Biomedicine & Pharmacotherapy 101 (2018) 362–370

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

GIRK1-mediated inwardly rectifying potassium current suppresses the epileptiform burst activities and the potential antiepileptic effect of ML297

Yian Huang[a,b,1], Yuwen Zhang[a,1], Shuzhen Kong[c], Kai Zang[a], Shize Jiang[d], Li Wan[a], Lulan Chen[a], Guoxiang Wang[a], Min Jiang[a], Xin Wang[a], Jie Hu[d,**], Yun Wang[a,*]

[a] Institutes of Brain Science and State Key Laboratory for Medical Neurobiology, Department of Neurology at Zhongshan Hospital, Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China
[b] State Key Laboratory of New Drug and Pharmaceutical Process, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai 200437, China
[c] College of Environment and Resource, Chongqing Technology and Business University, Chongqing 400067, China
[d] Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200032, China

ARTICLE INFO

Keywords:
GIRK channel
Epileptiform burst
ML297
GIRK1 subunit
Hippocampal cultured neuron

ABSTRACT

G protein-gated inwardly rectifying potassium (GIRK) channels are important inhibitory regulators of neuronal excitability in central nervous system, and the impairment of GIRK channel function has been reported to be associated with the susceptibility of epilepsy. However, the dynamics of GIRK channels in the pathogenesis of epilepsy are still unclear. In this study, our results showed that cyclothiazide, a potent convulsant, dose dependently increased the epileptiform bursting activities and suppressed the baclofen induced GIRK currents. In addition, TPQ, a selective GIRK antagonist, significantly decreased the total inwardly rectifying potassium (Kir) current, and increased the neuronal epileptiform activities. In contrast, ML297, a potent and selective GIRK channel agonist, reversed the cyclothiazide induced decrease of GIRK currents and the increase of neuronal excitability in cultured hippocampal neurons. Further investigation revealed that GIRK1, but not GIRK2, played a key role in suppressing epileptic activities. Finally, in pilocarpine mice seizure model, we demonstrated that ML297 significantly suppressed the seizure behavior. In summary, our current results indicate that GIRK channels, especially GIRK1-containing channels, are involved in epileptic activities and ML297 has a potential antiepileptic effect.

1. Introduction

Epilepsy is one of the most common and obstinate neurological diseases obsessing approximate 0.5–1% of the population around the world. One remarkable character of epilepsy is neuronal hyperexcitability, therefore reducing cell excitability is widely considered as potential method to treat epilepsy. Inwardly rectifying potassium (Kir) channels are important inhibitory regulators in reducing cell excitability through hyperpolarizing membrane potential in nervous system. In various Kir channels, G-protein-gated inwardly rectifying potassium (K^+) channels (GIRK/Kir3) play critical roles owing to their extensive expression both in the heart and the nervous systems [1,2]. GIRK channels are composed of inhibitory G-protein and are activated by binding of G protein Gβγ subunits to the intracellular domains [3]. There are four types of subunits in GIRK homo- or heterotetramers, including GIRK1, GIRK2, GIRK3 and GIRK4, also known as KCNJ3, 6, 9 and 5, respectively, each of which are comprised of several different splice variants [4,5]. In hippocampus, GIRK1 and GIRK2 are highly expressed in the region of CA1, CA3 and dentate gyrus, whereas the expression of GIRK4 is relatively low and therefore without substantial contribution to GIRK functions [6]. GIRK3 may only have a regulatory effect on GIRK channels in the brain [7,8]. Based on the above knowledge, GIRK1 and GIRK2 are supposed to be the most important subunits in the channel formation and functional regulation in hippocampal cells.

