1974) and is known to be synthesized from p-tyramine by β-hydroxylation.

3.3.1 Development :

Table-16 gives the developmental profile of p- and m-tyramine in the rat brain striatum. Both p- and m-tyramines were detected in the whole brain at embryonic day 15 (E15); p-tyramine level exceeded that of m-tyramine as found normally in the adult rat brain. At 10 day in the striatum, p-tyramine level was already above the adult level and decreased significantly between day 10 and 30. Between day 30 and 50 p-tyramine level did not change and the slight increase observed thereafter was not significant. Apparently m-tyramine also showed a similar pattern of development. As m-tyramine was not estimated at day 10, a clearcut picture about its developmental profile could not be ascertained. The m-tyramine concentration was found highest at day 50 and decreased thereafter; the difference in the level between day 30 and 50 was not significant. Interestingly, the developmental decrease of p-tyramine paralleled the decrease observed in the precursor p-tyrosine level in striatum i.e., very high concentration at day 10 with a sharp decline between day 10 and 30. This decline also seems to be related to the increased tyrosine-hydroxylase activity during this period. Jurio (1980) has

TABLE- 16 : The development of p- and m-tyramine in rat brain striatum.

Age (days)	p-tyramine	m-tyramine
E-15	2.47 ± 0.28 (6)	0.92 ± 0.07 (6)
10	14.69 ± 0.99 (13)	n.e.
30	10.88 ± 0.54 (12)*	4.59 ± 0.45 (7)
50	10.96 ± 0.61 (6)	5.48 ± 0.37 (6)
Adult (>90)	12.57 ± 0.53 (13)	3.30 ± 0.28 (13)

The values are given in ng g^{-1} of fresh tissue, ± S.E. of means with number of experiments in parenthesis and n.e. refers to not estimated.

* Significantly different from day 10 at $P<0.01$. Note E-15 refers to embryonic day 15 whole brain.

suggested such a probability with respect to the reciprocal relationship, mentioned earlier, between p-tyramine and DA turnover. This suggestion was based on the observation that activation of tyrosine hydroxylase lead to a shift towards the hydroxylation of p-tyrosine to form L-DOPA (Bartholini and Pletscher, 1969) that then would be decarboxylated to DA and consequently decreased availability of p-tyrosine for decarboxylation to p-tyramine (Fig. 15). Information todate in this regard during development is not reported.

3.3.2 Effect of corticosterone treatment

Table-17 shows the effect of subcutaneous administration of corticosterone on p-, m-tyramine and DA at embryonic day 15 whole brain and adult striatum. Acute treatment did not show any change in embryonic day 15 brain and at postnatal day 10 and 30 striatum (15.10 $\pm$ 0.99 Vs. 16.18 $\pm$ 1.0 and 10.30 $\pm$ 0.7 vs 11.58 $\pm$ 0.7 respectively).

In adult striatum acute corticosterone treatment showed a significant decrease (83% of control) in p-tyramine levels and a trend towards increase in m-tyramine, albeit not significant. Chronic corticosterone treatment did not show any change in either p- or m-tyramine levels in the striatum suggesting a differential effect of corticosterone on p-tyramine levels in the rat striatum. Such decrement in the p-tyramine could be seen only in adult animals whereas neither fetal brain nor 10 and 30 day old striatum showed any differences indicating an age specific effect of corticosterone treatment.

TABLE- 17 : Effect of corticosterone treatment on p- and m-tyramine, p-tyrosine, DA, DOPAC and HVA concentrations in the embryonic day 15 brain and adult striatum.

Age		Treatment	p-TA	m-TA	p-Tyrosine	DA	DOPAC	HVA
E 15	Acute	Control	2.47 ± 0.20 (6)	0.92 ± 0.07 (5)	29703±986 (7)	77 ± 2.6 (7)	137 ± 4.8 (7)	40 ± 2.2 (7)
		Treated	2.89 ± 0.37 (5)	1.20 ± 0.20 (5)	33783±880* (6)	79 ± 3.7 (6)	188 ± 2.3* (6)	35.3±2.9 (6)
Adult (Striatum)	Acute	Control	11.65 ± 0.05 (12)	3.30 ± 0.28 (13)	18540±610 (6)	9390±80 (6)	930 ± 20 (5)	600 ± 20 (6)
		Treated	10.4 ± 0.34** (13)	3.74 ± 25 (12)	13060±690*** (6)	9480±90 (6)	980 ± 30 (6)	660 ± 20 (6)
	Chronic	Control	11.48 ± 0.72 (7)	3.30 ± 0.66 (7)	15420±510 (11)	9200±250 (12)	1100 ± 40 (11)	750 ± 30 (12)
		Treated	11.1 ± 0.08 (7)	3.59 ± 0.35 (7)	13270±550** (11)	9840±420 (12)	1120 ± 50 (12)	790 ± 40 (12)

Values are expressed as ng/g tissue ± S.E. with number of observations in parenthesis.
E-15 refers to embryonic day 15 whole brain.

* P <0.05
** P <0.01 } Significantly different from controls.
*** P <0.001

Acute corticosterone treatment did not show any effect on the tryptamine level (Table- 18) in the striatum both when treated with MAO inhibitor (Pargyline) prior to steroid treatment or without pargyline injection and suggest a specific effect of acute corticosterone treatment on p-tyramine.

Tables-17,18 also shows the effect of acute and chronic treatment on the neurotransmitters DA, NE, 5HT, their precursors p-tyrosine and metabolite DOPAC both in the whole brain of embryonic day 15 and adult striatum. Acute corticosterone treatment in embryonic day 15 whole brain showed a significantly increased p-tyrosine levels but no change in DA, NE or 5HT levels (Table-17,18). The neurotransmitter metabolites DOPAC (41%) and 5HIAA (10%) increased significantly suggesting an increased dopaminergic and serotonergic activity with acute corticosterone treatment.

In adult striatum, although a significant decrease in the p-tyrosine level was found with acute corticosterone treatment, no significant change in the neurotransmitter levels occurred. DOPAC and HVA levels, however, showed a tendency towards increase with acute treatment and just fell short of significance limits. This indicates an increased dopaminergic activity with acute treatment. In both acute and chronic treatment none of the parameters related to serotonergic metabolism were affected.

TABLE- 18 : Effect of acute corticosterone treatment on tryptamine, tryptophan, 5HT and 5HIAA concentration ($ng\ g^{-1}$ tissue) in the embryonic day 15 rat brain and adult striatum.

