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Neurobiology of Aging 32 (2011) 811–820

NEUROBIOLOGY OF AGING

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Age-related changes in glutamate release in the CA3 and dentate gyrus of the rat hippocampus

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Received 13 February 2009; received in revised form 1 May 2009; accepted 8 May 2009
Available online 17 June 2009

Abstract

The present studies employed a novel microelectrode array recording technology to study glutamate release and uptake in the dentate gyrus, CA3 and CA1 hippocampal subregions in anesthetized young, late-middle aged and aged male Fischer 344 rats. The mossy fiber terminals in CA3 showed a significantly decreased amount of KCl-evoked glutamate release in aged rats compared to both young and late-middle-aged rats. Significantly more KCl-evoked glutamate release was seen from perforant path terminals in the DG of late-middle-aged rats compared young and aged rats. The DG of aged rats developed an increased glutamate uptake rate compared to the DG of young animals, indicating a possible age-related change in glutamate regulation to deal with increased glutamate release that occurred in late-middle age. No age-related changes in resting levels of glutamate were observed in the DG, CA3 and CA1. Taken together, these data support dynamic changes to glutamate regulation during aging in subregions of the mammalian hippocampus that are critical for learning and memory.

Keywords: L-Glutamate; Hippocampus; Voltammetry

1. Introduction

An understanding about how morphological and electrophysiological changes in brain regions critical for learning and memory translate into deficits in the cognitive functions of the hippocampus in aging is needed (for reviews see Rosenzweig and Barnes, 2003; Chawla and Barnes, 2007). In addition to preserved cell counts (West, 1993; West et al., 1993; Peters et al., 1996; Rapp and Gallagher, 1996; Rasmussen et al., 1996; Merrill et al., 2000; Merrill et al., 2001), most of the basic cellular characteristics of hippocampal principal cells do not change with advanced age including but not limited to resting membrane potential and amplitude and duration of Na^+-mediated action potentials (for review see Rosenzweig and Barnes, 2003). Therefore, changes in hippocampal cognitive functions during aging do not appear to be attributable to changes in neuron number or basic cellular physiology. It is believed that structural changes in synaptic connections during aging affect functional connectivity in the hippocampus (reviewed by Rosenzweig and Barnes, 2003; Chawla and Barnes, 2007); however, age-associated alterations in neurotransmission and subsequent effects on function of the hippocampal circuitry are far less defined.

In the hippocampus, the chief excitatory neurotransmitter is L-glutamate. The NMDA and AMPA ionotropic glutamate receptor subtypes are critical for long-term potentiation and hippocampal-dependent learning and memory (Riedel et al., 2003). A reduction in ionotropic receptors and their constituent subunits with age has been correlated with a decline in memory function (Magnusson, 1998a,b; Adams et al., 2001; Tang et al., 2001; Clayton et al., 2002), which can be modulated with pharmacological agents that facilitate activation of the glutamate receptors (Müller et al., 1994; Wu et al., 2002; Rosenzweig and Barnes, 2003). Thus, age-related changes in presynaptic and glial regulation of glutamate release may be needed to help explain age-related changes in hippocampal neuron function during aging.

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0197-4580/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2009.05.009

Glutamate regulation in the hippocampus has been extensively investigated using *ex vivo* methods like brain slices, tissue homogenates, and synaptosomes (for review see Segovia et al., 2001). Due to the limitations of these methods the literature has been filled with contradicting reports about increases, decreases or no changes in glutamate regulation with aging. The dentate gyrus (DG), cornu ammonis 3 (CA3) and cornu ammonis 1 (CA1) hippocampal subregions are distinct on a cellular, molecular, and functional basis, and are intricately connected via the trisynaptic circuit (for review Miller and O'Callaghan, 2005; Greene et al., 2008). Isolating the hippocampus from its extrinsic connections and disrupting the intrinsic connections could account for the inconsistency in previous aging studies. Only two prior microdialysis studies have reported on hippocampal glutamate regulation during aging using intact animals and the results are contradicting. Zhang et al. (1991) showed a decrease and Massieu and Tapia (1997) showed an increase in tonic glutamate levels in aged as compared to young rats. Though microdialysis does allow for *in vivo* measurements of tonic glutamate, controversy exists regarding the neuronal origin of the signals and its slow temporal resolution limits the ability to investigate the rapid release and clearance dynamics associated with glutamate neurotransmission (Timmerman and Westerink, 1997; Segovia et al., 2001). This is important because alterations in release and/or clearance may not manifest with changes in tonic resting glutamate levels due to biological compensation, and therefore must be studied directly. Furthermore, due to the size of microdialysis probes (mm), the previous studies are limited because they do not address discrete subregional variation within the hippocampus.

