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Research paper

Alterations of the electrophysiological properties from cortical layer 5 pyramidal neurons in temporary rapamycin-treated rodent brain slices

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HIGHLIGHTS

- Acute rapamycin-treatment affected cortical L5 pyramidal neurons' *I*/*V* curves and RMP.
- Rapamycin affected cortical L5 pyramidal neuron's spontaneous activities.
- Rapamycin switched cortical L5 burst-spiking modes to regular-spiking ones.

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ABSTRACT

The mammalian target of rapamycin (mTOR) signaling pathway is involved in neuro-developmental/degenerative and neuropsychiatric abnormalities. Rapamycin, a specific and potent inhibitor of mTOR signaling, could regulate synaptic plasticity and synaptic transmission of glutamatergic neurons following prolonged treatment. Its immediate effects on electrophysiological properties of cortical layer 5 (L5) pyramidal neurons where the information undergoes a sophisticated processing remain unknown. Here, we found that acute (within 2 min) bath-application of rapamycin (0.5 $\mu g\,ml^{-1}$) was able to depolarize the current-clamp baseline potentials significantly at postnatal day (P) 4, P10 in rats and P90 in mice ($P<0.05$), and altered the membrane current/voltage (*I*/*V*) curves in an age-dependent manner. Rapamycin not only increased the standard deviation or the peak amplitude of baseline membrane potential, but also increased the frequencies of spontaneous action potentials in more mature neurons (P10 and P90). In addition, rapamycin decreased the burst-firing frequencies of cortical L5 burst-spiking neurons from mature brains, and further switched their firing modes to regular-spiking ones. These findings suggest that acute inhibition of mTOR signaling by rapamycin induces an immediate impact on L5 pyramidal neurons' electrophysiological properties, indicating that its effects might involve mechanisms of ion channel's regulation.

1. Introduction

Compelling evidence indicates that the mammalian target of rapamycin (mTOR) signaling pathway is involved in cellular senescence, organismal aging and age-dependent diseases. In multicellular organisms, mTOR regulates cell growth and metabolism in response to nutrients, growth factors and cellular energy conditions [1–5]. In central nervous system, mTOR signaling impacts neurons' plasticity both functionally and anatomically [6], and it is also involved in neuro-developmental/degenerative and neuropsychiatric abnormalities [7,8].

Pyramidal neurons comprise the majority of the cell population in neocortex [9], particularly within the cortical layer 5 (L5), where information processing occurs via axons running as well as

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through axons projecting through the white matter to other neocortical areas and subcortical brain regions. From a developmental perspective, cortical L5 pyramidal cells undergo substantial remodeling after birth [10]. Furthermore, most of the cortical L5 pyramidal mature neurons are burst-spiking (BS) cells rather than regular-spiking (RS) neurons [11]. They play pivot roles in modulating the behavioral state of an animal [12]. Within L5, RS and intrinsically BS neurons co-exist. Because RS and intrinsically BS neurons in L5 are morphologically distinct from each other [11], their spiking patterns could be used to differentiate between two discrete classes of L5 pyramidal neurons. The features of L5 pyramidal neurons provide an excellent model for studying the neuro-therapy associated with development [10].

Rapamycin, a clinically important immunosuppressant, is a specific and potent inhibitor of mTOR signaling recently demonstrated to be an anticonvulsant [13]. Prolonged rapamycin treatment is known to regulate synaptic plasticity and synaptic transmission of glutamatergic neurons [14]. However, its immediate effects on electrophysiological properties of cortical L5 pyramidal neurons remain unknown. In the present study, by using whole-cell recording in temporary rapamycin-treated rodent brain slices, we found that inhibition of mTOR signaling induces an immediate impact on L5 pyramidal neurons' electrophysiological properties, indicating that rapamycin's effects might involve mechanisms of ion channel's regulation.

2. Materials and methods

2.1. Approval

These experimental procedures were approved by the Ethical Committee of Animal Experiments of the Zhejiang University School of Medicine.

2.2. Animal

The male and female Sprague–Dawley rats or male C57BL/6 mice were provided by the Experimental Animal Center of Zhejiang Province, China. The day of birth was considered as postnatal day 1 (P1), and experiments were performed with Sprague–Dawley rats on P4 and P10 and C57BL/6 mice on P90 respectively. A litter size of P4 or P10 rats were controlled to 3–5 pups. Animals were housed under a 12-h light/dark cycle with access to food and water *at libitum*. Rat pups were kept with their mother in the same cage.