Age	Treatment	Tryptamine		Tryptophan	5HT	5HIAA
		- Pargyline	+ Pargyline			
E-15	Control	n.e.	n.e.	n.e.	67.3 ± 3.5 (7)	109.9 ± 5.3 (7)
	Treated	n.e.	n.e.	n.e.	84.0 ± 2.8** (6)	122.3 ± 3.7** (6)
Adult striatum	Control	4.31 ± 1.33 (4)	100.7 ± 7.1 (7)	5080 ± 130 (6)	500 ± 20 (6)	570 ± 20 (6)
	Treated	5.74 ± 1.08 (6)	107.7 ± 10.3 (4)	5250 ± 250 (6)	470 ± 20 (6)	600 ± 20 (6)

Values are expressed as mean ± S.E. with number of observations in parenthesis;

** Significantly different from respective controls at $P<0.01$ level.

E-15 refers to embryonic day 15 whole brain. n.e. - not estimated.

The elevated level of glucocorticoid, in this case corticosterone, seems to affect the p-tyramine level and dopaminergic activity in the adult rat striatum. This seems to be the first attempt to elucidate the effect of exogenously administered corticosterone in the rat striatal trace amine concentrations.

Acute corticosterone administration to pregnant females on gestation day 15 did not affect the fetal brain p- and m-tyramine concentrations. The p-tyramine and m-tyramine concentrations in the whole brain of embryonic day 15 have not yet been reported and seem to be present in considerable amount. In this connection, other trace amines such as Octopamine and phenylethanolamine have been reported to be present as early as gestation day 15 in higher concentration than in adults. This high concentration of octopamine and phenylethanolamine at gestational age 15 till 18 is correlated with the low activity of MAO with an increase towards gestation (Saavedra et al, 1974).

In the present study, although p- and m-tyramine concentrations did not change with acute corticosterone treatment at embryonic day 15, increased p-tyrosine levels associated with increased dopamine turnover in terms of DOPAC and HVA levels suggest no direct relationship between these parameters. This does not, however, discount the possibility of a relationship between the p- and m-tyramine turnover with the increased dopaminergic activity.

Acute treatment in adult striatum decreased p-tyramine (17%) concentration. This decrement, although seems small, is quite appreciable because of the very high turnover rate of this trace amine. The values of p- and m-tyramine are consistent with the earlier reports (Boulton and Juorio, 1982). The decreased p-tyramine in striatum is associated with decreased p-tyrosine levels. This could be due to the fact that the p-tyramine is formed to some extent by decarboxylation of p-tyrosine in the rat (Boulton and Wu, 1973) and that the decreased p-tyrosine might have caused the p-tyramine decrement. Alternatively, a trend of near significant increase in HVA levels under these conditions suggesting an increased dopamine turnover might have caused the decreased p-tyrosine levels. The reciprocal relationship of decreased p-tyramine level with increased DAergic activity and a trend towards increase in m-tyramine level are consistent with the earlier reports with aggregate stress in mouse striatum (Juorio, 1980). The fact that such a relationship has been reported earlier under various experimental conditions manipulating DAergic system (Juorio, 1979) suggest a possible modulatory influence of p- and m-tyramines on dopamine system. Such a role of p- and m-tyramine seems plausible even with corticosterone treatment as chronic treatment did not alter the levels of these trace amines and that the neuromodulation is a relatively short term effect. Under chronic treatment the possibility of a change in the turnover rate of p- and m-tyramine cannot be excluded although no concomittant change in dopaminergic turnover has been observed. In view of the finding that acute corticosterone treatment showed the reciprocal relationship between p-tyramine and DA turnover only at adult stage and no such relationship at very early stage of brain development, it is tempting to speculate that the neuromodulatory role of p- and m-tyramine may develop as the complete neurotransmitter systems development occurs in the rat striatum.

p-Octopamine in rat brain regions has been shown to be highly sensitive to stress of electric shock suggesting its involvement in emotional and neurovegetative response to stress (Ennaceur <u>et al</u>, 1986). As it has been shown by earlier findings that p-Octopamine is formed by β-hydroxylation of p-tyramine (Boulton, 1979), the decrease associated with the p-tyramine levels with corticosterone treatment may also reflect on the suggested changes in octopamine under stress conditions.

The present finding of no change in the striatal tryptamine with acute treatment seems logical as no change in the L-tryptophan and 5HT levels was observed.

The fact that these tyramines have been implicated in various neurological and psychiatric diseases, including Parkinson's disease, Schizophrenia, depression and migraine-invariably associated with increased corticosteroid levels in blood, and the present finding of a decreased p-tyramine level and increased dopaminergic activity with corticosterone treatment, suggests a possible role for these tyramines under such disorders although, it cannot be said whether such relationship is a causitive or resultant phenomenon.

3.4 CORTICOSTERONE ADMINISTRATION AND LIPID CHANGES DURING THE BRAIN DEVELOPMENT

The alterations in enzyme activities related with GABA and glutamate metabolism and also in bioamine metabolism to the corticosterone treatment have shown that the effects vary from region to region and the age of the animals. As reviewed earlier, the steroid effect related to neurotransmitters have been performed on adult animals while certain other aspects exclusively on young animals. A case in point is the studies on the effect of steroid manipulations on myelinogenesis, a characteristic feature of the developing brain, mainly during early postnatal period in rats. Based on ultrastructural studies as well as several biochemical markers such as cerebrosides, sulfatides, CNPase activity etc., it has been suggested that early glucocorticoid treatment, although transiently, retards the rate of myelin deposition (Meyer, 1985).

Studies have also been reported on the role of glucocorticoids in myelinogenesis during early period of brain development. Several attempts by using early adrenalectomy have produced conflicting results. For instance, Meyer and Fairman (1985) adrenalectomised the rats on 11th day of life and estimated myelin content of the brain on 63rd day. They have shown increased myelin content of the cerebrum and that the myelin formed under these conditions was not completely normal in its composition i.e., deficient in total galactolipid and phospholipid concentrations with unchanged cholesterol concentration. In addition, specific activity of CNPase was not altered. Such a paradoxical situation of increased myelin yield with adrenalectomy but with out any change in one of its prime components, cholesterol, added to a rather decreased galactolipids and phospholipids may perhaps, as rightly mentioned by the authors, suggest incomplete recovery of lipids from the two myelin fractions. In slight contradiction to this report, adrenalectomy of rats at 14 days decreased the myelin contents of the cerebrum and the brain stem associated with a decreased GPDH activity but there was no change in specific activity of CNPase at 21 days (Preston and Mc Morris, 1984). Adrenalectomy at later ages did not yield such results suggesting a possible role of glucocorticoids during the early period of rapid myelination. This study, however, did not estimate the individual lipid components from the myelin (Preston and Mc Morris, 1984). The comparison between these two reports becomes difficult owing to two major differences in the experimental design - (a) apart from the different time of adrenalectomy performed, the estimation of biochemical parameters were done at 61 days by Meyer and Fairman (1985) while in the latter case at 21 and 22 days; (b) supplementation of mineralocorticoid for such a long period of time after adrenalectomy in the former case might have yielded a higher myelin content since it has been recently suggested that mineralocorticoid (ALD) itself may occupy glucocorticoid binding sites under depleted corticosterone conditions (Yongue and Roy, 1987).

