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Neurobiology of Aging 31 (2010) 605–613

NEUROBIOLOGY OF AGING

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Experience-dependent regulation of vesicular zinc in male and female 3xTg-AD mice

Amy S. Nakashima [a], Salvatore Oddo [c], Frank M. LaFerla [c], Richard H. Dyck [a,b,*]

[a] *Department of Psychology, University of Calgary, 2500 University Drive, NW, Calgary, Alberta, Canada T2N 1N4*

[b] *Department of Cell Biology and Anatomy, Hotchkiss Brain Institute, University of Calgary, 3300 Hospital Drive, NW, Calgary, Alberta, Canada T2N 4N1*

[c] *Department of Neurobiology and Behavior, University of California, Irvine, CA 92697-4545, USA*

Received 29 March 2008; received in revised form 8 May 2008; accepted 18 May 2008
Available online 7 July 2008

Abstract

In the mammalian cerebral cortex, zinc is an important modulator of synaptic transmission and conversely, plasticity. Zinc is also involved, in a sex-dependent manner, in the pathogenesis of Alzheimer's disease (AD), where substantial declines in plasticity may occur. To examine this relationship further, the regulation of vesicular zinc was examined after the induction of cortical plasticity through vibrissae plucking in male and female C57Bl/6 and 3xTg-AD mice at various age points. Female C57Bl/6 mice were found to have an elevated response compared to male C57Bl/6 mice through mid-adult ages, a sex-difference likely mediated by the differential regulation of vesicular zinc by the sex hormones. Male 3xTg-AD mice had a significantly greater zincergic response compared to C57Bl/6 mice, which is likely indicative of a compensatory mechanism utilized by the male 3xTg-AD mice to combat the decline in plasticity associated with the AD state. These results exemplify how the regulation of vesicular zinc may be a significant component in the progression of AD, especially regarding the sex-dependent element.

Keywords: Zinc; Aging; Alzheimer's disease; Sex-differences; Cortical plasticity; Barrel cortex; Vibrissae

1. Introduction

The functional malleability of the cerebral cortex is a property of the brain that persists throughout life. However, the degree to which these changes occur is not consistent—with aging, significant declines in plasticity occur, which may manifest with changes in behaviour. In neurodegenerative diseases such as Alzheimer's disease (AD), the decline in plasticity is accelerated and widespread, leading to dramatic alterations in cognitive functioning. Although much remains to be elucidated concerning the mechanisms of plasticity, as well as its deterioration with aging and AD, increasing evidence suggests a role for zinc in the manifestation and decline of plasticity in both normal aging and in AD.

Cortical zinc is found within two populations. Metabolic zinc consists of zinc tightly bound to proteins, performing regulatory roles (Frederickson, 1989). The second population, vesicular zinc, is contained in synaptic vesicles within a subset of glutamatergic neurons (Beaulieu et al., 1992). This zinc is released in a voltage- and activity-dependent manner and has been shown to have potent modulatory effects on postsynaptic receptors, most notably *N*-methyl-D-aspartate (NMDA) receptors (Assaf and Chung, 1984; Howell et al., 1984; Izumi et al., 2006). Given the importance of glutamate and NMDA receptors in the expression of plasticity, vesicular zinc is well positioned to modulate plasticity. Sev-

* Corresponding author at: Department of Psychology, University of Calgary, 2500 University Drive, NW, Calgary, Alberta, Canada T2N 1N4. Tel.: +1 403 220 4206; fax: +1 403 282 8249.
E-mail address: rdyck@ucalgary.ca (R.H. Dyck).

0197-4580/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2008.05.028

eral studies have demonstrated the importance of zinc for the induction of long-term potentiation (LTP) and depression (LTD) *in vivo*, giving further support for the role of zinc in plasticity (Izumi et al., 2006; Kodirov et al., 2006; Li et al., 2001). In addition, the induction of endogenous cortical plasticity through the trimming or plucking of vibrissae results in an increase in vesicular zinc within the corresponding regions of the somatosensory cortex (Brown and Dyck, 2002; Czupryn and Skangiel-Kramska, 2001). This response is bidirectional such that the stimulation of the vibrissae leads to a corresponding decrease in vesicular zinc concentration (Brown and Dyck, 2005). Interestingly, this change in vesicular zinc concentration may mediate the decline in plasticity that occurs with aging as this response is severely attenuated in aged mice (Brown and Dyck, 2003).

