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Effect of postweaning protein deficiency on the content and lipid composition of gray and white matter in neonatally undernourished rat brain

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Abstract. The effects of neonatal undernutrition and postweaning protein deficiency on the content and lipid composition of gray and white matter of 63 days old rat brain have been studied. The concentrations of different lipids remain the same, but the relative proportion of gray and white matter changes thus reflecting the differences in the concentration of whole brain lipids.

Keywords. Protein deficiency; white matter; gray matter; lipids.

Introduction

Reddy and Horrocks (1982) and Reddy *et al.* (1982) have recently reported that neonatal undernutrition is associated with significant changes in the lipid composition and the contents of gray and white matter in the rate brain. Further, they have also shown that nutritional rehabilitation for 6 weeks reverses the deficits observed in gray matter whereas in the case of white matter the lipid concentrations but not the contents come back to normal (Reddy *et al.*, 1982). The present study is concerned with the effects of undernutrition during suckling period superimposed by the postweaning protein deficiency on the lipid composition as well as the proportions of gray and white matter.

Materials and methods

Albino rats of the Charles-Foster Strain inbred in our animal house for several generations were used in these studies. Pups born on the same day in the stock colony were pooled together and assigned in litters of eight to mothers fed either stock diet (18% protein, control) or a low protein diet (5% protein, undernourished). The pups were weaned at 21 days of age in both the groups. At 21 days of age the pups from control group were given a high protein diet (20%) and the pups from undernourished group received a low protein diet (4%) for 6 weeks. All the rats were caged individually and food and water were given *ad libitum* throughout the experimental period. The composition of different diets used in this study are given elsewhere (Rajalakshmi and Nakhasi, 1974, 1974a, 1975).

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At the end of the experimental period (i.e. 63 days of age), all the rats were killed, brains removed and gray and white matter separated as described previously (Reddy and Horrocks, 1982). Lipids were extracted from the separated gray and white matter by the method of Folch *et al.* (1957). The methods for the estimation of different lipids were as described previously (Reddy and Horrocks, 1982; Reddy *et al.,* 1982).

All the chemicals used in various estimations were of Analar grade.

Results and discussion

When the neonatally undernourished rats were nutritionally stressed by feeding a low protein diet during postweaning period the body and brain weights were decreased further (table 1). A decrease of 71 and 24% respectively for body and brain weights at 3 weeks of age and 82 and 30% at 9 weeks of age were observed. Similar observations have been made by other workers (Guthrie and Brown, 1968; Fishman *et al.,* 1971; Krigman and Hogan, 1976; Reddy and Sastry, 1978). However, no significant differences were obtained between the control and protein deficient rats with regard to the concentration of different lipids either in gray matter or in white matter (table 1). It is interesting to note that the decrease

Table 1. Effect of neonatal undernutrition superimposed by postweaning protein deficiency on the lipid concentrations of rat brain gray and white matter at 63 days.

	Gray matter		White matter	
	Control	Protein deficient	Control	Protein deficient
	mg/g fresh weight*			
Cholesterol	14.7±0.43[a]	14.7±0.23	28.1±0.73	28.2±0.10
Galactolipids	4.5±0.20	4.4±0.10	28.2±0.56	27.1±0.93
Phospholipids	39.1±0.42	39.9±0.65	57.0±0.82	55.4±1.10
Plasmalogens	5.0±0.21	5.0±0.14	16.8±0.19	16.3±0.50
Ganglioside NANA (μg/g)	925±26	893±38	360±6	370±9
Phosphatidyl ethanolamine	14.5±0.43	15.0±0.29	23.4±0.56	22.6±0.58
Phosphatidyl choline	15.3±0.24	15.9±0.15	17.4±0.85	17.6±0.26
Sphingomyelin	2.4±0.02	2.5±0.12	3.9±0.33	4.3±0.20
Phosphatidyl serine+ phosphatidyl inositol	6.7±0.20	6.7±0.10	11.1±0.62	11.5±0.40

Body weights (g) were 179 ± 10 and 33 ± 1.1 and brain weights (g) were 1.73 ±0.02 and 1.21 ±0.02 for control and protein deficient groups respectively.