Apart from these differences adrenalectomy itself results in a complex set of endocrine changes including loss of cortical steroids, medullary catechols and an increase in circulating ACTH due to the absence of negative feedback. A combination of these factors could in itself have profound effect producing variable results.

Several lines of investigations presented below, however, would suggest that corticosteroids can also enhance lipid synthesis depending on the time of treatment -

(a) Casper et al.(1967) observed a significant increase in cerebroside concentration in rats treated with cortisol during 6th till 10th day of postnatal life. The spinal cord and cerebral lipids estimated on postnatal day 12 showed an increased cerebroside only in the spinal cord. Unfortunately this study was restricted to one age group only.

(b) PAPS; galactosyl ceramide sulfotransferase and UDP-galactose; ceramide galactosyl transferase, key enzymes for the synthesis of cerebrosides and sulfatides have been shown to be induced by hydrocortisone treatment in mouse glioma cell line G-26 (Dawson and Kernes, 1978, 1979; Sapirstein et al, 1980).

(c) Glycerol-3-phosphate dehydrogenase (GPDH) catalyses the conversion of dihydroxyacetone phosphate to glycerol-3-phosphate, a step in the main biochemical pathway producing phosphatidic acid, a key intermediate in the synthesis of glycerophospholipids (White et al, 1973). Phospholipids, are major components of CNS myelin comprising one third of the lipid of myelin on a molar basis (Norton, 1977). Glucocorticoids stimulate the activity of cytoplasmic GPDH in the rat brain in an age specific manner (De Vellis and Inglish, 1973).

(d) Hydroxymethyl-glutaryl-CoA reductase (3-HMGCoA reductase), a key enzyme in the synthesis of cholesterol has been shown to be responsive to the corticosteroid treatment depending on the circadian cycle (Lin and Snodgross, 1982). Although this study pertains to rat liver, such a possibility of regulation in the brain cannot be ruled out.

(e) Another important enzyme, Na^+-K^+-ATPase has been shown to be activated in the brain of chick embryo by cortisol injection into the eggs (Lisy et al, 1980) and in mouse brain regions by hydrocortisone (Sadasivudu, 1977). This enzyme associated with the movement of Na^+ and K^+ ions across plasma membrane has been known to be highly dependent on its lipid environment, e.g., accumulation of steroid molecules, cholesterol and testosterone (10^{-6} - 10^{-9} M) in the vicinity of cell membrane may increase Na^+-K^+-ATPase activity in synaptosomes (Alivisatos et al, 1981).

In view of these observations and in continuation with the present study, it was thought of interest to see how corticosterone treatment affect lipids in different brain regions and whether the sensitivity of these regions vary with respect to age and lipid parameter studied. For this purpose two parameters, cholesterol, as a lipid component of all the membranes and galactolipid, as a marker of myelin were studied at day 10, 20, 40 and adult stages. While reviewing the literature on these lines, as mentioned earlier, most of the studies were found to be pertaining to whole brain or on gross regions such as, the brain stem, the forebrain or the cortex. No studies are available on the striatum and the hippocampus even with respect to the developmental aspect of cholesterol and galactolipid. Therefore, the result on the developmental aspects of these parameters in the rat hippocampus, the striatum and the cerebellum are discussed although they were carried out as controls of the corticosterone treated animals.

3:4:1 Development of Cholesterol and Galactolipids in Different Regions of the Rat Brain.

Concentration of cholesterol and galactolipid at various ages are given in Table-19 . At day 10 after birth, cholesterol concentrations in the cerebellum, hippocampus and striatum were similar. Concentration of cerebellar cholesterol at 10 day was comparable with studies from this department as well as others (Lalitha, 1988; Gittoni and Reveglia,1977). Galactolipids were not detectable at this age in any of the regions studied and is consistent with the fact that myelination starts only after this age in the whole brain (Norton, 1977).

At postnatal day 20, concentration of cholesterol in the cerebellum was significantly higher than that of the hippocampus and the striatum. No differences in the cholesterol concentration between the latter two were found. An appreciable

TABLE- 19 : **Developmental pattern of cholesterol and galactolipid concentration in rat brain regions.**

Age (days)	Parameters	Cerebellum	Hippocampus	Striatum
10	Cholesterol	5.8 ± 0.48	5.4 ± 0.28	5.8 ± 0.44
	Galactolipid	n.d.	n.d.	n.d.
20	Cholesterol	11.6 ± 0.68	7.8 ± 0.50	7.9 ± 0.68
	Galactolipid	9.2 ± 0.49	4.9 ± 0.54	5.1 ± 0.40
40	Cholesterol	16.17 ± 0.91	21.1 ± 0.60	19.7 ± 1.20
	Galactolipid	9.1 ± 0.56	6.0 ± 0.28	8.2 ± 0.56
90	Cholesterol	22.7 ± 1.10	22.9 ± 0.9	24.8 ± 0.52
	Galactolipid	17.3 ± 0.84	10.1 ± 0.87	17.0 ± 0.90

Values are expressed as mg/g tissue ± S.E. with 7-10 number of observations.

n.d. - not detectable.

amount of galactolipid was present by day 20 in all the three regions.

Between day 20 and 40 concentration of cholesterol increased in all the regions; the hippocampus seems to have attained adult level of cholesterol while the cerebellum and the striatum showed further increase. Galactolipid also increased simultaneously but the rise in this parameter was not so high, especially in the hippocampus followed by the striatum.

At day 90, the concentration of cholesterol in all three regions were comparable although the striatum showed slightly higher value. Interestingly, a remarkable increase in the galactolipid concentration is observed in all the three regions although they differed in absolute amounts. Hippocampus has the least and cerebellum and striatum have almost similar amount of galactolipid. If 90 day value is considered as adult value for these two parameters, the ratio of galactolipid to cholesterol varies considerably between hippocampus and rest of the two regions. These differences could be partly due to differences in the myelinated fibres arising or reaching these areas.

Looking at the developmental pattern of cholesterol and galactolipids in all the three regions, it is clear that the rate of increase vary considerably for each parameter. The differences in the developmental pattern of cholesterol as well as galactolipid becomes apparent with the differences in rate of increments amongst the time periods studied (Table-20) whereas the cerebellum showed a maximal increase (100%) in cholesterol between 10 and 20 day followed by a gradual decrease between 20-90 days; the hippocampus and the striatum showed maximal rate of cholesterol accretion between 20-40 days. The difference between the hippocampus and striatum could be seen in that the rate of accretion was almost negligible (9%) at 40-90 days in the hippocampus whereas in the striatum it was 25% for the corresponding period. Thus cholesterol concentration in the three regions being same at 10 days continued to increase till 90 days in the cerebellum and the striatum while it remained plateau between 20-40 days in the hippocampus.