It is reported that GIRK2-knockout mice ($GIRK2^{-/-}$) develop

Abbreviations: AMPAR, AMPA receptor; CTZ, cyclothiazide; GABA, γ-aminobutyric acid; GIRK channel, G protein-gated inwardly rectifying potassium channel; KA, kainic acid; KCC2, potassium-chloride cotransporter 2; Kir channel, inwardly rectifying potassium channel; PTZ, pentylenetetrazol; TPQ, tertiapin Q; TTX, tetrodotoxin

* Corresponding author at: Institutes of Brain Science, Fudan University, 131 Dongan Road, Shanghai 200032, China.
** Corresponding author.
E-mail addresses: jiehu68@fudan.edu.cn (J. Hu), yunwang@fudan.edu.cn (Y. Wang).
[1] These author contributed equally in this work.

https://doi.org/10.1016/j.biopha.2018.02.114
Received 7 November 2017; Received in revised form 23 February 2018; Accepted 23 February 2018

spontaneous convulsion and show a propensity for generalized epileptic seizures in the condition of $GABA_A$ receptor antagonist injection. Higher severity score with shorter seizure latency time was induced by pentylenetetrazol (PTZ), a common convulsant, in GIRK2-knockout mice than in wild type and heterozygote ($GIRK2^{+/-}$) groups [9]. Tertiapin Q (TPQ), a GIRK antagonist, decreased the stimulation numbers required to induce spontaneous seizures in the kindling model [10]. These reports concentrated on the effects of GIRK-deficit (drug or transgenic model) on seizure behaviors. However, the functional and expressional dynamics of GIRK channels are not known in the process of epilepsy. ML297 is the first potent and selective GIRK agonist, which both decreased the convulsion percentage and increased the seizure latency and survival rate in PTZ and lethal electrical shock models [11]. However, the signaling pathway underlying its anti-epileptic effects are still unclear at present.

Given the substantiated relation between the GIRK deficiency and the disorder of cell excitability both in postnatal early period and adult stage [12,13], we hypothesize that GIRK channels, in particular those containing GIRK1 and GIRK2 subunits, play critical roles in the pathogenesis of epilepsy, and that the functional promotion of these GIRK channels may, at least in part, decrease the convulsion level during epileptic seizures. Therefore, the purpose of our research is to detect the functional and expressional changes of GIRK channels in epileptic models in order to examine hypothesis mentioned above.

In this report, by applying electrophysiological technology, GIRK antagonist TPQ significantly decreased the instant Kir current and steady-state Kir current; the percentage of neurons with epileptiform bursts increased and GIRK currents were attenuated when treated by two sorts of convulsant, CTZ and KA; in addition, burst frequency in TPQ incubation groups displayed a time-dependent increasing compared to control. The attenuation of GIRK current was significantly reversed by co-incubation of GIRK agonist ML297 and CTZ, and ML297 perfusion decreased the action potential frequency of CTZ incubated neurons. In behavioral tests, ML297 per-treatment significantly reduced the average seizure score in treatment group, the latency of entering Racine grade V was notably longer in treatment group than in control group.

2. Materials and methods

2.1. Animals

The pilocarpine seizure model and brain slice incubation were the only parts of the experiments that needed to use the postnatal animals. The experiments were performed on 6 to 8 weeks old male C57/BL mice, weighing of 20 to 25 g. These mice were ordered from Slac Laboratory Animal, China and maintained in 20 to 25 ˚C air-conditioned rooms, under a stable circadian rhythm (7:00 a.m to 7:00 p.m, light on) with food and water *ad libitum*. All the experiment procedures were approved by the Committee of Animal Use for Research and Education of Fudan University. In the epileptic behavior experiments, we made all our efforts to minimize the number of animals used and their suffering, in accordance with the ethical guidelines for animal researches.

2.2. Reagents

Reagents of tertiapin Q (TPQ), ML297, cyclothiazide (CTZ) and kainic acid (KA) were purchased from Abcam. Tetrodotoxin (TTX), the blocker of voltage-dependent sodium channel and DNQX, an AMPAR blocker, were purchased from Chinese Academy of Fishery Sciences and Tocris, respectively. Atropine and midazolam, which were used in animal experiments, were obtained from Sinopharm. Pilocarpine was purchased from Sigma-Aldrich. All of the drugs for cell research were dissolved to prepare as stock solutions first (1000 ×), and then diluted to the required concentration (1:1000) in the bath solution.

