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Neurobiology of Aging 32 (2011) 956–965

NEUROBIOLOGY OF AGING

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Effect of ageing on CA3 interneuron sAHP and gamma oscillations is activity-dependent

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Received 27 February 2009; received in revised form 5 May 2009; accepted 7 May 2009
Available online 11 June 2009

Abstract

Normal ageing-associated spatial memory impairment has been linked to subtle changes in the hippocampal network. Here we test whether the age-dependent reduction in gamma oscillations can be explained by the changes in intrinsic properties of hippocampal interneurons.

Kainate-induced gamma oscillations, but not spontaneous gamma oscillations, were reduced in slices from aged mice. CA3 interneurons were recorded in slices from young and aged mice using Fura-2-filled pipettes. Passive membrane properties, firing properties, medium- and slow-afterhyperpolarisation amplitudes, basal $[Ca^{2+}]_i$ and firing-induced $[Ca^{2+}]_i$ transients were not different with ageing. Kainate caused a larger depolarisation and increase in $[Ca^{2+}]_i$ signal in aged interneurons than in young ones. In contrast to young interneurons, kainate increased the medium- and slow-afterhyperpolarisation and underlying $[Ca^{2+}]_i$ transient in aged interneurons.

Modulating the slow-afterhyperpolarisation by modulating L-type calcium channels with BAY K 8644 and nimodipine suppressed and potentiated, respectively, kainate-induced gamma oscillations in young slices.

The age-dependent and stimulation-dependent increase in basal $[Ca^{2+}]_i$, firing-induced $[Ca^{2+}]_i$ transient and associated afterhyperpolarisation may reduce interneuron excitability and contribute to an age-dependent impairment of hippocampal gamma oscillations.

Keywords: Kainate; GluR5; Ageing; Hippocampus; Interneuron; CA3; Gamma oscillation; BAY K 8644; Nimodipine; Fura-2; Slow-afterhyperpolarisation; Intracellular calcium concentration

1. Introduction

Normal brain ageing is associated with mild cognitive impairments, especially of memory that relies on spatial and/or contextual information (Moffat et al., 2001; Rosenzweig and Barnes, 2003), suggesting functional impairment of the hippocampus. In the absence of significant neurodegeneration (Rasmussen et al., 1996), age-dependent hippocampal dysfunction has been ascribed to more subtle changes in network and cellular functions (Toescu and Verkhratsky, 2000). One potential change is the increased slow-afterhyperpolarisation (sAHP) observed in aged pyramidal neurons from hippocampal area CA1 (Thibault et al., 2001), which correlated with the variability of spatial memory performance in the Morris water maze (Tombaugh et al., 2005). The increased sAHP results from enhanced firing-induced $[Ca^{2+}]_i$ transients due to the calcium influx through L-type voltage-gated calcium channels (Thibault et al., 2001). Reducing $[Ca^{2+}]_i$ transients and consequently sAHPs by increasing intracellular calcium buffering power ameliorates age-dependent impaired learning (Tonkikh et al., 2006). The enhanced sAHP in CA1 neurons reduces excitability and may increase the threshold for inducing synaptic plasticity (Disterhoft and Oh, 2006; Gant and Thibault, 2008; Tonkikh et al., 2006). Alternatively, an increased sAHP in CA3 neurons impairs cognition by suppressing the neuronal oscillatory activity at gamma band (30–100 Hz) frequencies (Driver et al., 2007). Synchronisation of neuronal activity at gamma frequencies provides a framework for a temporal coding scheme (Fries et al., 2007) and the natural millisecond time-scale synchrony required for effective integration and Hebbian plasticity in the absence of synchronising electrical stimulation. Gamma oscillation strength is reduced

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0197-4580/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2009.05.006

with normal ageing (Driver et al., 2007; Vreugdenhil and Toescu, 2005) and experimentally increasing the sAHP in young neurons mimics the aged condition (Driver et al., 2007). However, CA3 neurons fire sparsely during gamma oscillations (Fries et al., 2007; Traub et al., 2000) and single action potentials trigger only very small sAHPs that are unlikely to affect gamma oscillations. In contrast, many interneurons involved in the generation of gamma oscillations fire on most cycles (Mann et al., 2005). Although the sAHP- and calcium-dependent accommodation is relatively small in CA3 interneurons (Chitwood and Jaffe, 1998; Tombaugh, 1998), an age-dependent increase in sAHP could reduce interneuron excitability and result in impaired gamma oscillations (Joho et al., 1999).

Here we tested the age-dependent changes in sAHP in interneurons patched with the calcium indicator Fura-2 and report an age-dependent increase in the sAHP of interneurons in the presence of kainate, which may contribute to the reduced gamma oscillations in the presence of kainate and learning impairments associated with ageing.

