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BRAIN RESEARCH

Research Report

Effects of pre- and postnatal protein malnutrition in hypoxic–ischemic rats

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ABSTRACT

Neonatal hypoxic–ischemic encephalopathy (HI) is a major cause of nervous system damage and neurological morbidity. Perinatal malnutrition affects morphological, biochemical and behavioral aspects of neural development, including pathophysiological cascades of cell death triggered by ischemic events, so modifying resulting brain damage. Female Wistar rats were subjected to protein restriction during pregnancy and lactation (control group: 25% soybean protein; malnourished group: 7%). Seven days after delivery (PND7), their offspring were submitted to unilateral cerebral HI; rats were then tested for sensorimotor (PND7 and PND60) and memory (PND60) functions. Offspring of malnourished mothers showed marked reduction in body weight starting in lactation and persisting during the entire period of observation. There was a greater sensorimotor deficit after HI in malnourished (M) animals, in righting reflex and in home bedding task, indicating an interaction between diet and hypoxia–ischemia. At PND60, HI rats showed impaired performance when compared to controls in training and test sessions of rota-rod task, however there was no effect of malnutrition per se. In the open field, nourished HI (HI-N) presented an increase in crossings number; this effect was not present in HI-M group. Surprisingly, HI-M rats presented a better performance in inhibitory avoidance task and a smaller hemispheric brain damage as compared to HI-N animals. Our data points to a possible metabolic adaptation in hypoxic–ischemic animals receiving protein malnutrition during pregnancy and lactation; apparently we observed a neuroprotective effect of diet, possibly decreasing the brain energy demand, under a hypoxic–ischemic situation.

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Table 1 – Nutritional composition of diets (g/kg of diet).

Component	Soybean diet	
	25%	7%
Soybean protein (92%) [a]	271.4	76
Salt mix [b]	40	40
Vitamin mix [c]	10	10
Non-nutritive fiber	10	10
Carbohydrate (corn-starch)	512.5	707.5
Fat (soybean oil)	150	150

[a] Soybean protein (from Solae, São Paulo, Brazil), 92% purity supplemented with 0.15% L-methionine.
[b] Mineral mixture mg/100 g of ration: NaCl, 557; KCl, 3.2; KH2PO4, 1556; MgSO4, 229; CaCO3, 1526; FeSO4·7H2O, 108; MnSO4·H2O, 16; ZnSO4·7H2O, 2.2; CuSO4·5H2O, 1.9; CaCl2·6H2O, 0.09.
[c] Vitamin mixture (from Roche, São Paulo, Brazil) mg/100 g of ration: vitamin A, 4; vitamin D, 0.5; vitamin E, 10; menadione, 0.5; choline, 200; p-aminobenzoic acid (PABA), 10; inositol, 10; niacin, 4; pantothenic acid, 4; riboflavin, 0.8; thiamin, 0.5; pyridoxine, 0.5; folic acid, 0.2; biotin, 0.04; vitamin B12, 0.003. Energy for both diets: 4.3 kcal/g.

1. Introduction

Neonatal cerebral hypoxia–ischemia (HI) is an important cause of neurological deficits in humans leading survivors to exhibit permanent disabilities, including cerebral palsy, mental retardation and epilepsy (Vannucci and Hagberg, 2004; Volpe, 2008). The mechanisms involved in the pathophysiology of HI injury include cellular energy deficit, glutamatergic excitotoxicity and oxidative stress (McLean and Ferriero, 2004). Furthermore, cell damage can be exacerbated by cytokine activation and infiltration of inflammatory cells in response to the initial damage (McLean and Ferriero, 2004; Mishra and Delivoria-Papadopoulos, 1999).

The Levine brain anoxia–ischemia method, as modified by Rice et al. (1981) has been utilized as a rat model to study the neonatal hypoxic–ischemic encephalopathy (Levine, 1960; Rice et al., 1981). It produces unilateral brain injury to structures such as hippocampus, striatum and cortex of the hemisphere ipsilateral to arterial occlusion (Vannucci and Hagberg, 2004). This model of unilateral cerebral hypoxia–ischemia causes sensorimotor deficits (Bona et al., 1997; Jansen and Low, 1996), delays in maturation of reflexes, such as righting reflex, cliff aversion and negative geotaxis (Lubics et al., 2005). Behavioral studies also revealed cognitive disabilities, as for example short and long-term spatial and aversive memory impairments (Arteni et al., 2010).

Many nutritional deficiencies can influence brain maturation during early life, but protein malnutrition during perinatal period figures among the main non-genetic factors affecting later brain development (Morgane et al., 2002). Human studies indicate that exposure to prenatal malnutrition predisposes to neurocognitive deficits (Liu et al., 2004) and increases the risk for development of psychiatric disorders, such as depression (Susser et al., 1996) and schizophrenia (St. Clair et al., 2005).

Animal studies have demonstrated that protein malnutrition in early stages of life could alter neurogenesis, cell migration, differentiation, synaptogenesis and plasticity (Bonatto et al., 2005; Gressens et al., 1997; Morgane et al., 2002; Rotta et al., 2003). Prenatal protein malnutrition leads to a decrease in neuronal and glial density in the cerebral cortex and cerebellum (Dobbing and Hopewell, 1971) and reduces dendritic spines in the cingulate cortex and hippocampus of rodents (Diaz-Cintra et al., 1991; Garcia-Ruiz et al., 1993); changes in cholinergic, noradrenergic, dopaminergic, serotoninergic, glutamatergic and GABAergic systems, in antioxidant systems as well as in drug sensitivity have also been reported (Almeida et al., 1996a, 1996b; Bonatto et al., 2005; Rotta et al., 2003, 2008; Schweigert et al., 2005). In addition, protein malnutrition impairs the performance on some behavioral tasks, i.e., nest finding (Tonkiss et al., 1996), social interaction and spatial memory (Almeida et al., 1996a, 1996b; Bedi, 1992), light–dark task (Brioni and Orsingher, 1988) and reactivity to aversive stimuli (Lynch, 1976).

Table 2 – Body and brain weight measurement of pups.

	Normal nourished		Malnourished [a]	
Body weight				
whole litter (individual mean weights)				
PND1	49.8±2.2 (6.2±0.5)		42.8±2.2 (5.8±0.67)	
PND4	76.7±5.0 (9.8±1.1)		49.3±5.0 (8.0±0.4)	
PND7	112.0±7.1 (14.1±1.7)		59.0±7.1 (8.2±0.6)	
	SH-N	HI-N	SH-M [b]	HI-M [b]
PND11	21.2±0.9	18.3±0.8	9.0±1.1	8.8±0.8
PND21	37.7±1.4	35.5±1.3	18.0±1.8	18.4±1.3
PND30	79.8±3.9	70.3±3.7	51.0±4.8	50.0±3.7
PND60	226.7±13.9	203.2±13.2	184±17.0	194.6±13.2
Brain weight (PND60)	1.6±0.0	1.4±0.0	1.23±0.06 [c]	1.2±0.0 [d]

Data are expressed as mean±SE. The first 3 measurements (PND1, PND4 and PND7), data represent weight mean of whole litters (7–8 pups per litter) and the individual mean weight. PND11, PND21, PND30 and PND60 measurements represent the individual mean weight.
[a] Significant effect of diet before HI.
[b] Significant effect of diet after HI.
[c] Interaction between HI procedure and diet ($p<0.05$).
[d] Effect of HI procedure.