Neurobiology of Aging 40 (2016) 78–85

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Beta-hydroxy-beta-methylbutyrate ameliorates aging effects in the dendritic tree of pyramidal neurons in the medial prefrontal cortex of both male and female rats

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ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form 8 January 2016
Accepted 9 January 2016
Available online 18 January 2016

Keywords:
Dendritic spine
Dendrite
Sex difference
Ovariectomy
mTOR
Golgi

ABSTRACT

Beta-hydroxy-beta-methylbutyrate (HMB), a supplement commonly used to maintain muscle in elderly and clinical populations, has been unexplored in the aging brain. In both healthy aging humans and rat models, there are cognitive deficits associated with age-related dendritic shrinkage within the prefrontal cortex. The present study explores the effects of relatively short- and long-term (7 and 31 weeks) oral HMB supplementation starting at 12 months of age in male and female rats on the dendritic tree of layer 5 pyramidal neurons in the medial prefrontal cortex. Since female rats continue to secrete ovarian hormones after reaching reproductive senescence, middle-aged female rats were ovariectomized to model humans. As expected, there were fewer spines and a retraction of dendritic material in the apical and basilar trees in old age controls of both sexes compared with their middle-aged counterparts. However, these losses did not occur in the HMB-treated rats in either dendrites or the total number of dendritic spines. Thus, HMB forestalled the effects of aging on the dendritic tree of this population of neurons.

1. Introduction

With medical advances and increases in life expectancy, there is a large, rapidly growing population of people 65 years and older. As a result, there is a need to explore preventative measures directed at the cognitive and neural declines that accompany normal aging. Normal aging humans show deficits in several modalities of executive functioning such as memory, attention, decision making, and visuospatial abilities (reviewed in Erickson and Barnes, 2003; Gallagher and Rapp, 1997). This cognitive decline in normal healthy aging is unsurprisingly accompanied by age-related changes in neurons within the prefrontal cortex (PFC) and medial temporal lobe, which are brain areas associated with the aforementioned functions (Burke and Barnes, 2006). Notably, in the PFC there is a significant decrease in neuropil, specifically in dendritic arborization, spine density, and spine number (de Brabander et al., 1998; Jacobs et al., 1997), which contributes to the age-related decreases in brain and gray matter volume (Coffey et al., 1992; Tisserand et al., 2002).

Similar to humans, aging animals exhibit a decline in performance on tasks involving working memory, reversals, and spatial learning (reviewed in Erickson and Barnes, 2003; Gallagher and Rapp, 1997). In addition, this decline in performance is associated with age-related neural losses in the PFC. In particular, a loss of both dendrites and spines has been well-characterized in aging rhesus monkeys (Cupp and Uemura, 1980; Duan et al., 2003; Kabaso et al., 2009; Uemura, 1980). Likewise, there is a reduction in dendritic arborization with fewer dendritic spines in the medial PFC (mPFC) of aged rats (Allard et al., 2012; Markham and Juraska, 2002).

In an effort to combat age-related cognitive decline and the associated neuroanatomic changes, dietary nutrients are being investigated, and some appear to correlate with significant effects in both cognitive function and brain volume in normal aging humans (Bowman et al., 2012). One such nutrient, beta-hydroxy-beta-methylbutyrate (HMB), known to be beneficial for aging muscles, may have potential for ameliorating cognitive decline in the normal healthy aging population. Although HMB has been extensively studied in peripheral tissue (i.e., muscle), considerably less is known about its effects in the brain. This is a gap in knowledge that needs to be addressed as many peripheral mechanisms by which HMB exerts its positive effects parallel mechanisms that may

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http://dx.doi.org/10.1016/j.neurobiolaging.2016.01.004

be beneficial for the aging brain. These peripheral effects of HMB include saturating the rate-limiting enzyme in cholesterol synthesis (reviewed in Nissen and Abumrad, 1997), increasing protein synthesis via the mechanistic target of rapamycin (mTOR) pathway (Eley et al., 2007; Hoeffer and Klann, 2010), and upregulating the growth hormone and/or insulin-like growth factor-1 (GH/IGF-1) axis (Gerlinger-Romero et al., 2011). These are mechanisms that may be vulnerable to aging and contribute to deficits in the aging brain (Thornton et al., 2000; Yang et al., 2014).

Although most literature on the aging rat model focuses solely on males, it is pertinent to use both male and female rats to reflect the aging human population, which has proportionately more females. As human females age, they experience reproductive senescence (menopause), which is the cessation of gonadal hormone cyclicity accompanied by a dramatic loss of ovarian hormone production (Perheentupa and Huhtaniemi, 2009). In contrast, this cessation in aging female rats does not include such a dramatic decline of ovarian hormones (Huang et al., 1978; Wise and Ratner, 1980). Rather, female rats' ovaries continue to secrete low to moderate levels of estrogen and progesterone after their cycles have stopped, and the relative amounts depend on their estropausal status. Since there is evidence that circulating estrogens are neuroprotective in aged female rats (Chisholm and Juraska, 2012; Chisholm et al., 2012; Garcia-Segura et al., 2001), female rats in the present study were ovariectomized (OVX) in middle age to model normal human female aging following menopause, whereas males underwent sham surgery.