Galactolipid accretion followed a different pattern in the three regions - i.e., maximal rate of accretion occurred in all the three regions between 40-90 days. Between 20-40 days, however, a large increment was seen in the striatum while almost no change in the cerebellum during the same period was apparent.

Cholesterol is a critical constituent of cellular membranes and is found in particularly high concentration in neural tissue (Suzuki, 1976). Cholesterol accumulation in brain is particularly active during development (Wells and Ditmer, 1967; Cuzner and Davison, 1968), which comprises mainly of cell proliferation (occurring mainly during postnatal stage for glial cells), elongation of axons, dendrite formation, myelination and synapse formation. The pattern, in general, TABLE- 20 : Percent increment in cholesterol and galactolipids in the rat brain regions during development.

Parameters	Brain regions	Age (days)		
		10-20	20-40	40-90
CHOLESTEROL	Cerebellum	100	40	40
	Hippocampus	44	200	9
	Striatum	36	149	25
GALACTOLIPID	Cerebellum	-	-	90
	Hippocampus	-	22	68
	Striatum	-	61	107

obtained in the present study of a low concentration of cholesterol in all the three regions appears to be associated with membrane of the cells and axon outgrowth. The rapid increase in cholesterol after 10 days coincides with the onset of myelination in the rat brain associated with the multiplication of oligodendroglial cells. This myelination process continues even till 90 days. In the case of the hippocampus and the striatum, the process of myelination although started at early stages increased rapidly only after 20 days and seemsto be over by 40 days in the former. Since cholesterol in brain is derived principally from synthesis in situ (Kabara, 1973) rather than from transport from blood (Pardridge and Meitus, 1980). Several attempts to correlate the developmental pattern of cholesterol with its biosynthetic enzymes have been reported. A temporal correlation between HMG-CoA reductase, a rate directing enzyme in sterol biosynthesis, with cholesterol biosynthesis in dissociated cell cultures of fetal rat brain during development does suggest such a link in association with oligodendroglial differentiation (Volpe et al, 1985).

Galactolipid, another important constituent of myelin in the CNS, parallelsthe development of cholesterol albiet in a delayed manner. The non-detectable amount of galactolipid at 10 days followed by a most rapid increase in accretion after 40 days in all the three regions compared well with that of cortex galactolipid accretion.
The slow progressive accumulation of galactolipids between

10 and 40 days followed by a rapid accumulation observed in the present study parallelsthe deposition of compact myelin as reported for myelination pattern in rat subcortical white matter (Bass et al, 1969).

Thus these observations of different pattern of cholesterol and galactolipid accumulation in the three brain regions studied, suggest possible differences in the susceptibility, if any, of these processes to corticosterone treatment. Furthermore, significantly lower galactolipid level in the hippocampus in comparison with the cerebellum and the striatum could suggest differences in the amount of myelinated axons in these regions, which is not reported so far.

3:4:2 Effect of Corticosterone Treatment

The effect of chronic corticosterone administration (40 mg/kg b.w. for 3 days prior to sacrifice) on cholesterol and galactolipid concentration in the cerebellum, hippocampus and striatum is given in the Fig.16. Corticosterone treatment increased the cholesterol concentration in the hippocampus and the striatum but not in the cerebellum. A significant increase was observed in the concentration of cholesterol at 10 and 20 days in the hippocampus but not at 40 and 90 days of age, whereas in the striatum increase was evident only at day 20. The magnitude of increase in cholesterol was more at day 20 (45%) compared to 29% at day 10 in the hippocampus while in striatum the increase was only 20% at