2. Materials and methods

2.1. Tissue preparation

Young (3–5 months) and aged (22–28 months) C57bl/j6 mice (ageing colony of the University of Newcastle, UK) were anesthetized by medetomidine (1 mg/kg) and ketamine (76 mg/kg) IP and killed by cervical dislocation. All procedures conformed to the UK Animals (Scientific Procedures) Act 1986 and were approved by institutional ethics review board. The brain was chilled in oxygenated sucrose-based cutting solution composed of (in mM) 189 sucrose, 26 $NaHCO_3$, 1.2 NaH_2PO_4, 2.5 KCl, 0.1 $CaCl_2$, 5 $MgCl_2$, and 10 glucose. Horizontal hippocampal slices were cut using an Integraslice (Campden Instruments, Loughborough, UK) and maintained at room temperature in an interface storage chamber containing 95%O_2–5%CO_2 oxygenated artificial CSF (aCSF) composed of (in mM) 135 NaCl, 16 $NaHCO_3$, 1.25 NaH_2PO_4, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 glucose, pH 7.4. 400 μm slices were transferred to an interface type recording chamber and 300 μm slices to a submerged recording chamber. The interface type recording chamber was perfused (7 ml/min) with warm (32 °C) aCSF, saturated with carbogen (95%O_2–5%CO_2) and covered with moist carbogen (0.3 l/min). The submerged recording chamber was perfused (7 ml/min) with warm (29 °C) carbogen-saturated aCSF.

Drugs were added to the aCSF from frozen stock solutions: R-(+)-1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid methyl ester (BAY K 8644); 1 mM in dimethyl sulfoxide (DMSO, final concentration 0.1%, with DMSO present in control solutions), isopropyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylate, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl 1-methylethyl ester (nimodipine); 100 mM in DMSO, kainate; 250 μM in H_2O, to protect the very light sensitive BAY K 8644 and nimodipine from light, the set-up and all tubing was blacked out. All chemicals were purchased from Sigma (Poole, UK).

2.2. Extracellular recordings

Extracellular recordings were made from stratum radiatum of area CA3c (just outside the dentate gyrus) in 400 μm thick slices, using borosilicate glass electrodes (4–7 MΩ) filled with aCSF. The signal was amplified with a Neurolog NL-104 unit (Digitimer, Welwyn Garden City, UK), bandpass (2–500 Hz) Bessel filtered with a Neurolog NL-125 unit, digitized at 2 kHz using a 1401 plus A-D converter (Cambridge Electronic Design, Cambridge, UK) controlled by Spike-2 software (Cambridge Electronic Design), which was also used for the analysis.

2.3. Patch-clamp recordings

Whole-cell patch-clamp current recordings were made from neurons at the stratum pyramidale/stratum oriens border of CA3c, using a Zeiss Axioskop (Carl Zeiss Ltd., Welwyn Garden City, UK) microscope with infrared differential interference contrast at 40× magnification. To improve visibility the slice thickness was reduced to 300 μm. Borosilicate glass patch electrodes (4–7 MΩ) were filled with intracellular solution composed of (in mM) 135 KCH_3SO_4, 8 NaCl, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP and 0.05 Fura-2 pentapotassium salt (Invitrogen Ltd., Paisley, UK) adjusted to pH 7.3 with KOH. The liquid junction potential between the bath and the electrode was −8 mV, as determined experimentally and was not corrected for.

Membrane potentials were recorded using an Axopatch 2B amplifier (Molecular Devices, Sunnyvale, USA), low-pass Bessel filtered at 2 kHz by a Neurolog NL-125 filter unit and digitized at 10 kHz using a 1401 plus A-D converter controlled by Signal software (Cambridge Electronic Design), which was also used for analysis. Taking into account a series resistance of 8–12 MΩ, after the standard 80% compensation, there remained a 2 mV error for 1 nA of current.

2.4. Intracellular calcium concentration recording

Measurements of free intracellular calcium concentration ($[Ca^{2+}]_i$) were performed using the Ca^{2+} indicator Fura-2; the excitation light (340 nm and 380 nm) was supplied by an Optoscan monochromator (Cairn Research, Faversham, Kent, UK) controlled by the software (MetaFluor, Molecular Devices, Berkshire, UK). Emitted light was selected using a 510 ± 20 nm filter, captured using an intensified GenIV camera (Photometrics UK, Maidenhead, UK) and analysed using MetaFluor/MetaMorph software (Molecular Devices, Berkshire, UK). Fluorescence images corresponding to the 340 nm and 380 nm excitation light were acquired