In this study, middle-aged (12-month old) rats were supplemented with an HMB solution or vehicle for either a relatively short (7 weeks) or long (31 weeks) period of time. We use a middle-age time point to assess whether HMB has any direct effects on neuronal morphology and an aged time point to determine whether HMB supplementation mitigates the normal neuroanatomic changes that are associated with aging. We focused our investigation within the mPFC on neuronal morphology to dendritic arborization, spine density, and spine number measurements of layer 5 pyramidal neurons as this layer appears to be vulnerable to age-related changes in both humans and rats (de Brabander et al., 1998; Markham and Juraska, 2002).

2. Materials and methods

2.1. Subjects

Subjects were male (n = 37) and female (n = 32) Long Evans hooded rats obtained from Harlan Laboratories (Indianapolis, IN, USA) at approximately 10 months of age. The subjects were housed individually in standard clear Plexiglas laboratory cages, fed and hydrated ad libitum, and weighed weekly. The colony was maintained on a 12-hour light/dark cycle, with lights on at 0800 hours. At 11 months, all subjects were anesthetized with isoflurane vapors and underwent surgery. In accordance with animal care policy, rats were administered the analgesic carprofen (5 mg/kg delivered subcutaneously) immediately after anesthetization and again 6- to 12-hour later. Female rats were OVX via bilateral dorsal incisions, whereas male rats underwent a sham surgery and retained their gonads.

2.2. Dosing regimen

HMB levels peak in the circulation by 1–2 hours after ingestion and reach baseline levels by 9 hours (Vukovich et al., 2001). Thus, HMB was administered twice daily with a target dose of 450 mg calcium-HMB (Ca-HMB)/kg body weight (BW), a dose shown to aid aging muscle (Wilson et al., 2012). Sipper tubes containing the vehicle (32 mg/mL calcium lactate + 20% sucrose) or HMB solution (50 mg/mL Ca-HMB + 20% sucrose) were placed into rats' home cages twice daily (~727 mg HMB/kg BW/day) Monday–Saturday (at approximately 0800 and 1800 hours) and once (~364 mg HMB/kg BW/Sunday) on Sunday (between 1800 and 2000 hours). Most rats began consuming their solution immediately and finished within 60 minutes of it being placed in their cage. However, tubes remained in cages until the next dose to maximize consumption. For each rat, the amount consumed was recorded, and the actual dose ingested was estimated.

Dosing with either vehicle or HMB solution began at 12 months and continued until day of sacrifice. The short-term treatment (middle-age) group was dosed for a month before the start of behavioral testing in the water maze (not reported here), whereas the long-term treatment (aged) group was dosed for 7 months before behavioral testing. Dosing continued through the week of behavioral testing in a water maze (a day of pretraining, a day of rest, and then 4 consecutive days of testing) and for the 2 weeks after training until sacrifice. Rats were injected with sodium pentobarbital (100 mg/kg; Sigma, St. Louis, MO, USA), and their brains were harvested for Golgi Cox staining and processing. Within the short-term treatment (middle-age) group, 7 males and 9 females were administered vehicle, and 10 males and 9 females were administered HMB. Within the long-term treatment (aged) group, 11 males and 8 females were administered vehicle, and 9 males and 6 females were administered HMB. Animal care and experimental procedures were in accordance and approved by the Institutional Animal Care and Use Committee at the University of Illinois Urbana-Champaign.

2.3. Neuroanatomy

2.3.1. Histology

All brains were Golgi impregnated by methods similar to those described previously (Glaser and Van der Loos, 1981) and in work from our laboratory (Koss et al., 2014; Markham and Juraska, 2002). Golgi-Cox solution was prepared from 5% solutions of potassium dichromate, mercuric chloride, and potassium chromate in a volume ratio of 5:5:4. At sacrifice, the brain was placed into Golgi-Cox solution for approximately 20 days and coded to keep the experimenter blind to the animal's identity. Coronal test-slices were cut periodically near the region of interest to ensure adequate staining. Once neurons were entirely filled, the brains were dehydrated, embedded in 12% celloidin solution, hardened in a desiccator, and stored in butanol until slicing. Brains were coronally sliced on a microtome at 150 μm to capture the entire extent of the dendritic tree of neurons. Tissue slices were developed in a series of solutions, mounted on slides with permount, and coverslipped.

2.3.2. Identification of layer 5 pyramidal neurons in the mPFC

Neurons were sampled in the mPFC from the initial appearance of cortical white matter (i.e., the forceps minor) to the first appearance of the genu of the corpus callosum. The ventral and dorsal boundaries of the prelimbic and infralimbic areas of the mPFC were conservatively identified based on previous work from our laboratory (Koss et al., 2014; Markham and Juraska, 2002), while referencing Nissl-stained brain tissue. Neurons from layer 5 were selected based on a pyramidal morphology within approximately 500–750 μm from the top of layer 1 that had a thick apical dendrite extending at least 250 μm toward the pial surface (Fig. 1).

2.3.3. Quantification of dendritic arbor

Three dimensional tracing of neurons was carried out using a 63X objective on a Zeiss Axioimager A1 light microscope with Neurolucida 9.0 software, whereas subsequent analyses of