We have recently demonstrated altered regulation of glutamate neurotransmission during aging in the striatum of rodents (Nickell et al., 2005, 2007) and the cortex of non-human primates (Quintero et al., 2007) using enzyme-based microelectrode arrays coupled to amperometry for *in vivo* glutamate recordings. In addition to a subsecond (2 Hz) temporal resolution that allows for rapid measurement of glutamate release and clearance in the extracellular space, our microelectrode technology measures from brain parenchyma with a spatial-resolution (μm) superior to that of microdialysis (Stephens et al., 2008) allowing accurate targeting of the DG, CA3 and CA1 subregions. These experiments investigated the capacity of glutamate release and uptake in subregions of the hippocampus (DG, CA1 and CA3) of young, late-middle aged and aged Fischer 344 (F344) rats. First, we studied the effects of aging on tonic (resting) levels of glutamate using a self-referencing enzyme-based microelectrode array recording technology (Day et al., 2006). Second, we used local application of a high potassium solution to evoke reproducible synaptic overflow of glutamate with a micropipette attached to the microelectrode arrays. This was carried out to simulate phasic bursts of glutamate release and determine the effects of aging on depolarization-induced release of glutamate. Finally, using the attached micropipette for evoked release we locally applied finite amounts of glutamate to study the effects of aging on glutamate clearance. The present studies are the first to provide insight into age-related changes in functional glutamatergic neurotransmission in subregions of the rat hippocampus.

2. Methods

2.1. Animals

Young adult (3–6 months, $n = 18$, mean weight = 337 ± 50 g), late-middle aged (18 months, $n = 14$, 452 ± 36 g) and aged (24 months, $n = 19$, 424 ± 32 g) male F344 rats were obtained from the National Institute on Aging colony (Harlan Sprague Dawley Inc., Indianapolis, IN) and used for all experiments. Protocols for animal use were approved by the Institutional Animal Care and Use Committee. In accordance with approved guidelines, animals were housed in a 12-h alternating light/dark cycle, with food and water available *ad libitum*.

2.2. Amperometric recordings for rapid measurements of L-glutamate

Ceramic microelectrode arrays consisting of four platinum recording surfaces (15 μm × 333 μm each) arranged geometrically in two pairs (30 μm spacing between recording surfaces) stacked in a dorsal–ventral orientation on the microelectrode array tip (100 μm spacing between the pairs), were used *in vivo* for discrete targeting of the DG, CA3 and CA1 subregions of the rat hippocampus. Microelectrode arrays were manufactured and configured for selective glutamate detection as per our previously published methods yielding one pair of 'glutamate-sensitive' recording surfaces and one pair of background 'sentinel' recordings surfaces (Burmeister et al., 2002; Pomerleau et al., 2003; Day et al., 2006; Hascup et al., 2007; Stephens et al., 2008). All platinum recording surfaces were electroplated with *meta*-phenylenediamine (5 mM, Acros Organics, New Jersey, USA), which creates a molecular exclusion layer blocking interferents such as dopamine (DA), 3-4-dihydroxyphenylacetic acid (DOPAC), ascorbic acid and other neurochemicals based upon their size. Constant voltage amperometry (+0.7 V vs. Ag/AgCl reference electrode) was performed using the Fast Analytical Sampling Technology high-speed electrochemistry instrument (FAST16, Quanteon, L.L.C., Nicholasville KY), and current from the four platinum recording sites was simultaneously recorded by the FAST software.

2.3. Microelectrode calibration

Individual recording specifications of each microelectrode, including the sensitivity to glutamate (current/concentration (pA/μM)) and the selectivity ratio of glutamate over an ascorbic acid interferent (glutamate:AA),