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Caloric restriction eliminates the aging-related decline in NMDA and AMPA receptor subunits in the rat hippocampus and induces homeostasis

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Abstract

Caloric restriction (CR) extends life span and ameliorates the aging-related decline in hippocampal-dependent cognitive function. In the present study, we compared subunit levels of NMDA and AMPA types of the glutamate receptor and quantified total synapses and multiple spine bouton (MSB) synapses in hippocampal CA1 from young (10 months), middle-aged (18 months), and old (29 months) Fischer 344×Brown Norway rats that were ad libitum (AL) fed or caloric restricted (CR) from 4 months of age. Each of these parameters has been reported to be a potential contributor to hippocampal function. Western blot analysis revealed that NMDA and AMPA receptor subunits in AL animals decrease between young and middle age to levels that are present at old age. Interestingly, young CR animals have significantly lower levels of glutamate receptor subunits than young AL animals and those lower levels are maintained across life span. In contrast, stereological quantification indicated that total synapses and MSB synapses are stable across life span in both AL and CR rats. These results indicate significant aging-related losses of hippocampal glutamate receptor subunits in AL rats that are consistent with altered synaptic function. CR eliminates that aging-related decline by inducing stable NMDA and AMPA receptor subunit levels.

Keywords: Dietary restriction; Fischer 344×Brown Norway rats; Glutamate receptor; Stratum radiatum; Electron microscopy; Multiple spine bouton (MSB) synapses

Introduction

Aging in rodents is associated with cognitive impairment as well as physiological, anatomical, and biochemical changes in the brain (Geinisman et al., 1995; Rosenzweig and Barnes, 2003). Aging-related cognitive and neural changes can be ameliorated by a variety of manipulations, including supplementation with trophic factors such as nerve growth factor and insulin-like growth factor 1 as well as the initiation of caloric restriction (CR) early in life (Ingram et al., 1987; Fischer et al., 1991; Tacconi et al., 1991; Sohal and Weindruch, 1996; Markowska et al., 1998; Sonntag et al., 1999). CR refers to the proportionate daily reduction of all dietary components and it can extend life expectancy by 20% to 40% in a broad range of species including rodents (Yu et al., 1985; Roth et al., 1995). CR also has been shown to maintain physiological parameters in different body systems at youthful levels and delays the onset of aging-associated diseases (Sohal and Weindruch, 1996; Major et al., 1997; Kalani et al., 2006).

Several studies have indicated that metabolic stability is a better predictor of longevity than metabolic rate. For example, it has been hypothesized that senescence-related loss of function is related to an impairment of homeostatic state and the capacity of an organism to maintain a steady metabolic state is a prime determinant of longevity (McCarter and McGee, 1989; Kirkwood and Shanley, 2005). As CR may enhance longevity by inducing metabolic stability (Demetrius, 2004), it may be that the ability of CR to prevent aging-related cognitive and neural changes is related to a CR-induced stability in the synaptic determinants of hippocampal function.

Earlier studies have revealed that principal neurons in the hippocampus are not lost in aged animals (Rapp and Gallagher, 1996; Morrison and Hof, 1997), suggesting that more subtle

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changes such as synaptic modifications may be occurring in the aging brain. Synapses are plastic structures and changes in the number of synapses or the ultrastructural composition of existing synapses can occur following microenvironmental alterations in the brain. Moreover, synaptic changes result in a continual refinement of neuronal circuitry (Adams et al., 2001a,b; Nicholson et al., 2004; Tata et al., 2006). Synaptic plasticity is essential for information storage, experience-dependent learning and memory as well as other phenomena associated with cognition (Martin et al., 2000; Burke and Barnes, 2006).

Previous reports have demonstrated aging-related changes in the structure, composition and functional capacity of synapses in CA1, CA3 and DG even when total synapse number is maintained. Specifically, both synaptic transmission (Barnes, 1994; Foster and Norris, 1997) and long-term potentiation (LTP), the synaptic phenomenon associated with spatial learning and memory, are compromised in aged rodents with cognitive impairments (Rosenzweig et al., 1997; Bach et al., 1999; Tombaugh et al., 2002). Interestingly, CR, which has been shown to protect neurons against excitotoxic, oxidative and metabolic insults (Ingram et al., 1987; Sohal and Weindruch, 1996; Guo and Mattson, 2000), also ameliorates aging-related changes in synaptic plasticity and neurotransmitter systems. Particularly, it prevents the aging-related deficits in LTP induction in hippocampal CA1, and thus may lessen the impairment of synaptic function in old animals (Eckles-Smith et al., 2000; Okada et al., 2003). CR might exert such beneficial effects by inducing stability of synaptic function across life span similar to the systemic benefits of eliminating the metabolic changes (Yu and Chung, 2001; Koubova and Guarente, 2003). In the presence of lifelong CR, a stable state in those parameters may be maintained across life span (Yu and Chung, 2001; Demetrius, 2004).