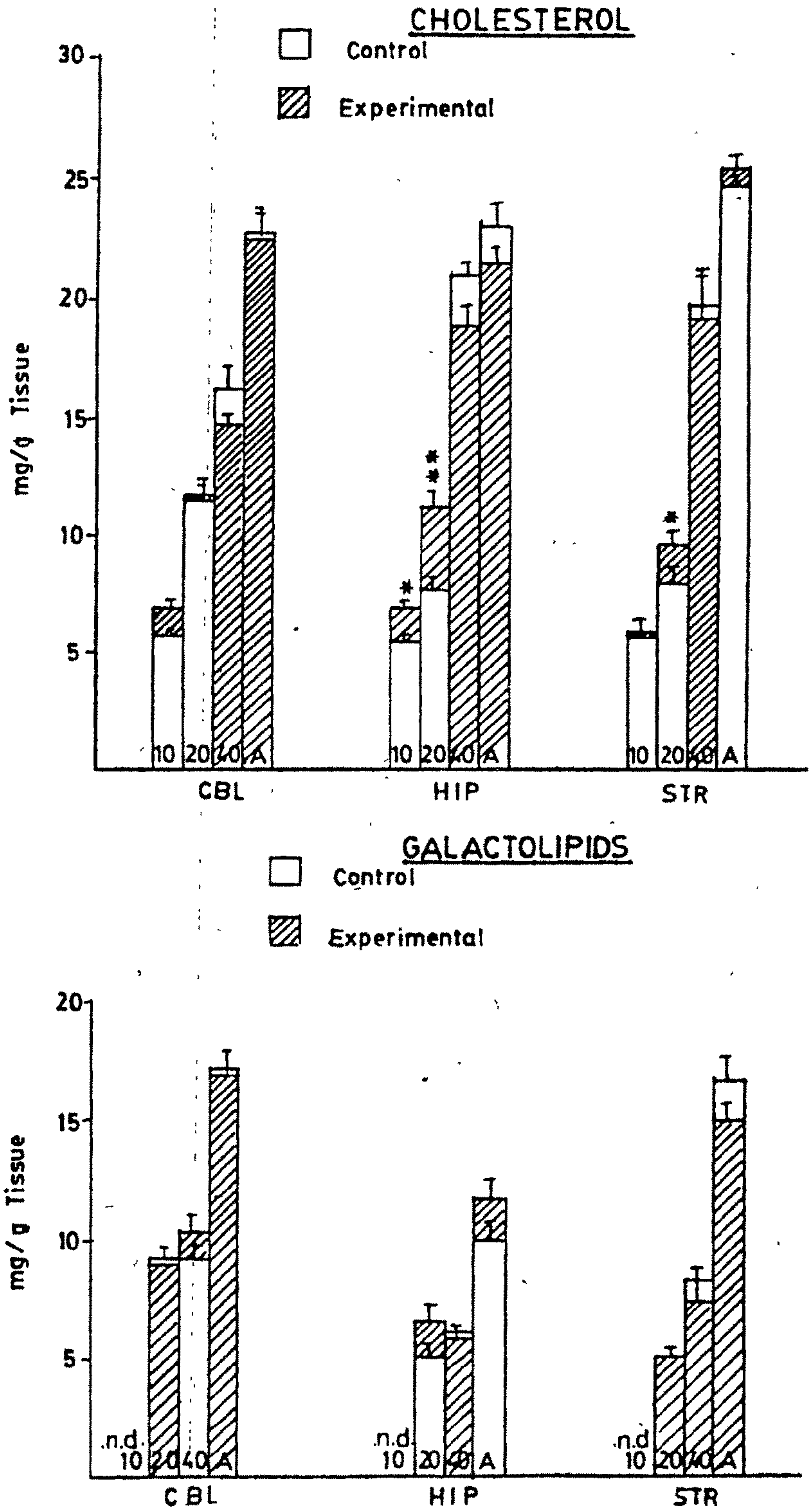


Fig. 16 **Effect of Chronic Corticosterone Administration on Cholesterol and Galactolipid concentrations In The Rat Brain Regions during Development**

Values are Mean of 8-10 observations

*Values are significantly different from Controls * $P < 0.05$, ** $P < 0.01$*

Number inside the bars refer to the age of animals in days. A refers to Adult (>90 days) animals day 20. The galactolipid concentration was not affected in any any of the regions studied irrespective of age.

The present result of increased cholesterol concentration at earlier ages following exogenous corticosterone treatment in the hippocampus and striatum contradicts the general trend of decreased cholesterol content in the several tissues (Cavenee _et al_, 1978; Picard _et al_, 1980). This could be due to several differences in the experimental design including that most of these studies were on liver tissue either _in vivo_ or _in vitro_ as well as the age and hormonal status of these tissue samples. A decrease in the cholesterol content per cell in the whole brain of rats implanted with steroid pellets and sacrificed at 7, 8 and 18 postnatal days has also been reported (Howard, 1965). The same study, however, reports an increased cholesterol concentration in the brain and reasons this increase as due to the loss of water from the tissue with corticosterone treatment. In view of the findings that adrenalectomy decreases myelin and GPDH activity only during early period of development (Preston and McMorris, 1984), along with the well established role of glucocorticoid induction of GPDH in the CNS during same period, the present finding of increased cholesterol in the hippocampus and striatum may not be unreasonable. Furthermore, the role of glucocorticoid in the regulation of 3-HMG CoA reductase in various tissues _in vivo_ and _in vitro_ have been investigated by many laboratories and the results are often contradictory (Edwards, 1973; Mitropaulos and Balasubramaniam, 1976; Lin and Snodgross, 1982). Lin and

Snodgross (1982) have shown both <u>in vivo</u> and also in cultured liver cells an increased cholesterol levels associated with an increased 3-HMG-CoA reductase activity dependent on the circadian cycle demonstrating the role of glucocorticoids in cholesterol synthesis.

Alternatively, the increased cholesterol concentration with steroid treatment could be due to the decreased lipid peroxidation (Hall and Braughler, 1982; Anderson and Means,1985) which is normally high in the CNS during development. This could only be a probability, since the enzymatic degradation of cholesterol in the brain is not likely (Horrocks and Harder,1983).

Thus the selective increase in cholesterol in specific regions and at specific age emphasizes the effectiveness of steroid treatment on these factors.

3:4:3 <u>^{14}C-Glucose Incorporation into Various Lipids in the Hippocampus and Striatum</u>

The increased cholesterol concentration in the young hippocampus and striatum with corticosterone administration observed in the previous experiment evoked an interest as to whether the increase in concentration is associated with increased rate of synthesis since cholesterol is synthesized in the brain itself. Two major precursors from which various lipids are known to be synthesized are glucose and ketone bodies during early postnatal life. Furthermore, several studies have indicated an increase in the blood glucose levels with glucocorticoid treatment. However, it is suggested that increase in blood glucose is due to decreased uptake of glucose by the peripheral tissues and some brain regions as well (Sapolsky, 1987). In view of these earlier observations, it became of interest to find out the role of glucocorticoid on the rate of synthesis of cholesterol from glucose as substrate in the hippocampus and striatum.

The steroid treated and control animals were given i.p. U-^{14}C glucose, 2 hrs prior to decapitation (the details of which are discussed under 'Materials and Methods'), an aliquot for the chemical estimation of cholesterol was also taken from these samples. Additional studies were carried out on total phospholipids and fractions were separated on TLC and taken for the measurement of radioactivity.

The results indicate that steroid treatment resulted in significant decrease in the incorporation of ^{14}C-glucose into lipid fractions both in the hippocampus and striatum (Table- 21). In the hippocampus decrease in total lipid accounted for the decrease in cholesterol and total phospholipid fractions but not in galactolipid. Further within the phospholipid fractions only phosphotidyl choline (PC) and ethanolamine (PE) had significantly lower incorporation but not in PI + PS and sphingomyelin fractions. The per cent deficits were highest in PE (38%) followed by PC (34%) and cholesterol (31%).

TABLE- 21 : *In vivo* ^{14}C-glucose incorporation into various lipids in hippocampus and striatum at 20 days of age with corticosterone treatment.

Regions	Treatment	CHL	GAL	PI + PS	Sph	PC	PE
		CPM/g tissue (± S.E. from 7-10 observations)					
HIPPOCAMPUS	Control	4826 ± 358	1036 ± 106	5604 ± 976	1449 ± 298	10353 ± 738	4966 ± 369
	Experimental	3334 ± 354** (69)	892 ± 117	4592 ± 721	897 ± 122	6932 ± 692** (66)	3065 ± 283*** (62)
STRIATUM	Control	4730 ± 598	1817 ± 140	4264 ± 698	974 ± 136	8478 ± 1013	6212 ± 219
	Experimental	2663 ± 348** (56)	1058 ± 134*** (58)	4260 ± 263	1259 ± 206	5640 ± 856* (66)	4386 ± 781* (70)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ Significantly different from controls.

Per cent of control values are given in parenthesis.

Total phospholipid (mg/g) : Hippocampus - 27.7 ± 0.9 Vs. 28.7 ± 1.2

Striatum - 34.8 ± 0.9 Vs. 36.6 ± 0.6

In the striatum decreased incorporation was due to decrease in all the three fractions - cholesterol, phospholipids and galactolipids. As in the case of the hippocampus, within the fractions of phospholipids PC and PE were the only two fractions decreased. The maximum deficits were in cholesterol (44%) followed by galactolipids (42%), PC (34%), and PE (30%).

