The Effect of Riboflavin Deficiency on Cerebrum and Cerebellum of Developing Rat Brain

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Summary After 21-day-old weanling rats were maintained on diets deficient in riboflavin the weights of their brains were 19.8% less than those of rats on control diets. In riboflavin deficiency, the myelin lipids, cerebrosides, and sphingomyelin, as well as phosphatidylethanolamine, a significant component of the myelin membrane, were considerably reduced in proportion. It is considered that riboflavin plays some role in the metabolism of essential fatty acids in brain lipids and the pathological effect of its deficiency is similar to that of essential fatty acid deficiency, causing a fast impairment to brain development and maturation.

Key Words riboflavin, deficiency, lipids, brain and development

The role of riboflavin in the diet of animals has been reviewed by Foy and Mbaya (1). Lack of riboflavin manifests itself first by retardation and later by complete cessation of growth (2, 3). This vitamin is an integral part of the prosthetic group of acyl-CoA dehydrogenase (an enzyme of importance in the B-oxidation of fatty acids) and so a deficiency of this vitamin will affect the lipid content of organs such as the liver, kidney, adrenals, and arterial walls.

In mammals, the concentrations of brain lipids have been found to change in some dietary deficiencies (4–6). In rats, riboflavin deficiency has been associated with myelin degeneration (1). There is, however, a paucity of information on the effect of riboflavin deficiency on brain lipid composition. A study of the changes in the lipid composition of riboflavin-deficient cerebrum and cerebellum of rat brain has, therefore, been carried out.

MATERIALS AND METHODS

Forty male Wistar rats were divided into two groups each containing twenty animals and maintained respectively on the following diets: (1) riboflavin-deficient diet; and (2) control diet, containing adequate riboflavin.

The composition of the diets is shown in Table 1. The riboflavin-deficient diet contained no riboflavin. Riboflavin (177.6 mg/kg diet) was added to the control diet. The animals were placed on the diet at 21 days of age and weighed weekly. Owing to high mortality in the deficient group, the experiment was terminated at

Table 1. Composition of riboflavin-deficient diet.

Ingredient	g/kg Diet
Corn starch	516
Casein (vitamin free)	250
DL-Methionine	4
Cellulose	40
Sucrose	100
Corn oil	40
Vitamin mix ^a	40
Mineral mix ^b	10

	1,000

^aThe vitamin mixture supplied by Pfizer Livestock Feeds (Nig.) Ltd. contained the following (g/per kg): ascorbic acid (1.0); choline chloride (1.665); calcium panthothenate (0.067); i-inositol (0.110); menadione (0.499); niacin (0.0999); *p*-amino benzoic acid (0.1110); pyridoxine-Hol (0.0222); thiamin (0.0222); biotin (0.0004); folic acid (0.002); vitamin B₁₂ (0.00003); vitamin A-acetate (0.02); D₂-calciferol (0.0022); and α -tocopherol (0.614). ^bThe composition of the mineral mixture prepared in the Biochemistry Department, University of Ilorin, was as follows (g/kg): CaCO₃, 6.45; CuSO₄ · 5H₂O, 0.00072; ferric citrate, 0.64; MnSO₄ · H₂O, 0.055; (COOK)₂ · H₂O, 9.46; KI, 0.0016; NaCl, 4.32; KH₂PO₄, 3.11; MgSO₄, 1.13; CaHPO₄, 14.22 and ZnCO₃, 0.0176; Na₂SeO₃, 0.017; CoCl₂ · 6H₂O, 0.001.

5 weeks.

The brains were removed, weighed and if lipids were not extracted immediately, they were stored for up to 6 days at -20°C . Dry weights, total lipids, lipid fractions and individual lipids were obtained as previously described (7-13). Analyses of variance were carried out to determine the statistical significance of results.

RESULTS AND DISCUSSION

Riboflavin-deficient animals exhibited pathological signs (such as hair, foot, and tail lesions) from the 12th day of the experiment. The body and brain weights of the pair-fed rats deficient in riboflavin were reduced to 65.6 and 88.2%, respectively, of the control value (Table 2). Prentice and Bates (2) reported a considerably smaller increase in body weight than that observed in the present study for riboflavin-deficient rats. The differences in the results may partly be due to the type of rats used. They used male weanling Norwegian hooded rats while male Wistar rats were employed in the present study.

The higher ratio of brain to body weight observed in the deficiency state would probably indicate that the brain was more relatively spared than other organs. This observed quantitative protection of the brain is consistent with previous reports that the brain is normally protected from the effect of malnourishment imposed

Table 2. Body and brain weights (g) of riboflavin-deficient rats.

Analysis	Pair-fed	
	+ Rib	- Rib
Initial body wt.	29.9±0.5	30.5±0.7
Final body wt.	165.4±5.8	108.5±6.6
Brain weight	6.20±0.3	5.47±0.3
Ratio of brain/Final body wt.	0.037±0.002	0.050±0.003

The results are the mean values for ten rats in each group ±SD.

