Brain Research Bulletin 127 (2016) 119–125

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Effects of chlorogenic acid on voltage-gated potassium channels of trigeminal ganglion neurons in an inflammatory environment

Fei Liu[a], Xiao-Wen Lu[b], Yu-Jiao Zhang[a], Liang Kou[b], Ning Song[a], Min-Ke Wu[a], Min Wang[a], Hang Wang[a], Jie-Fei Shen (DDS & PhD)[a,*]

[a] State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan Province 610041, China
[b] Ningbo Dental Hospital, Ningbo, Zhejiang Province 315000, China

ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 31 August 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Trigeminal ganglion neurons
Chlorogenic acid
Prostaglandin E_2
Patch clamp
Voltage-gated potassium channels

ABSTRACT

Chlorogenic acid (CGA) composed of coffee acid and quinic acid is an effective ingredient of many foods and medicines and widely exhibits biological effects. Recently, it is reported to have analgesic effect. However, little is known about the analgesic mechanism of CGA. In this study, whole-cell patch-clamp recordings were performed on two main subtypes ($I_{K,A}$ and $I_{K,V}$ channels) of voltage-gated potassium (K_V) channels in small-diameter(<30 μm) trigemianl ganglion neurons to analyze the effects of CGA in an inflammatory environment created by Prostaglandin E_2 (PGE_2). On one hand, the activation and inactivation $V_{1/2}$ values of $I_{K,A}$ and $I_{K,V}$ channels showed an elevation towards a depolarizing shift caused by PGE_2. On the other hand, the activation and inactivation $V_{1/2}$ values of the two channels had a reduction towards a hyperpolarizing shift caused by CGA under PGE_2 pretreatment. Our results demonstrated that CGA may exhibited an analgesic effect by promoting K_V channels activation and inactivation under inflammatory condition, which provided a novel molecular and ionic mechanism underlying anti-inflammatory pain of CGA.

1. Introduction

As one of the most abundant dietary polyphenols, Chlorogenic acid (5-caffeoylquinic acid, CGA) has been reported to have various biological properties and therapeutic potentials, including anti-oxidant, anti-carcinogenic, anti-bacterial, anti-inflammatory and so on (Higdon and Frei, 2006; Kang et al., 2013; dos Santos et al., 2006; Sato et al., 2011). For a long time, almost previous studies supposed the mechanisms underlying CGA anti-inflammatory pain was the inhibitory effect of CGA on synthesis or release of some inflammatory mediators such as tumor necrosis factor-α, nitric oxide and interleukins from inflammatory tissues (dos Santos et al., 2006; Chauhan et al., 2012; Akkol et al., 2012). Novel CGA studies have focused on its profound anti-nociceptive effect on the neurons as an analgesic drug. For instance, several groups suggested CGA was to reduce the risk of ischemic damage for its neuroprotective effect in PC12 cells (Cho et al., 2009; Li et al., 2008; Pavlica and Gebhardt, 2005). (Mikami and Yamazawa, 2015) reported that CGA could prevent neurons from glutamate neurotoxicity by regulating Ca^{2+} entry into neurons. The latest study indicated that CGA may exert an inhibitory effect on the functional activity of native acid-sensing ion channels in rat dorsal root ganglion neurons to alleviate nociceptive response (Qu et al., 2014). According above discoveries, CGA may be useful for novel treatments of inflammatory pain or neuropathic pain through modulating ion channels in neurons. However, in the trigeminal nervous system, the mechanism underlying anti-nociceptive effect of CGA under an inflammatory environment is still little known.