Experimental details are given in the text. The mean recovery of phosphorus in these components from TLC plates was 99.5%. NANA-N-acetyl neuraminic acid.

* Represents results from 7 observations. [a] Mean ± S.E.

observed in the concentration of different lipids at 21 days of age (Reddy and Horrocks, 1982) are bridged inspite of continued nutritional stress during the postweaning period. Similar observations have been made in this laboratory with regard to glutamate decarboxylase (Rajalakshmi and Telang, 1975).

The lack of difference in the concentration of different lipids between control and protein deficient rats in both gray and white matter contrasts with the lack of similar phenomenon in the whole brain (Krigman and Hogan, 1976; Reddy and Sastry, 1978).This discrepancy could prehaps be accounted for by the changes in the proportions of gray and white matter. Attempts were therefore made to estimate the gray and white matter content using the formula proposed by Reddy *et al.* (1982) namely a X + b (1—X) = C, where a represents the galactolipid concentration of gray matter, b, the galactolipid concentration of white matter and c, the galactolipid concentration of whole brain. The data are presented in table 2.

Table 2. Effect of neonatal undernutrition and subsequent protein deficiency on the content of rat brain gray and white matter.

	21 Day*			63 Day		
	C	UN	$\frac{UN \times 100}{C}$	C	PD	$\frac{PD \times 100}{C}$
Whole brain (mg)	1440	1100	76	1730	1210	70
Gray matter (mg)	1172	957	82	1111	926	83
White matter (mg)	264	141	53	619	288	47
% of whole brain						
Gray matter	81.6	87.2	—	64.2	76.3	—
White matter	18.4	12.8	—	35.8	23.7	—

* Values taken from Reddy *et al.* (1982)

C, Control; UN, undernourished; PD, protein deficient.

For experimental details see under materials and methods.

Values for gray and white matter were calculated as described in the text, using galactolipid concentration in whole brain, gray matter and white matter. The galactolipid values for whole brain were taken from the unpublished data of Mr. Hargit Singh, the values being 13.0 and 9.8 mg/g wet weight for control and protein deficient groups, respectively.

The decrease in the gray matter content (17%) is similar to that observed at 21 days of age. However, the increased reduction in the weight of whole brain observed at 63 days of age is reflected in greater reduction in the white matter content, the decrease being 24 and 30% respectively for the whole brain and 47 and 53% for the white matter at 21 and 63 days (table 2). The decrease in the content of gray matter correlates well with the delayed neuropil development (Cragg, 1972) and synaptogenesis (Gambetti *et al.,* 1974; Shoemaker and Bloom, 1977; Pysh *et al.,* 1979). Similarly the decreased myelin content in the undernourished rats

(Fishman *et al.,* 1971; Nakahasi, *et al.,* 1975; Wiggins and Fuller, 1978; Reddy *et al.,* 1979). The content of different lipids seems to be affected both in gray and white matter but the effects seems to be more on white matter (table 3).

Table 3. Effect of neonatal undernutrition and postweaning protein deficiency on the content of different lipids in rat brain gray and white matter.

	Whole brain*	Gray matter	White matter
		(% of control values)	
Cholesterol	62	83	47
Galactolipids	43	82	45
Phospholipds	65	85	45
Plasmalogens	64	84	45
Gangliosides	75	80	48
Phosphatidyl ethanolamine	59	86	48
Phosphatidyl choline	64	87	47
Sphingomyelin	66	87	47
Phosphatidyl serine+ phosphatidyl inositol	63	82	48

* Values taken from Reddy and Sastry (1978).

Values for gray and white matter were calculated from the mean values given in tables 1 and 2.

In conclusion, the neonatal undernutrition superimposed by postweaning protein deficiency seems to affect the quantity of both gray and white matter. The effects seems to be more on white matter. However, the quality of gray and white matter seems to be spared with regard to lipid concentrations inspite of the continued stress during postweaning period. Thus the qualitative and quantitative differences reported between control and undernourished rat brain (Krigman and Hogen, 1976; Reddy and Sastry, 1978) seems to be due to the changes in the proportions of gray and white matter.

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