Glutamate is the major excitatory neurotransmitter in the brain and NMDA and AMPA subtypes of glutamate receptors are the primary mediators of excitatory synaptic transmission in the hippocampus (Hollmann and Heinemann, 1994). Both NMDA and AMPA receptor subunits are essential for LTP induction and maintenance (Hayashi et al., 2000; Clayton et al., 2002; Malinow and Malenka, 2002) and are required for hippocampal synaptic plasticity as well as spatial learning and memory (McHugh et al., 1996; Tsien et al., 1996; Adams et al., 2001b; Nakazawa et al., 2002; Riedel et al., 2003). Moreover, NMDA and AMPA receptors have been implicated in the structural changes associated with synaptic plasticity, including synapse formation, maintenance and remodeling (Fischer et al., 2000; Luscher et al., 2000; Hering and Sheng, 2001). Studies in aging rodents have shown that functional impairments of these receptors are associated with spatial learning and memory deficits (Newcomer and Krystal, 2001; Clayton et al., 2002). CR can affect the aging-related loss and/or functional impairment of NMDA and AMPA receptors. Specifically, the CR-associated amelioration of hippocampal LTP deficits in old F344 rats has been suggested to result from enhanced NMDA-mediated transmission (Eckles-Smith et al., 2000; Okada et al., 2003). Moreover, the aging-related decrease in the NMDA receptor subunit NR1 was shown to be ameliorated by lifelong CR (Eckles-Smith et al., 2000; Magnusson, 2001).

Aging- and CR-induced changes in synaptic efficacy as well as in levels of NMDA and AMPA receptors may be reflected in modifications of specific features of synaptic morphology (Ziff, 1997; Luscher et al., 2000; Hering and Sheng, 2001). Particularly, multiple spine bouton (MSB) complexes, i.e., single presynaptic terminals contacting two or more postsynaptic targets, have been correlated with synaptic efficacy (Buchs and Muller, 1996; Toni et al., 2001) and the incidence of synapses in MSB complexes is an indicator of synaptic efficacy and thus plasticity (Sorra et al., 1998; Toni et al., 1999, 2001; Jones, 1999; Geinisman et al., 2001; Nikonenko et al., 2002). Moreover, MSBs increase after exposure to enriched environments (Jones et al., 1997) and LTP induction (Toni et al., 1999). Not only is the incidence of MSB synapses regulated by neuronal activity in a highly dynamic manner, this parameter also has been associated with more efficient synaptic transmission as well as enhanced learning and memory in rats (Sorra et al., 1998; Toni et al., 1999; Jones, 1999; Geinisman et al., 2001; Nikonenko et al., 2002).

The present study investigated the effect of lifelong CR in young, middle-aged and old Fischer 344×Brown Norway rats on NMDA (NR1, NR2A and NR2B) and AMPA (GluR1 and GluR2) subunits of glutamate receptors in hippocampal CA1, the output region of the hippocampal trisynaptic pathway. In addition, the effects of aging and CR on total synapses and MSB synapses in stratum radiatum of hippocampal CA1 were investigated. This is the first study to investigate the potential effect of CR on ultrastructurally identified synapses in hippocampal CA1 across life span.

Materials and methods

Animals and caloric restriction

A total of 84 ad libitum fed (AL) and CR Fischer 344×Brown Norway (F344×BN) F1 hybrid male rats were acquired from the National Institute on Aging (NIA) Caloric Restriction Colony (Harlan Industries, Indianapolis, IN). Six groups of rats were included in the present study: AL and CR rats at 10 (young), 18 (middle-aged), and 29 months (old) (n=14/group). Cohorts of animals containing each of the 6 groups were used for either Western blot or electron microscopic analyses. Forty-eight (n=8/group) rats were used for Western blot analyses of different NMDA and AMPA receptor subtypes and 36 (n=6/group) rats were used for quantitative electron microscopic analyses of synapses. All rats were housed individually on a 12-hour light–dark cycle in the animal facility of Wake Forest University School of Medicine and housed for 2 months prior to sacrifice.

At the NIA facility, all rats were AL fed (NIH-31 diet) from weaning until 14 weeks of age, at which time the CR regime was initiated by incremental reduction of 10% food intake per week over a 4-week period until CR rats received 60% of caloric intake compared to AL animals. The vitamin-fortified NIH-31 diet that CR rats received provided 60% of the calories