Table 3. Lipids of rats brain cerebrum and cerebellum. Values are expressed as percentage dry weight and are the mean values from ten brains for each dietary group (analyzed in duplicate)±SD.

	Anatomical region							
	Cerebrum				Cerebellum			
	+ Rib		- Rib		+ Rib		- Rib	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total lipids	51.2	3.7	49.5	2.9	47.3	2.4	47.8	2.9
Total phospholipid	21.7	1.6	20.6	2.0	20.8	1.9	19.6	1.9
Phosphatidylcholine	4.7	0.6	7.6	0.5	4.0	0.4	8.5	0.7
Sphingomyelin	3.6	0.5	2.5	0.4	3.8	0.3	2.6	0.3
Phosphatidylethanolamine	11.0	0.9	8.8	1.2	10.9	0.8	8.2	0.9
Phosphatidylserine	0.7	0.1	0.8	0.1	0.5	0.1	0.5	0.1
Phosphatidylinositol	0.7	0.1	0.7	0.1	0.4	0.04	0.7	0.1
Phosphatidic acid	0.3	0.01	0.3	0.01	0.3	0.01	0.3	0.01
Diphosphatidylglycerol	0.5	0.01	0.5	0.01	0.4	0.02	0.5	0.02
Total neutral lipids	18.8	1.7	21.6	2.1	19.2	1.1	22.5	1.3
Free fatty acids	1.1	0.1	7.2	0.4	0.9	0.1	6.8	0.5
Cerebrosides	10.1	0.5	7.0	0.3	6.6	0.6	5.0	0.3
Gangliosides	0.6	0.1	0.3	0.1	0.7	0.1	0.4	0.1
Dry weight (mg)	329.4	18	241.2	11	76.6	5.5	67.8	4.0
Wet weight (mg)	1,647	70	1,254	45	385	14	301	8.4

after weaning in the rat (6, 14).

Results of the present study have shown that the total lipid concentration remained fairly constant (Table 3). Similarly, Odutuga (5) observed no change in total lipid concentration when rats were fed a diet deficient in essential fatty acids.

There were considerable changes in the concentration of the glycolipids in the deficiency state. Compared with the controls, when rats were fed riboflavin-deficient

diet, cerebroside were reduced to 69.3 and 75.8% in the brain cerebrum and cerebellum respectively. The values for gangliosides were 50 and 57.1% in the brain cerebrum and cerebellum, respectively. These values are statistically significant ($p < 0.001$). The cerebroside and sphingomyelin are myelin lipids and have been used as an index of myelination (7). It has been observed in this study, that in riboflavin deficiency, there was a greater reduction in cerebral compared to cerebellar cerebroside. This might indicate a slightly greater reduction in myelination. This difference in the effect of riboflavin deficiency on cerebrum as compared to cerebellum was not, however, as pronounced as that previously observed in EFA deficiency (6).

Phosphatidylcholine was significantly increased ($p < 0.01$) while phosphatidylethanolamine was reduced ($p < 0.01$) when rats were fed riboflavin-deficient diet (Table 3). It has been suggested that phosphatidylcholine may have a role to play in non-myelin membrane formation during the early part of development (8). A considerable reduction (20.0 and 24.8% in cerebrum and cerebellum respectively), within 5 weeks, in the concentration of phosphatidylethanolamine, which is a major component of the myelin membrane, may indicate fast changes in membrane structures and function. Sphingomyelin, another myelin lipid, was significantly reduced to 69.4 and 68.4% ($p < 0.001$) in cerebrum and cerebellum respectively when rats were maintained on riboflavin-deficient diet. Such a reduction has been previously reported for essential fatty acid deficiency (5).

The present study has clearly shown that there are interesting similarities between the pathology of essential fatty acid deficiency (5, 15) and riboflavin deficiency, particularly as regards brain composition. Foy and Mbaya (1) have suggested that the enormous increase in the size of liver mitochondria in riboflavin-deficient mice may be associated with diminished mitochondrial function. They also contended that the deficiency of essential fatty acids produced enlarged mitochondria which reverted to normal with riboflavin.

The accumulation of free fatty acids in both cerebrum and cerebellum observed in the present study would probably indicate inhibition of either lipolysis or prostaglandin synthesis. Moreover, the observed dermal lesions may indicate that essential fatty acids were not converted into prostaglandins in riboflavin deficiency. Similarly, it has been shown that prostaglandin synthesis in adipose tissue was increased by lipolytic hormones and reduced by essential fatty acid deficiency (16).

Since mitochondrial activities are associated with lipid metabolism, it is postulated that riboflavin is essential for phospholipid metabolism and the utilization of essential fatty acids. The altered lipid composition in riboflavin deficiency found in the present studies is considered to be a direct result of riboflavin deficiency (17, 18) affecting lipids, especially the essential fatty acids and phospholipids which are involved in the membrane structure metabolism, and altering the structure and function of brain cells and membranes (5, 19) and impairing the proper coupling of oxidative phosphorylation.

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