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ScienceDirect
Neuroscience Letters 431 (2008) 118–122
Neuroscience Letters
www.elsevier.com/locate/neulet

Adult-onset calorie restriction attenuates kainic acid excitotoxicity in the rat hippocampal slice

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Received 3 September 2007; received in revised form 4 November 2007; accepted 11 November 2007

Abstract

Lifelong calorie restriction is the only known intervention that has been shown to consistently increase life span and reduce the effects of aging on the brain. Given the difficulties of replicating lifelong calorie restriction within human populations, we have sought to assess the effects of short-term adult-onset calorie restriction upon acute excitotoxic insults in the rat hippocampus. Adult animals (approximately 6 months of age) underwent calorie restriction (alternate day feeding) for 7–10 weeks. Utilizing both electrophysiological and immunocytochemical techniques, we report that calorie restriction had no effect upon long-term potentiation (LTP), a measure of neuronal function. In control animals, application of kainic acid (20 μM) resulted in only 35% recovery of CA1 population spikes post-insult. However calorie-restricted animals showed significantly improved recovery after kainic acid treatment (64%). This data was supported by immunocytochemical studies which noted widespread loss of microtubule-associated protein (MAP 2) immunoreactivity in control slices following treatment with kainic acid; however MAP 2 staining was preserved in the CA1 and CA3 regions of calorie-restricted animals. Interestingly there was no significant difference in the recovery of population spikes between groups when slices were treated with *N*-methyl-D-aspartate (15 μM). We conclude that short-term adult-onset calorie restriction does not alter normal neuronal function and serves to protect the hippocampus from acute kainic acid excitotoxicity.

Keywords: Long-term potentiation; Kainic acid receptors; NMDA receptors; Microtubule-associated protein

In most of the developed world and many developing nations the past 50 years has seen an increase in life expectancy that has resulted in a shift in population demographics. This is now manifesting in increasingly aged populations with concomitant increases in diseases that affect the elderly. Given this shift, much research is now geared towards understanding the physiological changes that take place during aging and how these may be modulated. One possibility that has offered much hope is dietary or calorie restriction (CR). CR has been shown to extend life span, both in rodents and higher animals [9,14].

Some epidemiological data suggests that CR can reduce the risk for Alzheimer's dementia (AD) [18,20] and Parkinson's disease [16]. In rat and mouse models, CR has been shown to reduce the cognitive decline associated with AD [12], slow the cognitive decline associated with aging [17] and reverse the age-related deficits in long-term potentiation (LTP) [8]. Not all studies have observed such beneficial effects of CR and as such there is still considerable debate regarding these findings [1,33]. CR has also been shown to attenuate the predisposition to epilepsy [3] and the excitotoxic cell damage associated with epilepsy [4] and other lesions [5,7].

While animals that have been placed upon CR shortly after weaning demonstrated increased life expectancy, it is also reported that the effects of CR are reduced (i) with delayed onset of CR and (ii) if CR is stopped [28]. This has particular relevance as we seek to extrapolate these results and apply them to human populations. Humans are unlikely to be maintained on prolonged CR without compliance becoming a significant issue. Given these limitations there is now much interest in initiating CR at later stages of life (adult-onset calorie restriction) and for shorter durations of time.

We therefore sought to investigate the effects of CR in young adult animals (adult-onset CR) and for a relatively short duration (7–10 weeks). We demonstrate that short-term adult-onset CR appeared to have no effect upon normal neuronal function, but it did attenuate the effects of acute kainic acid (KA) excitotoxicity.

Experiments were carried out in accordance with procedures established by the local Ethics Committee. The 16 animals

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doi:10.1016/j.neulet.2007.11.064

(male Sprague–Dawley rats) used for the study were approximately 5–6 months of age at inception. Prior to the beginning of these experiments, all had received food ad libitum and had been housed under standard conditions in the Faculty Animal Care Facility. Eight animals each were randomly assigned to the control group and experimental group (underwent calorie restriction). The mean starting weight of the control group was 452 ± 17 g and that of the experimental group was 448 ± 16 g; there was no significant difference between the starting weights ($p > 0.05$, Student's *t*-test).

CR was achieved by using alternate day feeding for a period of 7–10 weeks depending upon when the animal was sacrificed. Animals underwent periodic fasting in which they were deprived of food for 24 h starting at 4 p.m. every other day; the control group was fed ad libitum. Water was available ad libitum to all animals. Previous studies have shown that rodents maintained on such a diet will consume 30–40% less calories over time compared to controls and have a reduced body weight of approximately 20% compared to animals fed ad libitum [10,29]. This protocol has been successfully used to induce changes in several biochemical and physiological parameters [7,25].