2.3. Cell culture

In previous works, the expression of major GIRK subunits, in particular GIRK2 and GIRK3, has been proved that begins at embryonic period [12,13]. On the basis of these works, we confirmed the practicability and chose cultured neurons as one of our research models. Hippocampal cultured cells were obtained from embryonic day 17 to 18 Sprague Dawley rat embryos, as our previous work [14]. Briefly, the embryos were dissected and the hippocampus was divided first. Tissue was rinsed in the cold HBSS and then digested in the trypsin-EDTA solution (0.05%) in 37 ˚C incubator for 15–20 min. Then FBS (fatal bovine serum, 10%) was used to end the digesting process. Single neurons were collected in the plating medium DF12 (DMEM, 10% FBS, 10% F12) and then seeded on the coverslips which were pre-coated by poly-D-lysine (0.1 mg/ml, Sigma-Aldrich). The seeding density of the hippocampal cells was $4*10^4$ cells/cm^2. The cultured cells were maintained in the 37 ˚C, 5% CO_2 incubator. On the 1, 3, 6, 10 days in vitro (DIV 1, 3, 6, 10) after seeding, the culture medium was half-changed by NB27 culture medium (Neurobasal, 2% B27, 2mM GlutaMAX). Beta-D-arabinofuranoside cytosine (AraC, 1 μM, Sigma-Aldrich) was used on the DIV 6 to end the glia cell proliferation. On the DIV 14 to 16, electrophysiological recording and immunocytochemistry were performed on these cultured cells. All culture reagents were ordered from Invitrogen.

2.4. Preparation of brain slices

Animals were deeply anesthetized by 1% pentobarbital, intraperitoneal injection, in the dose of 0.6 ml/100 g body weight, and then sacrificed. The brain was quickly removed into ice cold cutting solution, which contained (in mM): sucrose 215; KCl 2.5; D-glucose 20; $NaHCO_3$ 26; NaH_2PO_4 1.6; $CaCl_2$ 1; $MgCl_2$ 4; $MgSO_4$ 4. pH was adjusted to 7.4 with 0.5 M HCl, the osmolarity was adjusted at 340 mOsm and bubbled with 95% O_2/5% CO_2. Coronal 350 μm slices were obtained by a vibrating blade microtome (Leica VT-1200S). Slices were removed into artificial cerebrospinal fluid (aCSF), which consisted of (in mM): NaCl 119; KCl 2.3; $NaHCO_3$ 26.2; D-glucose 11; $CaCl_2$ 2.5; NaH_2PO_4 1; $MgSO_4$ 1.3. pH was adjusted to 7.3 with 0.5 M HCl, the osmolarity was adjusted at 320 mOsm and bubbled with 95% O_2/5% CO_2 for at least 30 min at 33 ˚C to recover. After that, tissues were divided into control and null-magnesium groups, incubated in normal aCSF and the same fluid without $MgSO_4$, respectively. After 2 h incubation, hippocampus section of the slices were carefully divided and collected for the western blotting experiments.

2.5. Electrophysiology

The major electrophysiological experiments were divided into three parts: GIRK current, total Kir current, and action potential recording. GIRK current recording was performed through puffing ligand by using a pressure micro-injector (PMI-100, Dagan, USA). Baclofen, as a G-protein coupled receptor activator, was used to open GIRK channel in our research. In the experiment, a glass micro-pipette, with the resistance around 0.5–1 MΩ, puffed baclofen to the local surrounding area of the recording neuron, under a stable pressure (50 kPa) and time course (0.5 s). Voltage clamp was used to hold the membrane potential in −50 mV during GIRK current recording, to normalize and maximize the current amplitude. Tetrodotoxin (0.5 μM) was used to block the action potentials during GIRK current recording. When recording total Kir currents, voltage clamp was also used to hold the membrane potential in −120 mV, for the difference comparison between different groups. Lastly, those experiments with action potential and membrane potential recordings were performed with the current clamp, holding the membrane potential around −70 mV, except those recordings under the resting membrane potential.

All recordings were performed with Multiclamp 700B amplifier