ELSEVIER

Neurobiology of Aging 31 (2010) 328–338

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Hippocampal 'zipper' slice studies reveal a necessary role for calcineurin in the increased activity of L-type Ca^{2+} channels with aging

Christopher M. Norris [a,b,*], Eric M. Blalock [b], Kuey-Chu Chen [b], Nada M. Porter [b], Olivier Thibault [b], Susan D. Kraner [b], Philip W. Landfield [b]

[a] *Sanders-Brown Center on Aging, University of Kentucky College of Medicine, Lexington, KY 40536, USA*
[b] *Department of Molecular and Biomedical Pharmacology, University of Kentucky College of Medicine, Lexington, KY 40536, USA*

Received 28 September 2007; received in revised form 18 February 2008; accepted 26 March 2008
Available online 8 May 2008

Abstract

Previous studies have shown that inhibition of the Ca^{2+}-/calmodulin-dependent protein phosphatase calcineurin (CN) blocks L-type voltage sensitive Ca^{2+} channel (L-VSCC) activity in cultured hippocampal neurons. However, it is not known whether CN contributes to the increase in hippocampal L-VSCC activity that occurs with aging in at least some mammalian species. It is also unclear whether CN's necessary role in VSCC activity is simply permissive or is directly enhancing. To resolve these questions, we used partially dissociated hippocampal "zipper" slices to conduct cell-attached patch recording and RT-PCR on largely intact single neurons from young-adult, mid-aged, and aged rats. Further, we tested for direct CN enhancement of L-VSCCs using virally mediated infection of cultured neurons with an activated form of CN. Similar to previous work, L-VSCC activity was elevated in CA1 neurons of mid-aged and aged rats relative to young adults. The CN inhibitor, FK-506 (5 μM) completely blocked the aging-related increase in VSCC activity, reducing the activity level in aged rat neurons to that in younger rat neurons. However, aging was not associated with an increase in neuronal CN mRNA expression, nor was CN expression correlated with VSCC activity. Delivery of activated CN to primary hippocampal cultures induced an increase in neuronal L-VSCC activity but did not elevate L-VSCC protein levels. Together, the results provide the first evidence that CN activity, but not increased expression, plays a selective and necessary role in the aging-related increase in available L-VSCCs, possibly by direct activation. Thus, these studies point to altered CN function as a novel and potentially key factor in aging-dependent neuronal Ca^{2+} dysregulation.

Keywords: L-type; Phosphatase; Hippocampus; Aging

1. Introduction

Neuronal L-type voltage sensitive Ca^{2+} channels (L-VSCCs) are potent and tightly regulated signaling molecules involved in the control of membrane excitability, Ca^{2+}-induced Ca^{2+} release (CICR), synaptic plasticity, and gene expression, among other processes (Gallin and Greenberg, 1995; Lipscombe et al., 2002; Surmeier, 2007; Thibault et al., 2007; Tsien et al., 1986). Activity of L-VSCCs is increased in hippocampal CA1 neurons during aging (Thibault and Landfield, 1996) and may underlie, in part, numerous age-related alterations in hippocampal Ca^{2+} signaling and regulation (Disterhoft and Oh, 2007; Murphy et al., 2006; Thibault et al., 2007; Toescu and Verkhratsky, 2007). Blockade of L-VSCCs reverses or ameliorates many of the most consistent bio-behavioral markers of brain aging including the enhanced Ca^{2+}-dependent slow afterhyperpolarization (AHP) and reduced neuronal excitability (Moyer et al., 1992), impaired frequency facilitation (Thibault et al., 2001), increased susceptibility to long-term depression (Norris et al., 1998b), and diminished spatial cognition (Batuecas et al., 1998; Deyo et al., 1989; Disterhoft and Oh, 2006; Veng et al., 2003). Moreover, increased L-VSCC activity may result in or interact with increased CICR with aging (Gant et al., 2006; Kumar and Foster, 2004; Thibault et al., 2007).

* Corresponding author at: 800 South Limestone Street, 131 Sanders-Brown Building, University of Kentucky College of Medicine, Lexington, KY 40536, USA. Tel.: +1 859 257 1412x249; fax: +1 859 323 2866.
E-mail address: cnorr2@email.uky.edu (C.M. Norris).

0197-4580/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2008.03.026

Despite the accumulating evidence of a role for increased L-VSCC activity in hippocampal aging, little is yet known about the mechanisms that mediate this increase. Evidence has been found for modestly elevated expression of the less abundant isoform ($Ca_V1.3$) of the pore-forming L-VSCC subunit in hippocampus of aged rats (Herman et al., 1998; Veng et al., 2003). However, these changes in expression do not appear sufficient to account fully for age-differences in L-VSCC activity (Hell et al., 1993; Davare and Hell, 2003), especially in light of recent evidence suggesting that expression of the predominant $Ca_V1.2$ isoform may be reduced with aging (Rowe et al., 2007). Moreover, an age-related increase in $Ca_V1.2$ phosphorylation by the cAMP-dependent protein kinase (PKA), which appeared initially to account for increased L-VSCC activity (Davare and Hell, 2003), was not observed in a subsequent study (Murphy et al., 2006).