Thus the decrease in glucose incorporation in to lipids was more affected in the striatum than in the hippocampus.

Contrary to expectation, the results on ^{14}C-glucose incorporation showed a decreased rate of cholesterol synthesis from glucose. The increase in cholesterol concentration noticed in the earlier experiment due to corticosterone treatment, now raised the probability of other alternative substrates for increased cholesterol synthesis. Interestingly, decrease in glucose incorporation in both these regions with such a remarkable decrease would indicate that the supply of glucose to these regions would have decreased as the deficits were not in a specific lipid constituent, but was rather a uniform one in all lipid fractions. As will be discussed later this decreased incorporation cannot be attributed to blood glucose level as it was found to be significantly elevated with the treatment (Table- 22)- such a rise has been demonstrated by other workers as well (Sapolsky, 1987). This decreased incorporation of ^{14}C-glucose could be due to the decreased uptake of the substrate by the hippocampus and striatum as such an effect of corticosterone has been shown earlier

(Landgraf et al, 1978). The mechanism of inhibition of glucose uptake by corticosterone, although not clear in brain, has been shown to be by decreasing the available glucose transporters from cell surface into the intracellular membrane fractions in the case of fibroblast cells in culture (Horner et al, 1987).

Amongst the two regions, striatum showed greater deficits compared to hippocampus. This difference, partly can be accounted for the difference in the total amount of lipid present in the two with striatum having higher amounts of lipid compared to hippocampus. In addition, striatal galactolipid concentration, although was not decreased, there was significant decrease in the ^{14}C-glucose incorporation into this fraction. Thus these results suggest that corticosterone treatment during early stages of brain development decrease the glucose incorporation into lipids in the hippocampus and striatum, probably by decreasing its uptake by these regions.

Thus the contradictory results of increased cholesterol concentration and decreased glucose incorporation into the various lipid fractions following corticosterone treatment from 17-19th postnatal day raised the possibility of altered utilization of other substrate for the purpose of lipid synthesis. As mentioned earlier, at or around the postnatal age of 20, ketone bodies contribute to a substantial proportion for lipid synthesis (Webber and Edmond, 1977). Perhaps the decrease in glucose uptake by these regions might have altered the proportion of ketone bodies being utilized for this purpose. In this connection, it has been reported that, at this age - 45-80% carbon derived from acetoacetate or β-hydroxybutyrate goes for lipid synthesis as against 14-40% from glucose (Yeh et al, 1977). Amongst different fractions of lipids, acetyl groups derived from ketone bodies are used for sterol synthesis to a larger extent than acetyl groups originating from glucose (Koper et al, 1981). Therefore, it is possible that the discrepancy observed with regard to the increased concentration of cholesterol and a decreased glucose incorporation could be due to increased proportion of ketone bodies being utilized for this purpose. Since the utilization of ketone bodies by brain depends on the circulating levels of ketone bodies in the blood which is known to be high during this developmental stage. (Hawkins et al, 1971).

Glucocorticoids are known to increase hepatic gluconeogenesis and increase plasma free fatty acids. It was, therefore, thought to check the blood levels of glucose and ketone bodies. Surprisingly, there are several reports on the blood glucose levels which have consistently reported an increase in blood glucose with corticosteroid treatment (acute) while information in this regard with respect to ketone body is scarce. The studies of Aranguez et al. (1986) have injected cortisol on day 1, monitored blood glucose and β-hydroxybutyrate levels at different postnatal ages (8, 12 and 22 days) and reported high levels of ketone bodies on day 8 but not subsequently whereas blood glucose level increased till day 4 and declined thereafter at all the ages - 6, 8, 12 and 24 days.

3:4:4 Effect on Ketone Body Utilizing Enzyme (BDH)

In the present study there was significant decrease in the blood ketone body levels (Table- 22), perhaps due to increased utilization by the peripheral tissues. Unfortunately, owing to the lack of availability of labelled ketone bodies it was not possible to check this possibility. Alternatively, it was thought to check the activity of ketone body utilizing enzyme, mainly β-hydroxybutyrate dehydrogenase (BDH). The reasons for suspecting alteration in enzyme activity are (i) activity of this enzyme can be induced by blood levels of ketone bodies, (ii) the maximal response in induction can be observed around 18-20 days and (iii) it was observed that neonatal undernutrition achieved by feeding dams a low protein diet, although did not alter blood ketone bodies, increased significantly the activity of BDH only at postnatal day 18 whereas at day 10 and 30, time points at which in spite of blood levels of ketone bodies being very high, BDH activity was not altered. This indicates that other factors could possibly induce ketone bodies metabolizing enzyme in under-nourished animals (Juliet, 1988). Elevation of corticosterone levels with undernutrition has been reported by several investigators. Taking these points into consideration, it was of interest to check whether there is alteration in the BDH activity with corticosterone treatment.

TABLE- 22 : Effect of chronic corticosterone treatment on BDH activity (units/g tissue $\pm$ S.E.) in 20 day old rat brain regions.

Treatment	[a] Blood ketone levels (umoles/ml)	BDH Activity		
		Cerebellum	Hippocampus	Striatum
Control	0.339 ± 0.002 (13)	1.634 ± 0.092 (6)	1.598 ± 0.009 (5)	1.586 ± 0.034 (6)
Experimental	$0.255 \pm 0.019^{***}$ (16)	$1.206 \pm 0.114^{**}$ (7)	$1.900 \pm 0.05^{**}$ (7)	$2.11 \pm 0.166^{***}$ (7)

** $P < 0.01$; *** $P < 0.001$, Significantly different from controls. * $P < 0.05$.

[a] Blood glucose values (mg %) - Control 79.0 ± 3.0 Experimental $99.6 \pm 7.0^{*}$ (n=6)

The results presented in Table- 22 showed significant increase in the activity of BDH in the hippocampus and striatum whereas a decrease in the cerebellum with chronic corticosterone treatment at postnatal day 20. The results when seen in terms of specific activity in the hippocampus and striatum exhibited a similar trend. Activity of BDH in cerebellum and striatum in the present study are higher than the values reported in literature for the corresponding regions (Leong and Clark, 1984b). Whereas for hippocampus value is not available in the literature for comparison.

In conclusion, chronic corticosterone administration affect predominantly the hippocampus and striatum during third week of postnatal life by increasing cholesterol concentration. This increase in concentration was associated with decreased carbon atoms going from glucose to sterol synthesis, perhaps due to decreased uptake of glucose in these regions. The increased BDH activity in brain regions associated with decreased blood ketone body levels provide circumstantial evidence for the increased ketone body utilization culminating in the increased cholesterol concentration with corticosteroid treatment.