Accumulating evidence also suggests that zinc is involved in the pathogenesis of AD. Total zinc levels are found to be elevated in the brains of AD patients and zinc homeostasis can be severely altered by the disease (Religa et al., 2006; Sensi et al., 2007). In addition, senile plaques are histochemically reactive to zinc; that is, some staining methods for vesicular zinc will also result in the staining of senile plaques in AD brains (Lee et al., 1999; Suh et al., 2000). The zinc observed within senile plaques likely facilitates the formation of these entities as it has been shown that zinc can precipitate soluble beta-amyloid (Aβ; Bush et al., 1994; Esler et al., 1996).

Conversely, the removal of vesicular zinc through genetic manipulations in a murine model of AD leads to a drastic reduction in the number of Aβ accumulations (Lee et al., 2002). Similarly, administrating a chelator for zinc has been shown to be effective in reducing the pathology and symptomology in a murine model of AD (Cherny et al., 2001). The removal of zinc is promising as a treatment for AD; in clinical trials, the administration of a zinc chelator to AD patients minimized the magnitude of cognitive decline (Ritchie et al., 2003). Intriguingly, there is also evidence to suggest that vesicular zinc is involved in the processes that may give rise to a sex-difference in the incidence and severity of AD. The genetic removal of vesicular zinc in a murine model of AD has been shown to eliminate pre-existing sex-differences in the degree of histopathology observed (Lee et al., 2002).

Substantial declines in cortical plasticity define AD. Animal models of AD show deficits in LTP/LTD processes and the behavioural manifestations of the disease, namely alterations in learning and memory, exemplify how plasticity becomes abnormal in AD (Klyubin et al., 2005; Rowan et al., 2004, 2005). Given the strong support for a role of zinc in AD, especially the sex-mediated differences, as well as the involvement of zinc in cortical plasticity, we examined how these factors might intersect. The vibrissae plucking paradigm was utilized in order to examine how the dynamics of vesicular zinc homeostasis in response to the induction of plasticity could become altered, in a sex-dependent manner, in a murine model of AD.

2. Methods

2.1. Animals and treatment groups

The 3xTg-AD mouse was utilized as a model of AD. Mice were generated as previously described (Oddo et al., 2003b). Unlike most other animal models of AD that only develop neurofibrillary tangles or senile plaques; this model utilizes several transgenes (APP, PS1 and tau) to generate a phenotype with both pathologies. As with human AD, the progression of symptomology is age-dependent, with Aβ deposits being observed initially at 6 months whereas tau aggregates do not become apparent until 12 months (Oddo et al., 2003a,b).

To investigate the effect of sensory deprivation on cortical plasticity of zincergic circuits in a model of AD, 96 mice were utilized. Male and female 3xTg-AD mice as well as age-matched male and female control mice were examined at 1, 3, 6, 9, 12, or 18 months of age n = 4 per strain/sex/age. Time points were chosen to reflect the majority of the lifespan of the mice. All mice were provided food and water *ad libitum* and were housed in standard laboratory housing under a 12:12 light/dark cycle for the duration of the experiment. All procedures were authorized by the Animal Care Committee of the University of Calgary and mice were handled in accordance to the guidelines dictated by the Canadian Council for Animal Care.

To induce plasticity within the barrel cortex, vibrissae were bilaterally removed. Previous study has demonstrated that the unilateral removal of vibrissae does not affect the level of vesicular zinc in the barrel cortex ipsilateral to the vibrissae plucking (Brown and Dyck, 2002). Thus, hemispheres were considered independent of each other. Under light isoflurane anesthesia, the 'c-row' of vibrissae was removed, using tweezers to pluck them individually in a manner as to minimize bleeding and prevent damage to the follicle. Mice were then returned to their home cage and 48 h later, were prepared for vesicular zinc histochemistry.

2.2. Tissue preparation and histochemistry

Mice were weighed, then injected with sodium selenite intraperitoneally (i.p.; 15 mg/kg, Sigma, St. Louis, MO) and after 1.5 h, were killed using an overdose of sodium pentobarbital. In order to visualize the barrel cortex in a single plane, tissue was prepared for tangential sectioning. After extraction, the brains were bisected and the cortices gently removed. Cortices were then flattened between glass slides and rapidly frozen using dry ice and stored at −80 °C. The tissue was then sectioned at 20 μm using a Cryostar HM560 cryostat.

Sections were stained for vesicular zinc via autometallography using the Timm–Danscher method (Danscher, 1982). Briefly, after thawing the sections at room temperature, sections were fixed in 95% ethanol for 15 min. Sections were hydrated in a descending series of ethanol and water (75%, 2 min; 50%, 2 min; distilled water 3× 2 min), then dipped