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Activities of myelin bound cytidine 5′-diphosphate-choline 1, 2 diacyl-glycerol choline phosphotransferase and uridine 5′-diphosphate-galactose-ceramide galactosyltransferase under restricted food intake

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Abstract. Activities of cytidine 5'-diphosphate-choline glycerol choline phosphotransferase and uridine 5'-diphosphate galactose-ceramide galactosyltransferase were determined in isolated myelin in different brain regions of control, and rats with restricted food intake. Kinetic experiments indicated an increase in K_m value of phosphocholinetransferase in brain stem of undernourished rats, without significant change in the specific activity of this enzyme. Stimulation of this myelin bound enzyme activity was also evident in the animals when myelin was treated with the detergent: Tween CF. 54. Though specific activities of galactosyl transferase in myelin of undernourished rats were significantly diminished, the K_m of this enzyme was unaltered. These studies point to an adverse effect of early nutritional stress on the activities of enzymes bound to myelin membrane which has hitherto been considered metabolically inert.

Keywords. Myelin; UDP-galactose-ceramide galactosyltransferase; CDP-choline; nutritional stress.

Introduction

Maternal feed inadequacy instituted during gestation, and continued through lactation in dams, was found to result in marked impairments in the activities of microsomal cytidine 5′-diphosphate (CDP) choline: l,2-diacyl-sn-glycerol choline phosphotransferase (EC 2·7·8·2) and uridine 5′-diphosphate (UDP) galactose: ceramide galactosyltransferase (EC 2·4·1·45) in developing brains of the offspring nursed by such feed restricted animals. The presence of enzymes needed to convert diacylglycerol to phosphatidylcholine and ethanolamine, in highly purified myelin is well established. In view of the proposed role of some of these enzymes in myelin maintainence and remodelling (Leeden *et al.*, 1985), a detailed study was undertaken to enable us to obtain an insight into the developmental profiles of these 2 myelin enzymes in different regions of the rat brain under nutritional stress.

Materials and methods

Typically, undernutrition was imposed by feed restriction (experimental animals fed 50% of the amount consumed by control animals on an ad-lib regimen of a 22% protein diet adequate with respect to all other constituents) through gestation and lactation, in 10-day old pregnant Wistar strain rats. Pups nursed by these 2 groups of dams were considered as experimental and control respectively, litters pooled to a size of 8 numbers at birth, and used at 14 and 21 days of postnatal age. Myelin and microsomes were isolated from pooled rat brain regions by the method of Wu and Ledeen (1980). A second procedure utilizing EGTA (DeVries, 1976) to obtain

Abbreviations used: CDP, Cytidine 5'-diphosphate; UDP, uridine 5'-diphosphate.

myelin free from axolemmal contamination was also employed. Choline phosphotransferase and galactosyltransferase were assayed by the method of Wu and Ledeen (1980) and Costantino-Ceccarini *et al.* (1979) respectively. Myelin membrane subfractions were routinely checked for purity by assaying succinate dehydrogenase (mitochondrial marker) glucose-6-phosphatase and NADPH-cytochrome C reductases (microsomal markers) by the methods of Morre (1971) and Takeshita *et al.* (1982).

Results and discussion

The myelin choline phosphotransferase activity was activated in the presence of Triton CF-54 in both groups of animals. Interestingly, however, the activation was higher in myelin obtained from control cerebella and brain stems; myelin from control brain stems exhibited an 8-fold increase in the presence of 60 μg of Triton CF-54, while experimental brain-stem myelin yielded around 6·5-fold stimulation (figure 1). The results of varying substrate concentrations (table 1) revealed

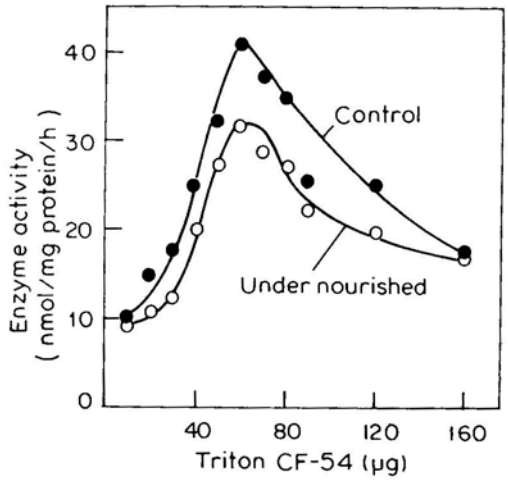

Figure 1. Effect of Triton CF-54 concentration on brain stem myelin choline phosphotransferase activity.

Table 1. K_m values for choline phosphotransferase and UDP-galactosyl transferase of brain stem-myelin of control and undernourished weanling rats.

Enzyme	Substrate		K_m
Choline phosphotransferase	Diolein	C	$2{\cdot}6 \pm 0{\cdot}3 \times 10^{-4}$M
		E	$4{\cdot}4 \pm 0{\cdot}3 \times 10^{-4}$M
	CDP-choline	C	$3{\cdot}6 \pm 0{\cdot}6 \times 10^{-4}$M
		E	$6{\cdot}5 \pm 0{\cdot}6 \times 10^{-4}$M
UDP-galactosyl transferase	Hydroxy ceramide	C	$2{\cdot}1 - 2{\cdot}4 \times 10^{-4}$M
		E	$2{\cdot}2 \pm 2{\cdot}4 \times 10^{-4}$M
		C	$2{\cdot}7 \pm 3{\cdot}4 \times 10^{-5}$M
		E	$2{\cdot}6 - 3{\cdot}5 \times 10^{-5}$M

C, Control; E, experimental,

Table 2. Myelin UDP galactose: ceramide galactosyltransferase in the developing rat brain

	Enzyme activity (nmol/mg protein/h)		
	Cerebrum	Cerebellum	Brain stem
14 day control	24·5 ± 2·66	27·1 ± 2·07	32·3 ± 1·07
14 day experimental	17·7 ± 2·96*	18·4 ± 2·65***	22·8 ± 1·80***
21 day control	16·7 ± 1·44	19·1 ± 1·84	21·5 ± 3·04
21 day experimental	10·1 ± 1·67**	9·7 ± 1·49**	10·6 ± 2·99***

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

significant differences in the K_m of the myelin enzyme for both, diolein and CDP-choline in experimental brain stems at the 21-day stage, as compared to corresponding control values. One plausible explanation of the differences (in response to detergent activation) of enzyme activity, between myelin derived from control and experimental animals could be attributed to the fact that myelin membrane acyl phospholipid composition is appreciably different in brain stem from undernourished rat pups (Shantaram and Srinivasa Rao, 1989).

The activity of myelin choline phosphotransferase is not altered in experimental animals. In contrast to this, the specific activities of myelin galactosyltransferase were drastically diminished (table 2) as compared to control values, in all 3 regions of the brain, during the third week of postnatal life. The K_m of myelin galactosyltransferase for either UDP-galactose or hydroxy fatty acid ceramides did not vary in myelin membrane from control and experimental brains (table 1) irrespective of age or regions. This observation supports the hypothesis that hypomyelination observed in the developing brains of undernourished animals may be a consequence of decreased synthesis of galactosylceramides, since these lipids are localised on the external surface of myelin membrane and are known to contribute to the stability of the myelin sheath.

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