3.5 Overview :

Increased corticosteroid secretion by adrenal glands and its regulation by hypothalamo-pituitary-adrenal axis in response to various physiological stressors has been very well documented. The key to normal functioning of the pituitary-adrenal axis appears to be prompt and efficient turning on and shutting off of the adrenocortical stress response i.e., the rapid production of glucocorticoids appears to be consistent with good adjustment to the environment, as long as efficient shut off of secretion can also occur. The efficient shut off of corticosteroid secretion that ensues as stress response envisages an important role for hippocampus (Sapolsky et al, 1984) that could be influenced by experiences during early life (Meaney et al, 1988). An excessive cumulative exposure to glucocorticoid can initiate a cascade of degenerative processes in the brain which include hippocampal neuron loss and cognitive impairments (Meaney et al, 1988). Glucocorticoids also affect many neurochemical systems within the brain, including monoamines, amino acid transmitters and lipids etc. Their biochemical effects include regulation of neurotransmitter receptor levels, generation of second messengers, and alteration of some enzyme activities. Thus it is not at all surprising that glucocorticoids influence mood, perception, attention, recognition, learning and extinction and that excess of glucocorticoids can precipitate psychotic reactions and insomnia.

The heterogeniety in distribution and development of various neurochemical systems in brain regions is very well known.

In spite of this, several gaps exists in the information available to date regarding the development of lipids, certain enzymes such as GABA-T and GS in discrete brain regions, the hippocampus and striatum. The present study has attempted to obtain information in this regard and suggest differences in the pattern of accumulation of cholesterol and galactolipid as well as the enzymes of GABA metabolism. One of the notable observation made during this study is that the striatum showed a delayed pattern of maturation in terms of GAD and GABA-T activities, concentrations of 5HT and DA and in cholesterol and galactolipid accumulation - all of which continued to increase beyond day 40. On the other hand, hippocampus attained adult level of GAD, GS and GDH activities and the concentrations of p-Tyr, Try and 5HT before day 30. Even in terms of galactolipid accretion, striatum showed a maximal rate of accumulation beyond day 40 suggesting a delayed pattern of maturation in this region.

From the results on effect of corticosterone on administration these above mentioned parameters, several interesting observations could be made. The effect of corticosterone (summarised in Tables 23 & 24) could be observed mainly in young animals, especially around day 20 as even cholesterol increase was found at this age. With respect to GABA related enzymes and cholesterol increase, hippocampus and striatum seem to be affected most with steroid treatment suggesting a region specificity. The studies on bioamine metabolism indicate that the region specificity in terms of biochemical changes occur, apparently, after the age of 30 days.

TABLE-23 : Summary of the effects of chronic corticosterone treatment on the enzymes of GABA metabolism during development.

	10	20	40	Adult (>90 days)
		GAD		
Cerebellum	-	-	-	-
Hypothalamus	-	-	-	-
Hippocampus	-	↑	-	-
Striatum	-	↑	-	n.d.
		GABA-T		
Cerebellum	-	-	-	-
Hypothalamus	-	↑	↑	-
Hippocampus	-	↑	↑	-
Striatum	-	↓	↓	-
		GS		
Cerebellum	↑	↑	n.d	↑
Hypothalamus	↑	-	-	-
Hippocampus	-	↑	-	-
Striatum	-	-	n.d	n.d
		GDH		
Cerebellum	↓	-	-	-
Hypothalamus	-	-	-	-
Hippocampus	↓	-	-	-
Striatum	-	-	-	↑

↑ increased; ↓ decreased

\- no change; n.d. not determined

TABLE-24 : Summary of the effects of chronic and acute cortico-sterone treatment on bioamine concretration and their precursors and metabolites.

Treatment	Region	Days	Tyr.	DA	DOPAC	HVA	NA	Try.	5-HT	5-HIAA
Chronic treatment	HYPOTHALAMUS	10	↓	-	↓	-	↑	↓	-	↓
		30	↓	-	-	-	↑	-	-	-
		A	↓	-	↓	-	-	↓	-	-
	STRIATUM	10	↓	-	↓	↑	-	↓	-	↓
		30	-	-	-	-	-	-	-	-
		A	↓	-	-	-	-	-	-	-
	HIPPOCAMPUS	10	↓	-	↓	n.d	-	↓	-	↓
		30	-	-	-	n.d.	-	↓	-	-
		A	n.e	-	-	n.d	-	-	↓	↓
Acute treatment	HYPOTHALAMUS	10	-	-	-	-	-	-	-	-
		A	↓	-	↑	-	-	-	-	
	HIPPOCAMPUS	10	-	n.d	-	-	-	↑	-	-
		A	↓	n.d.	-	-	-	↑	-	↑

↑ = increase. ↓ = decrease

- = no change. n.d. = not determined

The alterations in enzyme activities (GAD and GABA-T) indicate a possible increased GABA in the striatum at day 20 and 40; in the hypothalamus a decrease at day 40 is possible under corticosterone treatment (Table-23). It is interesting to note here that the effects of ACTH and corticosteroids on epilepsy are related to the state of maturation of the nervous system. Little or no anticonvulsant activity has been associated with these compounds in adult. Thus it would appear that their effectiveness is limited to the immature nervous system (De Lorenzo and Dashefsky, 1985) and possibly associated with the age specific responses of the GABA-T and GAD enzyme activities observed in the present study.

