Neuroscience Letters 580 (2014) 62–67

Contents lists available at ScienceDirect

Neuroscience Letters

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

Persistent sodium currents contribute to Aβ$_{1-42}$-induced hyperexcitation of hippocampal CA1 pyramidal neurons

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HIGHLIGHTS

- We investigated the effects of soluble Aβ$_{1-42}$ on neuronal excitability.
- Soluble Aβ$_{1-42}$ increased the mean frequency of hippocampal spontaneous discharges.
- Soluble Aβ$_{1-42}$ also increased the amplitude of persistent sodium current.
- Riluzole inhibited the Aβ$_{1-42}$-induced neuronal hyperexcitation.

ARTICLE INFO

Article history:
Received 5 March 2014
Received in revised form 3 July 2014
Accepted 25 July 2014
Available online 4 August 2014

Keywords:
Alzheimer's disease
Amyloid β
Persistent sodium current
Hyperexcitation
Riluzole

ABSTRACT

Patients with Alzheimer's disease (AD) have elevated incidence of epilepsy. Moreover, neuronal hyperexcitation occurs in transgenic mouse models overexpressing amyloid precursor protein and its pathogenic product, amyloid β protein (Aβ). However, the cellular mechanisms of how Aβ causes neuronal hyperexcitation are largely unknown. We hypothesize that the persistent sodium current (I_{NaP}), a subthreshold sodium current that can increase neuronal excitability, may in part account for the Aβ-induced neuronal hyperexcitation. The present study was designed to evaluate the involvement of I_{NaP} in Aβ-induced hyperexcitation of hippocampal CA1 pyramidal neurons using a whole-cell patch-clamp recording technique. Our results showed that bath application of soluble Aβ$_{1-42}$ increased neuronal excitability in a concentration-dependent manner. Soluble Aβ$_{1-42}$ also increased the amplitude of I_{NaP} without significantly affecting its activation properties. In the presence of riluzole (RLZ), an antagonist of I_{NaP}, the Aβ$_{1-42}$-induced neuronal hyperexcitation and I_{NaP} augmentation were significantly inhibited. These findings suggest that soluble Aβ$_{1-42}$ may induce neuronal hyperexcitation by increasing the amplitude of I_{NaP} and that RLZ can inhibit the Aβ$_{1-42}$-induced abnormal neuronal activity.

1. Introduction

Alzheimer's disease (AD), the most common type of dementia, is characterized by progressive memory impairment and cognitive decline. Excessive accumulation of amyloid β (Aβ) is thought to be a causal factor in producing the cognitive deficits [7]. It is still unclear how Aβ accumulation leads to impaired memory and cognitive function. Recent studies indicated that Aβ-related neuronal hyperexcitation and aberrant network activity may contribute to the cognitive deficits in AD [16]. Mouse models of AD that have elevated levels of Aβ exhibit altered neuronal activity, spontaneous seizures and epileptiform discharges [14], which further contribute to memory impairments and cognitive deficits [23]. In addition, blocking Aβ-induced epileptiform discharges can ameliorate cognitive decline and behavior dysfunction in transgenic AD mouse models [18]. Accumulating evidence further suggests that soluble Aβ, rather than amyloid plaques, correlates well with the severity of cognitive decline and crucially leads to malfunction of neurons [19]. The hippocampal region of the AD transgenic mouse has an increased proportion of hyperactive neurons prior to the formation of Aβ plaques. Moreover, extracellular application of soluble

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http://dx.doi.org/10.1016/j.neulet.2014.07.050
0304-3940/

$A\beta_{1\text{-}42}$ induces hyperactivity of hippocampal CA1 neurons in wild-type mice [2]. These findings link Aβ to neuronal hyperexcitation, aberrant network activity and cognitive impairment. However, the underlying cellular mechanisms and pathways that mediate Aβ-induced neuronal hyperexcitation are poorly understood.

Voltage-gated sodium channels control neuronal excitability by initiating and propagating action potentials. Persistent sodium current (I_{NaP}), a slow inactivating component of TTX-sensitive sodium current, is important for regulating neuronal excitability [4,6]. As a low-voltage-activated current, I_{NaP} depolarizes membrane potential toward the threshold for action potential initiation. Despite its small magnitude compared with the peak of transient sodium current (I_{NaT}), I_{NaP} has important effects on neuronal functions, including generating subthreshold oscillatory activity, amplifying synaptic potentials, and facilitating repetitive firing patterns [26]. I_{NaP} keeps the membrane depolarized longer to facilitate epileptic firings; thus, it is involved in both acquired and genetically determined epilepsy [20]. In animal models of temporal lobe epilepsy, a particular type of epilepsy that exhibits both the memory impairment and the hippocampal neuropathology that occur in AD, increased I_{NaP} has been found in neurons with elevated excitability [8]. Furthermore, an antiepileptic drug that blocks I_{NaP} has been demonstrated to be effective in reducing epileptiform discharges in a mouse model of AD [28]. Thus, it is tempting to speculate that I_{NaP} may participate in Aβ-induced neuronal hyperexcitation and epileptiform neuronal activity in AD.

In the present study, we first investigated the effects of soluble $A\beta_{1\text{-}42}$ on the excitability of hippocampal CA1 pyramidal neurons. We then examined the alteration of I_{NaP} in the presence of soluble $A\beta_{1\text{-}42}$. Lastly, we tested the effect of riluzole (RLZ), an inhibitor of I_{NaP} [22], on $A\beta_{1\text{-}42}$-induced abnormal neuronal activity in hippocampal neurons.