The Ca^{2+}-/calmodulin-dependent protein phosphatase calcineurin (CN) is another potential candidate mechanism for mediating the aging-related increase in L-VSCC activity. The relationship between CN and VSCC activity varies considerably, depending on cell type. Laboratories employing different neuronal and non-neuronal cultures have found evidence either for CN-mediated inhibition of L-VSCCs (Armstrong, 1989; Day et al., 2002; Hernandez-Lopez et al., 2000; Lukyanetz et al., 1998; Oliveria et al., 2007; Schuhmann et al., 1997), or that CN has no effect, or increases L-VSCC activity, particularly in cardiomyocytes (Branchaw et al., 1997; Frace and Hartzell, 1993; Victor et al., 1997; Wang et al., 2001; Yatani et al., 2001; Zeilhofer et al., 2000). A possible role for CN in modulating hippocampal L-VSCCs emerged from our prior studies showing that inhibition of CN function blocked L-VSCC activity in cultured hippocampal neurons (Norris et al., 2002). However, it is not clear from previous work whether CN also modulates L-VSCC activity in aged animal brain cells.

With regard to aging, several biochemical studies indicate that CN activity and CN-dependent processes are enhanced in the hippocampus of aged rats (Foster et al., 2001; Jouvenceau and Dutar, 2006; Norris et al., 1998a; but see Agbas et al., 2005). In addition, overexpression of activated CN in forebrain neurons of adult mice precipitates aging-like alterations in hippocampal synaptic plasticity and cognition (Mansuy et al., 1998; Winder et al., 1998), whereas blockade of CN or CN-dependent signaling cascades in aged rodents restores synaptic and cognitive function to young-adult levels (Genoux et al., 2002; Norris et al., 1998a). However, CN gene expression in brain neurons may be reduced with aging (Rowe et al., 2007). Conversely, CN expression in reactive astrocytes is apparently elevated with aging and neuropathology (Norris et al., 2005).

Nevertheless, as noted, it remains unclear whether CN modulates L-VSCC activity in adult hippocampal neurons, as it does in embryonic cultures, or whether CN contributes to the aging-related increase in L-VSCC activity. Moreover, the nature of CN modulation of VSCC function in hippocampal neurons is poorly understood.

To address these questions here, we employed the partially dissociated hippocampal "zipper" slice preparation (so named for its tendency to dissociate gradually along the main cell body layers), which was developed by Gray et al. (1990) to enhance single-channel recording in adult animal brain. We have found that the preparation is highly advantageous for aging studies (Thibault and Landfield, 1996), and also allows the extraction of largely intact, physiologically characterized neurons for subsequent molecular analysis (Chen et al., 2000; Blalock et al., 2001). Blockade of CN function with FK-506 was used to determine whether CN activity contributes to increased L-VSCC activity during brain aging. Further, an activated form of CN was delivered in a viral vector (Norris et al., 2005) to cultured hippocampal neurons to test whether CN activity enhances as well as is necessary for L-VSCC activity. The results directly implicate CN activity as a candidate mechanism in the aging-related increase in L-VSCC function (and, consequently, in multiple Ca^{2+}-related biomarkers, e.g., Disterhoft and Oh, 2007; Thibault et al., 2007). Moreover, they show that activation of CN is capable of amplifying as well as permitting L-VSCC activity.

2. Materials and methods

2.1. Preparation of hippocampal "zipper" slices

Adult (6–9-month-old, mean = 8.34 months), mid-aged (15–19-month-old, mean = 17.63 months), and aged (20–29-month-old, mean = 23.44 months) male Fischer 344 rats were obtained from the National Institute on Aging's colony at Harlan. Rats were treated in accordance with the guidelines established by the Institutional Animal Care and Use Committee.

Procedures for obtaining partially dissociated hippocampal "zipper" slices from guinea pigs was developed by Gray et al., 1990 and modified by our research group for studies on aged rats and mRNA analysis of individual, largely intact neurons (Chen et al., 2000; Thibault and Landfield, 1996). Fig. 1 outlines the zipper slice protocol used in this study to obtain VSCC current activity and CN mRNA levels from CA1 neurons. Briefly, rats were euthanized with CO_2 gas and rapidly decapitated. Brains were extracted and stored for 1–2 min in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/5% CO_2 gas. Composition of the ACSF was (in mM): 114 NaCl, 2.5 KCl, 2 $MgCl_2$, 30 $NaHCO_3$, 10 glucose, and 0.1 $CaCl_2$. Hippocampi were dissected free and 350 μm transverse slices were prepared using a McIlwain Tissue Chopper. Slices were then transferred to prewarmed (32 °C), oxygenated ACSF containing 2 mM $CaCl_2$ and 0.7 mg/ml pronase to begin the partial dissociation process. After a 30-min incubation, pronase-containing ACSF was removed and slices were incubated for an additional 15 min in prewarmed ACSF containing 0.5 mg/ml thermolysine. Slices were washed twice in ACSF and were then bathed for 1–4 h in ACSF containing either 5 μM FK-506 (Cal-