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Neuroprotective potential of dietary restriction against kainate-induced excitotoxicity in adult male Wistar rats

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Abstract

The influence that dietary factors have on the nervous system and its susceptibility to disease, is an active area of biomedical research. Recent studies have shown that dietary restriction (DR) can have profound effect on brain function and vulnerability to injury and disease and can also enhance synaptic plasticity, which may increase the ability of brain to resist aging and restore function following injury. The dietary restriction may result in neuroprotection as suggested by marked reduction in neuronal cell death of the CA3 region of hippocampus after kainate administration in our study. We examined the effects of 3 months of DR (alternate day feeding regimen) on the antioxidants and antioxidant enzymes from different brain regions such as cerebral hemispheres, diencephalon, cerebellum and brain stem after kainate-induced excitotoxicity in adult male Wistar rats. The present study reports the beneficial effects of dietary restriction on different antioxidants and antioxidant enzymes against kainate-induced excitotoxicity in different brain regions of young adult male Wistar rats. The expression of stress response protein heat shock protein 70 (HSP 70) was also studied from discrete regions of rat brain under the same set of experimental conditions. DR significantly enhanced the expression of HSP 70 in kainic acid (KA)-treated rats, whereas KA treatment of ad libitum fed rats resulted in decreased HSP 70 expression. The DR was observed to exert neuroprotection by enhancing the expression of HSP 70 in kainic acid treated rats.

Keywords: Neuroprotection; Dietary restriction; Kainate; HSP 70; Oxidative stress; Seizures

1. Introduction

Dietary restriction (DR) a condition of reduced calorie intake with nutritional maintenance can extend life span in many organisms commonly used in biomedical research including the yeast *Saccharomyces cerevisiae* [29], the roundworm *Caenorhabditis elegans* [47], mice and rats [50] and this also appears to be the case in humans [41]. Conversely, overeating is a risk factor for cardiovascular diseases, many types of cancers, type-2 diabetes and stroke. Several such DR feeding regimens can extend lifespan, with the two most commonly used protocols being intermittent (every other day) feeding and paired feeding that employs feed pellets containing 30–40% less calories than pellets in control diet [33]. In addition to slowing the aging process, DR may increase resistance of cells to acute metabolic and oxidative insults. As evidence, rats maintained on DR showed reduced susceptibility to myocardial infarction [10], and DR in adult rats results in reduced hippocampal and striatal damage and improved behavioral outcome following excitotoxic and metabolic insults [6].

The impact of diet on brain function and susceptibility to neuropsychiatric and neurodegenerative disorders is increasingly appreciated. It has also been proposed that prolonged low calorie intake may result in neuroprotection with respect to both chronic and acute brain pathologies [10]. Recent experimental findings suggest profound neuroprotective effects of DR in animal models relevant to the pathogenesis of neurodegenerative disorders like Huntington's, Parkinson's, Stroke and Alzheimer's [12,32,54,56]. It has also been reported that rats maintained under dietary

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restriction from second to the eighth month of age are fully protected towards degeneration of GABAergic neurons in the hippocampus and olfactory-entorhinal cortex caused by the systemic administration of the convulsant toxin, kainic acid [9]. Available data suggests that much of the studies carried out involve long-term dietary restriction regimen in relation to aging. Recent studies involving short-term dietary restriction have documented the similar beneficial effects against vulnerability to excitotoxic and metabolic insults [6,26]. A genomic profiling study of short and long-term caloric restriction also showed that short-term calorie restriction (4 weeks) reproduced nearly 70% of the effects of long-term calorie restriction on genes that changed expression with age [8].