2. Materials and methods

2.1. Soluble $A\beta_{1\text{-}42}$ preparations

All reagents were obtained from Sigma-Aldrich, USA. Soluble $A\beta_{1\text{-}42}$ was prepared as described previously [10]. In brief, $A\beta_{1\text{-}42}$ was first dissolved in hexafluoro-2-propanol (HFIP) and aliquoted. HFIP was then removed by evaporation under vacuum, and the resulting clear peptide films were stored at −20 °C. Prior to use, an aliquot of $A\beta_{1\text{-}42}$ peptide film was dissolved in anhydrous dimethyl sulfoxide (DMSO) and then added to ice-cold artificial cerebral spinal fluid (ACSF) to obtain a working concentration of 100 μM. This solution was then incubated at 4 °C for 24 h without any disturbance and then centrifuged to obtain the supernatant. The supernatant included the soluble $A\beta_{1\text{-}42}$ preparation. Previous studies by western blotting and atomic force microscopy have demonstrated that the major species was $A\beta_{1\text{-}42}$ monomer and also included the trimer, tetramer, and, to a lesser extent, the dimer [10,11]. These $A\beta_{1\text{-}42}$ preparations were diluted in ACSF for immediate use, and the final concentration of DMSO was always ≤0.1%.

2.2. Slice preparation

Sprague-Dawley rats (P12-14) were used in this study. Animals were obtained from the Laboratory Animal Center at the Third Military Medical University in China, and all protocols and procedures were approved by the University Animal Care and Use Committee. Rats at this age range stably shows datable levels of I_{NaP}, but without significant fluctuation of I_{NaP} conductance [13]. After halothane anesthesia, animals were decapitated, and the brain was removed quickly. The brain was subsequently submerged in cold ACSF containing (in mM) 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 KH_2PO_4, 1.2 $MgSO_4$, 2 $CaCl_2$ and 10 dextrose, bubbled with 95% O_2–5% CO_2, pH 7.4. The brain was blocked, and an oscillating tissue slicer (Leica, VT1000, Wetzlar, Germany) was used to cut 400 μm-thick horizontal sections. Slices were initially incubated for a minimum of 90 min at room temperature (22–24 °C) in ACSF and were then transferred to and submerged in a recording chamber, where they were perfused continuously with carbogen buffered ACSF.

2.3. Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings were obtained from cell bodies of CA1 pyramidal neurons in the rat hippocampus. The cell bodies of these neurons were found using an upright microscope equipped with Leica differential interference contrast optics, a 40× water immersion objective, and an infrared video imaging camera. Data acquisition was conducted with EPC10 amplifiers (HEKA Elektronik, Lambrecht/Pfalz, Germany). The signal was stored for off-line analysis with Pulse/Pulse fit v.8.74 (HEKA Elektronik) and Igor Pro v.4.03 (WaveMatrics). Pipettes (4–8 MΩ) for whole-cell recordings were pulled on a horizontal micropipette puller (P-97, Sutter Instrument) from filamented capillary glass and were filled with a pipette solution containing (in mM) 145 K-gluconate, 0.5 EGTA, 2 $MgCl_2$, 5 HEPES, 5 K-ATP, and 0.4 Na-GTP, pH 7.4, 290–295 mOsm. Liquid junction potential was calculated to be −10 mV for our intrapipette solution and membrane voltages were corrected off-line. To record I_{NaP}, recording electrodes were filled with an internal solution containing (in mM) 110 CsCl, 5 NaCl, 3 $MgCl_2$, 1 $CaCl_2$, 3 EGTA and 40 HEPES, pH 7.4. The bath solution consisted of (in mM) 100 NaCl, 40 TEA-Cl, 3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, 10 HEPES, 1 $BaCl_2$, 1 CsCl, 2 4-AP and 0.1 $CdCl_2$, bubbled with 95% O_2-5% CO_2, pH 7.4. After seal formation and cell membrane rupturing, capacitance currents were minimized using the amplifier circuitry. Series resistance was compensated by 80% and was continually monitored throughout the experiment. Neurons were discarded if the series resistance changed by more than 15%. Under a holding potential of −60 mV, I_{NaP} was recorded in CA1 pyramidal neurons by applying a 3 s depolarization ramp current from −80 to 0 mV. To isolate I_{NaP}, current responses to depolarizing voltage ramps were recorded in the absence and presence of TTX (Tetrodotoxin, 1 μM) and then subtracted. To account for any differences in cell size, all current recordings were normalized to whole cell capacitance to get current density. Conductance–voltage (G–V) relationships of I_{Nap} were calculated from $G = I/(V - V_{rev})$, where I is the recorded I_{NaP} measured at potential V and V_{rev} is the Na reversal potential calculated from the Nernst equation. Normalized activation curves were fitted to Boltzmann relationships in the form $G/G_{max} = 1/\{1 + \exp[(V_{1/2} + V)/\kappa]\}$, where G_{max} is the maximal peak conductance, G is the peak conductance at each test voltage, $V_{1/2}$ is the voltage at which half-maximal activation is reached, and κ is the slope factor.

2.4. Data analysis

Statistical analysis was made using statistical analysis software Origin 8.0 (Microcal, Inc, Northampton, MA, USA) and SPSS 13.0 (IBM, New York, NY). The values were presented as the mean ± S.E.M. Differences in the mean values among groups were analyzed using Student's t-test and one-way ANOVA. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Soluble $A\beta_{1\text{-}42}$ induced hyperexcitation of hippocampal CA1 pyramidal neurons

Previous studies have demonstrated that soluble Aβ at nanomolar concentrations induced abnormal synaptic