Animals were randomly selected on the day of experimentation. After completion of the study it was noted that of the eight CR animals utilized, six had been fed immediately prior to sacrifice. Animals were anaesthetized with urethane (1.5–1.7 g/kg i.p.) and allowed to breathe 100% O_2. The rats were then killed by cervical dislocation and decapitated. The brain was rapidly removed into ice-cold artificial cerebrospinal fluid (aCSF) pregassed with 95% O_2/ 5% CO_2. The aCSF was made of (in mM): NaCl 115; KCl 2.0; KH_2PO_4 2.2; $NaHCO_3$ 25; D-glucose 10; $MgSO_4$ 1.2 and $CaCl_2$ 2.5. Slices of hippocampus were produced 400-μm thick using a McIlwain Tissue Chopper as described previously [34] and placed in an incubation chamber for at least 1 h at 23–24 °C before being transferred to the recording chamber.

A single slice was transferred to a recording chamber, fully submerged in pre-gassed (95% O_2/5% CO_2) aCSF and perfused at approximately 4 ml/min at 30–31 °C, using a temperature regulator (Warner Instruments). Orthodromic evoked population spike (PS) potentials and field excitatory post-synaptic potentials (fEPSPs) were recorded via a glass micro-electrode, tip diameter approximately 5 μm, containing 3 M sodium chloride placed in the stratum pyramidale and stratum radiatum, respectively. Stimulation of the Schaffer collateral pathway in the stratum radiatum was carried out every 15 s using a bipolar electrode (Clark Electromedical, Reading, UK), delivering a constant current stimulus (100–600 μA), duration 100 μs. Potentials were amplified and stored digitally on a microcomputer via a CED micro-1401 interface and CED Signal 2 software. The slope of the fEPSPs and the peak to peak amplitude of the PS in millivolts (mV) were used as a marker of synaptic transmission and cellular function within the CA1 region.

For each animal, an assessment was made of LTP induction, response to kainic acid excitotoxicity and NMDA excitotoxicity using a different slice. Prior to the induction of population spike LTP (PS-LTP), PS were reduced to 50–60% of the maximum size by reducing the stimulus strength. PS-LTP was induced by a train of stimuli (same stimulus strength as utilized to achieve baseline) at 100 Hz in 1 s, repeated three times at 60-s intervals. This protocol has been shown to successfully induce PS-LTP in our laboratory [35]. In assessing the effects of NMDA and KA upon neuronal transmission and recovery, a supra-maximal current was used in order to activate all axons. In all experiments, recordings were allowed to stabilize for 15–30 min before a baseline was recorded for 15 min.

Following each electrophysiological study, hippocampal slices were briefly fixed in 4% paraformaldehyde and cryoprotected in 20% sucrose and phosphate buffered saline (PBS) for 24 h before being frozen using compressed CO_2. The hippocampus was subsequently sectioned using a Bright cryostat. The 10-μm thick sections were placed on gelatin-coated slides and incubated overnight with antibodies directed against microtubule-associated protein (MAP 2) (Clone HM-2; Sigma–Aldrich, USA; 1:1000 dilution). The immunocytochemical procedure was completed the following day using appropriate biotinylated secondary antibodies (at a dilution of 1:200) and the ABC stain (Vector Burlingame, CA, USA). The reaction was visualized using a previously described glucose oxidase method [27]. Sections from a control-treated (KA or NMDA) and a CR (KA or NMDA)-treated animal were placed on the same slide to ensure that similar immunocytochemical observations were derived. Immunocytochemical staining was evaluated using a Nikon E 600 microscope and photographed with a Digital Cool Pix 950 Nikon Camera. All the chemicals used in the study were purchased from Sigma–Aldrich, USA unless otherwise stated in the text.

Values from several slices from different rats were pooled into groups and compared using Student's *t*-test or ANOVA. Significance was noted at the level of $p < 0.05$. Data are presented as mean ± standard error of mean (S.E.M.) and the '*n*' values reflect the number of slices used.

After 5 weeks of alternate day feeding the average weight of the experimental group was 17% less than the control group and this was significantly different ($p < 0.01$, Student's *t*-test). A similar pattern was seen with the average weights when the animals were sacrificed prior to harvesting the brains. The average weight of control animals was 540 ± 47 g and the average weight for CR animals was 472 ± 38 g; this was significantly different ($p < 0.05$, Student's *t*-test).

In control slices tetanic stimulation resulted in robust PS-LTP such that 30 min later PS were $167.7 \pm 10.3\%$ ($n = 6$) and 45 min later the PS were $165.6 \pm 10.5\%$ ($n = 6$) of the baseline (Fig. 1). This was significantly increased as compared to the baseline ($p < 0.01$, Student's *t*-test). In slices from CR animals tetanic stimulation resulted in PS-LTP at 30 min, $146.7 \pm 4.8\%$ ($n = 6$) and at 45 min $146.4 \pm 5.7\%$ ($n = 5$) (Fig. 1). There was no statistical significant difference between the induction of PS-LTP in control slices and CR slices (ANOVA, $p = 0.093$).

To assess the effects of excitotoxicity, KA (20 μM) was allowed to superfuse the slices for 20 min. This produced a rapid decrease in PS size and within 10 min the PS had totally disappeared. Recovery was monitored for 45 min after the removal of the insult, at which time the PS had plateaued. The mean recovery was $35.2 \pm 3.7\%$ of the baseline ($n = 6$) in control