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Prolonged protein deprivation, but not food restriction, affects parvalbumin-containing interneurons in the dentate gyrus of adult rats

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ABSTRACT

Several studies have demonstrated the vulnerability of the hippocampal formation to malnutrition. In this study, we compared the effects of food restriction and protein malnutrition in the total number of neurons of the dentate gyrus and in the number of parvalbumin-immunoreactive (PV-IR) interneurons, which are related to the control of calcium homeostasis and fine tuning of the hippocampal circuits. Two month-old rats were randomly assigned to control, food-restricted and low-protein diet groups. After 6 months, 10 rats from the low-protein diet group were selected at random and fed with a normal protein diet for 2 months. The total number of granule and hilar cells was reduced in protein-deprived rats and the nutritional reestablishment with a normal protein diet did not recover neuron numbers. Protein deprivation increased the number of PV-IR interneurons in the granule cell layer and hilus, but their number returned to values similar to controls after nutritional rehabilitation. Food restriction did not affect the total number of neurons or the density of PV-IR interneurons in the dentate gyrus. These results support the view that protein deprivation may disturb calcium homeostasis, leading to neuronal death. The up-regulation of PV-IR cells may reflect a protective mechanism to counteract the calcium overload and protect the remaining neurons of the dentate gyrus. This imbalance in cell-ratio favoring GABAergic interneurons may justify some learning and memory impairments described in protein-deprived animals. This contrast between the results of food restriction and protein deprivation should be further analyzed in future studies.

1. Introduction

Despite the well-known deleterious effects of starvation, increasing evidence suggests that moderate levels of food restriction have beneficial effects in many species (Fontana et al., 2010; Roth and Polotsky, 2012). Indeed, moderate food restriction increases the mean and maximum lifespan in some experimental animals and ameliorates several

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age-related diseases, including type 2 diabetes mellitus, cardiovascular diseases and cancer (Andrade et al., 2002, 2006; Colman et al., 2009; Fontana et al., 2010; Mattison et al., 2012). Furthermore, it reduces the susceptibility to the toxic effects of several chemicals (Hart et al., 1992), increases the resistance to epileptic seizures (Bough et al., 1999) and protects neurons from metabolic and ischemic insults (Andrade et al., 2002, 2006; Bruce-Keller et al., 1999; Duan and Mattson, 1999).

Contrary to food restriction, prolonged deprivation of proteins in the perinatal period or adulthood induces structural and biochemical alterations in different brain regions, particularly the hippocampal formation (HF) (Andrade et al., 1996, 2002; Andrade and Paula-Barbosa, 1996; Cintra et al., 1990, 1997; Debassio et al., 1996; Gressens et al., 1997; Lukoyanov and Andrade, 2000; Mesquita et al., 2002; Paula-Barbosa et al., 1989). It has been found that exposure of adult rats to a low-protein diet for 6 months results in neuronal loss in the main populations of the HF (Lukoyanov and Andrade, 2000) accompanied by degenerative alterations in the dendritic arborizations, loss of synaptic contacts and changes in the cholinergic and gamma-aminobutyric acid (GABA)-ergic systems, mainly in the hilar region (Andrade et al., 2002; Lukoyanov and Andrade, 2000). In agreement with this, behavioral studies indicate that cognitive functions are altered in perinatal and adult protein-malnourished animals (Alamy and Bengelloun, 2012; Duran et al., 2011; Laus et al., 2011; Lukoyanov and Andrade, 2000).