2.3. Whole-cell recordings

2.3.1. Slice preparation

Coronal slices containing cortex were prepared from P4 and P10 Sprague–Dawley rats, as well as from P90 C57BL/6 mice. Animals were decapitated immediately after deep anaesthetization with chloral hydrate (10% w/v, 400 mg/kg). Brains were removed quickly and then cooled in an ice-cold slicing solution consisting of (in mM): 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 10 $MgCl_2$, 10 D-glucose, 0.5$CaCl_2$, 240 sucrose, and then 300 μm-thick slices were sectioned with a Vibroslice (Leica VT 1000) and transferred to a nylon holder basket (AutoMate Scientific) immersed in artificial cerebrospinal fluid (aCSF) composed of (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2$CaCl_2$, 26 $NaHCO_3$ and 10 glucose. After recovery for ~60 min by incubation in aCSF at 37 °C, slices were kept at room temperature for ~60 min before recording. All solutions were saturated with 95% O_2/5% CO_2.

2.3.2. Electrophysiology

The slices were transferred into a submersion-type recording chamber and superfused with oxygenated standard solution (32–33 °C, 2–3 ml/min^{-1}) and anchored with platinum wires on the bottom of the recording chamber. L5 pyramidal neurons in prefrontal cortex were visually identified with an infrared-sensitive CCD camera with a 63 × water-immersion lens (ZEISS, Examiner A1), and whole-cell patch clamp recordings were carried out using a MultiClamp 700B amplifier controlled by pCLAMP 10 acquisition software (Molecular Devices). Pipettes were fabricated from borosilicate capillaries (OD: 1.5 mm/ID: 0.86 mm, Sutter Instrument) by a Flaming/Brown Micropipette Puller (Model P97, Sutter Instrument). Pipettes used for recordings had a tip resistance of 3–8 MΩ when filled with the internal solution containing (in mM): 40 potassium gluconate, 100 KCl, 2 NaCl, 10HEPES buffer, 4 EGTA, 4 Mg-ATP, 0.3 Na_2-GTP, corrected to pH 7.2–7.4 with KOH. Upon achieving whole-cell access, membrane test was run to measure parameters including membrane capacitance (C_m), membrane resistance (R_m), access resistance (R_a), time constant (Tau) and holding current (Hold). Resting membrane potential (RMP) was read in "$I = 0$" mode. Membrane current/voltage (I/V) curve was documented by injection of square-current steps (−200 pA to 1700 pA, in 100 pA increments for P4/P10 or 50 pA for P90, 500 ms). Threshold current for spike generation (I_{th}) was defined as the minimum depolarizing current required for eliciting at least one AP. Instantaneous firing frequency (IFF) was calculated as the inverse of time interval between first and second spikes in a train (Hz) evoked by a 500 ms suprathreshold current. To observe the spontaneous APs (sAPs), continuous current-clamp recording was performed before and after bath application of rapamycin (Sigma, 0.5 μg ml^{-1}, 2–3 ml min^{-1}). Rapamycin was firstly dissolved in DMSO and then added into aCSF at a demanded concentration (DMSO in the bath solutions is about 0.55 μM). Only neurons with an initial seal resistance larger than 2 GΩ were accepted for subsequent experiments. Access resistance was measured during each recording. Data were discarded if the access resistance was over 25 MΩ and changing >20% throughout the recording. Bridge balance was adjusted. Currents were filtered at 1 kHz and sampled at 5 kHz using a Digidata 1440A (Molecular Devices).

2.4. Statistical analysis

I/V curve, variance of baseline membrane potential, and sAP frequencies, I_{th} as well as IFF were analyzed using Clampfit 10.2 (Molecular Devices). Data were expressed as mean ± SEM (stand error of mean). Pair t-test was used for the comparison of same cell before and after treatment. $P < 0.05$ was considered of statistical significance.

3. Results

3.1. Temporary rapamycin treatment altered the electrophysiological properties of cortical L5 pyramidal neurons in P4 rat brain slices

The membrane I/V relationships were assessed by applying square-current injection (in 100 pA increments and lasting for 500 ms) from −200 pA to 700 −1700 pA. We found that 5-min rapamycin treatment did not significantly alter the voltage levels from −200 to −100 pA ($P > 0.05$), but increased the voltage levels from 0 to 600 pA ($P < 0.05$) with statistical significance (Fig. 1A). Interestingly, rapamycin treatment gradually depolarized the current-clamp baseline potentials within 2 min and the baseline levels after rapamycin were significantly increased (9.82 ± 4.75 mV; $P < 0.001$) (Fig. 1B1 and 2). Several slow inward potentials were observed after the rapamycin treatment (3–5 mV, 200–400 ms) (Fig. 1B1; see arrowheads). We further analyzed the effects of rapamycin on the spontaneous activities of the neurons.