C H A P T E R IV

S U M M A R Y

SUMMARY

It is well known that the developing brain is vulnerable to nutritional deficiencies in early life. Extensive studies on the effects of early deficiencies of protein, calorie and certain vitamins, in animals revealed the adverse effects on the brain maturation as judged by the observed deficits in brain protein, DNA, RNA and lipids and changes both in the activities of certain brain enzymes and morphology of CNS (Dobbing, 1968; Rajalakshmi and Ramakrishnan, 1972; Jones, 1976; Dakshinamurti, 1977; Subba Rao,1978). Early nutritional deprivations have also been associated with delayed neuromotor development and changes in various behavioural parameters (Barness et al., 1968; Frankova, 1974; Chase and Metcalf, 1975).

Cholinergic system is known to play a key role in a number of vital functions of CNS and also in behavioural modulations. However, large number of published studies seem to be directed towards understanding of cholinergic mechanisms vis-a-vis behavioural parameters. Studies on nutritional deprivation and cholinergic system as a whole are very few. Early undernutrition has been associated with altered activities of brain acetylcholine esterase (AChE)

and choline acetylase (ChAc) (Sereni et al., 1966; Adlard and Dobbing, 1971a; 1971b; Im et al., 1971; Eckhert et al., 1976a; 1976b; Tyzbir et al., 1977). Brain ACh levels were found to be altered by different stress conditions, drugs and also thiamine deficiency (Richter and Crossland,1949; Elliot et al., 1950; Naik et al., 1970; Vorhees et al., 1977). ACh levels in the brain were also found to be influenced by dietary levels of choline (Growdon and Wurtman, 1979). However, detailed studies on all individual components of cholinergic system in the developing brain of the undernourished and malnourished animals have not been catalogued.

The present studies were undertaken on the effects of nutritional deficiencies on the cholinergic system of the brain at different stages of development with special reference to the following aspects:

1. Effects of prenatal undernutrition induced by maternal protein deficiency during gestation.

2. Effects of different degrees of neonatal undernutrition induced by manipulating litter size and the maternal diet.

3. Comparative effects of deficiencies of calorie and protein during the postweaning period.

4. Effects of maternal thiamine deficiency during gestation and/or lactation on the progency.

5. Effects of thiamine deficiency during postweaning period.

6. The reversibility of any effects found with dietary rehabilitation.

The paramaeters studied were body and brain weights, brain protein, DNA, ACh AChE and ChAc. Preliminary studies on the incorporation of label from 1-^{14}C acetate and 3H (methyl) choline into brain ACh of the deficient rats were also initiated. Additional studies were also made of the effects of thiamine deficiency on thiamine and moisture content of the brain regions.

Prenatal undernutrition resulting from maternal protein deficiency during gestation was found to affect the body and brain weights significantly at birth. The activity of AChE per g brain was significantly lowered; whereas, ChAc activity remained unaffected.

Neonatal undernutrition induced by increasing the litter size was found to result in a reduction of body and brain weights at all ages studied but no significant deficits were found either in brain ACh levels or the enzyme

activities. A more severe degree of undernutrition was induced by feeding the mothers a protein deficient diet (5% protein) during only lactation (G^+L^-) or both gestation and lactation (G^-L^-) so that body and brain weight deficits were increased. The deficits in the weights were more drastic in G^-L^- group. The significant reduction was observed in brain ACh levels in both the groups at 21 days. However, the reduction was more in the G^-L^- group. ChAc activity per gram brain was reduced more in G^-L^- group as compared to G^+L^- group at 7, 14 and 21 days of age. AChE activity per g brain was reduced in G^-L^- group at all ages upto 21 days. However, the deficits were lower with the progress of deficiency suggesting a possible adaptation to the deficiency. AChE activity in G^+L^- group showed upward change, eventually resulting into a 13% increase at 21 days. This suggests a possible 'sparing' of cholinergic synapses subjected to neonatal undernutrition.

The subsequent dietary rehabilitation of the deficient rats resulted in partial 'catch-up' of body and brain weights associated with persistent significant deficits. However, brain ACh levels returned to normal. AChE activity was elevated in both groups whereas ChAc activity remained significantly lower as compared to the controls.

Severe deficiencies of protein and calories during post-weaning period were found to result in lowered body and brain weights. However, ACh levels were drastically reduced in protein deficient rats as compared to calorie deficient rats of the similar body weights. Nonetheless, severe calorie restriction resulted in significant deficits in brain ACh levels. The enzyme activities, however, remained unaffected. Protein deficiency for a period of 65 weeks did not affect the activities per gram brain of the cholinergic enzymes.

Preliminary studies on the incorporation of the label from 1-^{14}C acetate and 3H(methyl) choline into brain ACh revealed no significant differences in the protein deficient and the control groups.

In summary, undernutrition during early period of brain development seems to have adverse effect on brain ACh level and the activities of AChE and ChAc. However, the degree and timing of undernutrition seems to have differential effect on the individual components of the cholinergic system. It is felt that more detailed studies are required to throw more light on this differential effect and particularly on the correlation of ACh levels and the cholinergic enzymes.

Maternal thiamine deficiency during gestation and lactation resulted in significant deficits in body weight but not in the brain weight of the deficient pups as compared to those of pair fed controls. Brain ACh levels were significantly reduced at 21 and 28 days of life. However, the enzyme activities were unaffected. Thiamine deficiency during lactation alone resulted in lesser deficits in body weight. ACh level was reduced only at 28 days of life with the enzyme activities remaining unaffected. Subsequent dietary rehabilitation of the thiamine deficient pups revealed complete reversal of brain ACh levels but the body weight deficit still persisted.

Interestingly maternal thiamine deficiency during gestation and lactation was found to result in deficits of DNA and AChE activity specifically in a pooled regions of medulla + pons + stem, but not in cortex or cerebellum. However, more studies are required to augment these observation.

Thiamine deficiency during post-weaning period was associated with deficits in body weights but not in brain weight as compared to pair-fed controls. Moisture content in the brain regions remained unaffected. Thiamine concentration in liver, heart and the three brain regions

was significantly reduced in the deficient animals. However, there was no significant difference in the concentration between the three brain regions of the deficient animals. Brain ACh levels were reduced in the deficient animals as compared to pair-fed controls. However, cholinergic enzyme activity remained unaltered.

Preliminary studies on the incorporation of label from the two precursors did not reveal any significant differences in the deficient animals. However, as mentioned earlier, more observations would greatly augment the present findings.

In summary, thiamine deficiency during early period of life showed adverse effects on brain ACh levels but not on the cholinergic enzymes. The deficit appeared earlier in the progeny of rats reared on deficient diet during both gestation and lactation as compared to those of lactation alone. The ACh deficits were reversed on dietary rehabilitation. Regional studies revealed a peculiar deficit in AChE activity in one pooled region only. Postweaning thiamine deficiency resulted in significant deficits of brain ACh levels but not of the cholinergic enzymes.

In conclusion, the present studies demonstrate the effects of nutritional deficiencies during different stages of brain development on the cholinergic system of the brain. Early undernutrition seems to have differential effects on the individual components of the system. Thiamine deficiency during all the stages studied affected ACh levels but not the other components of the system.