With regard to the possible mechanism of altered activities of GABA related enzymes in this study, the increased GS activity could be due to the increased *de novo* protein synthesis as Patel *et al.* (1983b) have demonstrated such a possibility. The altered GABA-T activity although, was associated with corresponding alterations in specific activity, conclusion of increased *de novo* protein synthesis with corticosterone treatment cannot be drawn, as it has been demonstrated by Maitre *et al.* (1979) that the turnover number of GABA-T (expressed as the number of moles transformed per second per mole of enzyme) differed from one region to another and could be altered by certain effector molecules. In this connection, Ossola *et al.* (1979) have isolated and characterized a natural GABA-T inhibitor from astroblast primary cultures. Under such conditions, a possibility that the concentration of GABA-T inhibitor molecule could be modulated by corticosterone treatment and thereby altered GABA-T activity. Alternatively, glucocorticoid may be increasing the activity of GABA-T activity depending on the concentration of the inhibitors during developmental stages. GABA-T has been shown to exist in high concentration in glial cells and possess a higher affinity for GABA than does GABA-T of synaptic or postsynaptic compartments (Tardy et al, 1979). With the increased corticosterone by exogenous administration, the type-II glucocorticoids may be occupied by the hormone and result in the altered enzyme activity in glial cells. As mentioned earlier, all the three regions - hippocampus, hypothalamus and striatum have been shown to possess such receptors for corticosterone (De Fiore and Turner, 1983). With respect to increased GAD activity at day 20 in the hippocampus and striatum, the specific activity was not increased significantly and hence may reflect the activation of the enzyme. The region and age specificity of this effect, however, cannot be explained with the present data. Nevertheless, several potentially important factors have been identified that regulate the in vivo GAD activity. These include the presence of multiple forms of the enzyme in brain (Bayon et al, 1977; Denner and Wu, 1985), interconversion of apo and holo-GAD (Miller et al, 1978b), binding of the enzyme to membranes (Covarrubias and Tapia 1978) and protein phosphorylation process (Sze, 1979). Recently Spink et al. (1987) have resolved four molecular forms of GAD having similar molecular weights but differ in electropheretic mobility, subcellular distribution, affinity for glutamate and in their inactivation by glutamate and GABA in the absence of PLP, and suggested that these multiple forms differ in regulatory properties. In view of these findings, it may be possible that only a certain form of GAD respond to excess corticosterone which may predominantly be present in these regions at these specific age. Similarly, GDH, present in mitochondrial matrix is also present in substantial amount in membrane rich fractions and differ in their allosteric inhibition by GTP. The differential distribution may serve different metabolic requirements in the brain. A membrane localization of the enzyme may expose it to a particular metabolic compartment of neural or glial cells that may be distinct from that associated with the readily solubilized GDH (Colon et al,1986). In the present study, the availability of such different form of GDH to corticosterone during different stages of development might be important for its responsivity. Thus irrespective of the mechanism involved, enzymes of GABA and glutamate metabolism are responsive to corticosterone administration predominantly at around day 20. Interestingly, even in intestine certain enzymes have been shown to be responsive to corticosteroids during this period wherein most of the adaptive changes occur.

The studies on the effect of chronic corticosterone treatment indicate that an uniform decrease in the precursors, p-tyr and try concentration in all the brain regions at day 10 and also a decreased 5HT and DA metabolism as reflected by metabolite levels. The decreased DA metabolism, however, was observed only in hypothalamus and the 5HT metabolism in the hippocampus at adult stage suggesting a region specific effects at this stage. In contrast, acute treatment increased 5HT metabolism only in the hippocampus and DA metabolism only in adult hypothalamus. One of the observation made here is that, the decrease in p-tyr and tryptophan in the 10 day old brain regions, with corticosterone treatment. This assumes importance from two points of view - tryptophan being one of the scarce amino acids is drastically decreased and may lead to decreased protein synthesis during a period of high rate of protein synthesis and also on the 5HT metabolism. Tryptophan has been extensively studied, with reports of its effects on sleep, locomotor activity, pain sensitivity, aggression and eating behaviour. In general tryptophan has a calming or behavioural depressant action inducing sleep in low doses and reducing locomotor activity. The behavioural effects of tyrosine have been less extensively studied. Tyrosine administration reduces the hyperprolectinemia associated with chronic reserpine treatment (Cf. Gibson, 1985). Furthermore, tryptophan has been tested in many conditions including depression, myoclonus, migraine headaches, sleep disorders and tyrosine is carefully being investigated as an antidepressant (Wurtman et al, 1981). In the wake of the present finding that these precursors and neurotransmitter metabolites concentration is decreased with chronic corticosterone treatment, it would be interesting to explore their possible association with childhood depression as suggested by Cantwell (1983) and other psychiatric disorders that are accompanied by high level of corticosteroids.

It may be relavant to note here that at day 10, the plasma level of corticosteroids have been shown to be low and the feed-back regulation of the elevated corticosterone develops at a much later age (Sapolsky and Meany, 1986). With the administration of corticosterone to these pups, an elevation in its plasma level which was not regulated by inhibitory feed-back mechanism leading to proloned high level of steroid exposure of the brain regions. Thus exogenous administration of corticosterone to animals at this age would be similar to the extent of exaggerated plasma corticosteroid level and decreased efficiency of feed-back regulation observed in psychiatric disorders.

The region specificity with respect to bioamine changes observed only at adult stage may be attributed to two reasons - (i) As mentioned above, with the lack of feed-back regulation of plasma corticosterone at day 10, the exogenously administered corticosterone may have a greater eccess to all the regions and cells whereas with the presence of efficient feed-back regulation at adult stage sufficient amount of corticosterone may not be reaching all these regions. (ii) Associated with this may be the fact that complete maturation of vatious processes of bioamines occur only after day 30 as also of glucocorticoid receptor systems, thus leading to region specificity in adult stage.

Results on lipid changes with corticosterone treatment suggest a possibility of structural and functional alterations in the hippocampal and striatal membranes. This possibility arise from two observations made in this study -

(a) an increased cholesterol concentration in the hippocampus and striatum with chronic corticosterone exposure during early period of life (16-19 and 7-9 postnatal days);
(b) a decreased incorporation of ^{14}C derived from glucose into phospholipids (PE and PC) with no change in total phospholipids (Table-21). It has been suggested that cholesterol increases the rigidity of biological membranes and consequently many of its functional aspects (Mc Ilwain and Bachelard, 1985).
In addition, the decreased contribution of glucose to PE and PC observed in the present study, irrespective of the relative contribution from ketone bodies, may led to small but sudtle decrease in these components and therefore may alter the membrane fluidity in these regions. Under such conditions, the increased cholesterol concentration might be a case of adaptive change to prolonged corticosterone exposure. In this regard, glucocorticoids altered membrane fluidity in dog brain synaptosomes (Deliconstantinos, 1985) and in the primary neuronal-enriched cultures from fetal mouse spinal cords (Demeduik et al, 1985).

An increased Ca^{++} uptake (Vmax) by rat brain synaptosomes has been demonstrated under physiological concentration of corticosterone (Sze, 1980). In view of these findings, the altered incorporation of glucose into phospholipids and increased cholesterol concentration in region and age specific manner may suggest altered structure of hippocampal and striatal membrane and consequently its functions. The glucose incorporation study also indicate deleterious effects of elevated corticosterone level during early period of brain development by decreased glucose uptake in these regions, that may be important both as energy substrate as well as for the anabolic processes culminating in an increased vulnerability to further insults as proposed by Sapolsky (1987). This study also emphasizes the importance of other substrates for energy and tissue building purpose (ketone bodies) under the conditions of prolonged glucose depletion due to excess of corticosterone as evidenced by the increased BDH activity in the specific regions, hippocampus and striatum.

In conclusion, the present study demonstrates an age dependent effect of altered corticosterone levels on the hippocampus and striatum. The effect of corticosterone in these regions also demonstrate alteration in enzymes of GABA metabolism, biamine metabolism and cholesterol in younger animals and suggests a possible alteration in the metabolic processes that may lead to its vulnerability to other insults during development.