Working on similar lines, here we show that short-term DR of 3 months can protect against kainic acid induced oxidative stress in adult male Wistar rats. A period of 2–4 months of dietary restriction was required to observe neuroprotective effect of DR against excitotoxic insults, whereas shorter periods of DR were ineffective [27]. Kainic acid (KA) is an exogenous glutamate analogue frequently used as a tool to experimentally mimic human temporal lobe epilepsy. In kainic acid models, there is a great involvement of excitotoxic neuronal injury in vulnerable limbic structures, most notably the CA3 region of hippocampus. Reactive oxygen species production has been considered to be a part of mechanisms involved with the glutamatergic excitotoxicity 'in vitro' [5] and 'in vivo' [48]. Studies have also shown reduced levels of glutathione a free radical scavenger and increased levels of lipid peroxidation in rat brain after kainic acid administration [11,17]. It has also been reported that excitotoxicity may play a major role in the stimulation of the cellular stress response in the nervous system [46]. In models of excitotoxicity and seizures, KA administration resulted in widespread heat shock protein 70 (HSP 70) induction, particularly within cortical and hippocampal neurons [2,55]. HSPs and molecular chaperons have been known to protect cells against a wide variety of physiological stress conditions. HSP induction is not only a signal for detection of physiological stress, but is utilized by the cells in the repair process following a wide range of injuries to prevent damage [40]. HSP 70 is one of the important members of stress proteins induced in response to many metabolic disturbances and injuries. HSP 70 protein chaperones have been shown to prevent apoptotic death in a variety of cell types [3]. These proteins may bind to proteins either upstream (e.g. Akt kinase and Bcl-2 family members) or downstream (e.g. caspases and Apaf-1) of mitochondrial alterations involved in cell death cascades [44]. However, its neuroprotective role is highly debated [43,52].

The interactive role of dietary restriction and KA-induced exicitotoxicity in cellular defense has not been studied in details. The present investigation was aimed to evaluate the antioxidative defense response of brain of rats kept on DR regimen to KA insult. The experiments were carried out on four groups of rats such as ad libitum fed (AL) control and 3 months DR rats as well as KA-treated AL and DR rats to compare the effects of excitotoxic injury in AL and DR groups of animals. Histological analysis of hippocampal formation was done to assess the neuroprotective effect of DR against KA. Scavengers of oxidative stress such as catalase, copper zinc superoxide dismutase (Cu–Zn SOD) and glutathione peroxidase (GPx) activity, as well as glutathione (GSH) content and lipid peroxidation (LPx) were measured in these four groups. In addition, the expression of the stress protein HSP 70 was also studied in brain regions as this protein has been utilized as marker of cellular response to stress [18,31,51].

2. Materials and methods

2.1. Chemicals

The primary antibodies used for the Western blot analysis were monoclonal anti-HSP 70 (Clone BRM-22, Sigma) that recognizes both constitutive and inducible forms of HSP 70 and anti-α-tubulin (Clone Dm 1A, Sigma). The secondary antibody used was anti-mouse IgG, peroxidase linked antibody (Bangalore genei). Catalase, dithiotreitol (DTT), GPx, glutathione reductase, NADPH, thiobarbituric acid, Hoechst 33258 and kainic acid were procured from Sigma (St. Louis, MO, USA). All other chemicals and reagents were the purest, available commercially from local suppliers.

2.2. Experimental animals

Wistar strain young adult male albino rats in the age group of 3 months and weighing 130–150 gm were used for these experiments. Animal care and procedures were followed in accordance with the guidelines of Institutional Animal Ethical Committee. The paradigm of DR involved periodic fasting, in which 3 months old Wistar Strain male albino rats were deprived of food for a full day, every other day, and were fed ad libitum on the intervening day for 3 months. Food was provided or removed at 10 a.m. every day. Water was available ad libitum to all the animals. Another group of rats of similar age was fed ad libitum and used as control. The body weights were recorded every 15th day in DR and age matched AL rats.

2.3. Administration of kainic acid

After the completion of 3 months of dietary restriction, the DR and AL rats received intraperitoneal injection of KA (8 mg/kg body weight), dissolved in phosphate buffer saline (PBS). Sham-control rats of both ad libitum fed and diet restricted group received an equivalent injection of vehicle (PBS). The animals were kept for 7 days after administration of excititoxin KA and vehicle on respective paradigm of feeding. Only animals that reached status epilepticus were used in further studies. In this experiment, the dosage of the convulsant toxin injected had to be kept lower compared to the dosage used for younger animals of the same strain (10 mg/kg) [11]. We observed 8 mg/kg of KA administered for the present experiment resulted in low mortality among ad libitum-fed rats (2 out of 10 animals) and no mortality at all among dietary-restricted rats of similar age group. No relevant differences were apparent in the onset of latency (30–40 min after KA injection) and in the pattern of first epileptic signs (wet dog shakes) between rats belonging to different