Despite the studies showing reduction in the number of inhibitory GABAergic neurons (Andrade and Paula-Barbosa, 1996), it is not know which are the specific subpopulations affected by protein deprivation (Brady and Mufson, 1997; Celio, 1990; Lister et al., 2011). One of the most numerous GABAergic subpopulations is composed by interneurons that express parvalbumin (PV) (Celio, 1990; Lister et al., 2011), a member of the calcium-binding proteins group that also includes calbindin D-28K and calretinin (Celio, 1990; de Jong et al., 1996; Lawrence et al., 2010). PV-immunoreactive (IR) neurons are predominantly basket and chandelier cells that provide inhibition to the principal neurons at the cell body and axon initial segment, respectively, regulating the precise timing of principal cells activation (Freund, 2003; Lister et al., 2011; Szilagyi et al., 2011; Vreugdenhil et al., 2003). These interneurons are thus important to regulate the output from granule cells and changes in this population are likely to affect synaptic transmission through the HF with possible implications in cognitive functions, including learning and memory (Freund, 2003; Lister et al., 2011).

Therefore, we sought to evaluate and compare the effects of food restriction and protein malnutrition in the structure of the dentate gyrus of the HF of adult rats. To this end, we estimated the total number of granule and hilar neurons and the density of PV-IR interneurons. Furthermore, and bearing in mind that some of the protein malnutrition-induced alterations described in the HF of adult rats are reversible, we also found of interest to test whether animals previously exposed to protein deprivation followed by nutritional rehabilitation would recover from the anatomical and/or neurochemical changes.

2. Results

2.1. *Animals and diets*

Daily food intake, measured at 08.00 h every day, was 31.6 ± 1.25 g in control rats, 29.4 ± 4.10 g in low-protein diet animals and 19.2 ± 0.90 g in the food-restricted group. Nutritionally rehabilitated rats consumed 32.0 ± 1.50 g per day. The mean body weights of control, food-restricted, low-protein diet, and nutritionally rehabilitated rats at the end of experiment are shown in Table 1. By the end of the experiment, the mean body weight of low-protein and nutritionally rehabilitated rats was similar to control rats. On average, the body weight of food-restricted animals was 35% lower than that of control rats ($P < 0.001$). No significant difference was detected between the mean brain weights of control (1.54 ± 0.03 g), food-restricted (1.54 ± 0.04 g), low-protein diet and nutritionally rehabilitated (1.54 ± 0.05 g) animals.

2.2. *Morphometric analyses*

2.2.1. *Total neuronal numbers*

The total number of dentate gyrus granule cells and hilar cells estimated in the HF from control, food-restricted, low-protein diet and nutritionally rehabilitated groups are shown in Table 2. Analysis of these data revealed that there was a significant effect of the feeding regimen on cell numbers in the granule cell layer ($F_{3,16} = 6.958$, $p < 0.01$) and in the hilus ($F_{3,16} = 5.784$, $p < 0.01$). Post hoc comparisons showed that in the low-protein diet group the total number of granule cells was reduced by approximately 21% compared to the control group ($p < 0.01$). In the nutritionally rehabilitated group, a reduction of 20% was observed when compared to the control group ($p < 0.01$). A similar reduction was observed in the total number of hilar neurons of nutritional rehabilitated animals compared to controls. In the low-protein diet group, a reduction of approximately 15% and 20% was detected comparatively to control and food-restricted groups, respectively, ($p < 0.05$). In the nutritionally rehabilitated rats a reduction of approximately 17% was observed comparatively to the food-restricted rats ($p < 0.05$). Nutritional rehabilitated rats presented no statistical significant differences when compared to low-protein treated rats.

2.2.2. *Areal density of PV-immunoreactive neurons*

PV-immunoreactivity is observed in the soma, dendritic trees and axons of stained interneurons (Fig. 1). PV-IR cells are heterogeneous in shape and, in the dentate gyrus, most of the cells are larger than the unstained granule cells and are

Table 1 – Mean body weights (g) of control, food-restricted, low-protein diet, and nutritionally rehabilitated rats after 6 and 8 months of treatment.

	6 months	8 months
Control	770 ± 24.10	831 ± 25.22
Food-restricted	531 ± 10.82	550 ± 17.70
Low-protein diet	741 ± 12.31	791 ± 14.12
Nutritionally rehabilitated	–	852 ± 31.14