Many studies have revealed that potassium (K^+) channels play an important role in nociceptive processing, such as leak K^+ channels, which produce background K^+ currents that regulate cell excitability and are involved in pain signaling and behavior (Li and Toyoda, 2015). Recently, voltage-gated potassium (K_V) channels have also been supposed to be a potential drug therapeutic target of a variety of pain, such as neuropathic pain and inflammatory pain (Xu et al., 2006; Takeda et al., 2011). K_V channels are one of the important physiological regulator of resting membrane potential (RMP) and action potential (AP) in sensory neurons (Puil et al., 1989; Ficker and Heinemann, 1992). Patch-clamp studies found trigeminal ganglion (TG) neurons have two main types of K_V currents, including the fast inactivating transient A-current ($I_{K,A}$) and the dominant sustained K-current ($I_{K,V}$) (Takeda et al., 2014; Shen et al., 2007). $I_{K,A}$ which is only sensitive to 4-aminopyridine ($I_{K,A}$ blocker) could regulate the threshold potential and the fre-

* Corresponding author at: State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, No. 14, Section 3, Renminnan Road, Chengdu, Sichuan 610041, China.
E-mail addresses: shenjiefei@scu.edu.cn, jiefei.shen@gmail.com (J.-F. Shen).

http://dx.doi.org/10.1016/j.brainresbull.2016.09.005
0361-9230/

quency of action potentials (AP) of trigeminal ganglion neurons (TGNs), whereas $I_{K,V}$ only inhibited by tetraethylammonium (TEA) could regulate the duration of AP (Matsumoto et al., 2010a; Piao et al., 2006). TMJ inflammation could decrease the level of Kv 1.4 channels in the small diameter neurons and decrease $I_{K,A}$ (Takeda et al., 2008). K_V 1.4 channels that can mediate $I_{K,A}$ are expressed in the small-diameter (Aα, C-fibres) neurons, which are thought to transmit nociceptive signal (Takeda et al., 2006; Rasband et al., 2001a). Additionally, studies suggested that $I_{K,V}$ also contributes to the formation and maintaining of inflammatory hyperalgesia of the rat's whisker pad areas with inflammation (Takeda et al., 2008; Matsumoto et al., 2010b). According to these results, $I_{K,A}$ and $I_{K,V}$ channels may play an important role in the development and maintenance of inflammatory pain. In addition, our previous study demonstrated that CGA caused a significant reduction in the activation and inactivation $V_{1/2}$ values of $I_{K,A}$ and $I_{K,V}$ of trigeminal ganglion neurons (TGNs) under formal condition in vitro (Zhang et al., 2014).

In order to further explore the mechanism of CGA in the therapy of neuro-inflammatory pain, in present study, we continued to investigate the effect of CGA on the electrophysiological characteristics of $I_{K,A}$ and $I_{K,V}$ of rat TGNs in an inflammatory environment. PGE_2 produced by the cyclooxygenase (COX) enzymes, an important mediator of inflammation, was used to mimic an inflammatory condition in vitro because direct delivery of PGE_2 into tissues could initiate inflammation and high levels of PGE_2 were observed in inflammatory lesions such as arthritic joints (Anderson et al., 1996; Stock et al., 2001; Vane, 1971). The results demonstrated the activation and inactivation $V_{1/2}$ values showed congruously elevation towards a depolarizing direction caused by PGE_2 with an elevation in the peak $I_{K,A}$ and congruously reduction with a hyperpolarizing shift caused by CGA with an depressed peak $I_{K,A}$ under PGE_2 pretreatment. Our results provided a novel molecular and ionic mechanism of CGA analgesia in an inflammatory condition.

2. Materials and methods

2.1. Acute dissociation of trigeminal ganglion neurons

All animal procedures were approved by the State Key Laboratory of Oral Diseases, Sichuan University. Rat TG isolation and neurons dissociation were described in our previous report (Shen et al., 2007). Briefly, bilateral trigeminal ganglia were isolated from neonatal (3–5 days) *Sprague–Dawley* rats that were anaesthetized with 0.1% pentobarbital sodium by peritoneal injection. The extracted TGs were washed using ice-cold Hanks' balanced salt solution (pH = 7.4; Sigma-Aldrich China, China), minced into small pieces under a dissecting microscope and incubated in *Hanks*' balanced salt solution containing 20U/ml papain (Sigma-Aldrich China, China) at 37 °C for 40 min. After appropriate digestion, the cells were washed thrice with DMEM/F12 culture medium (1:1 vol; Gibco, Life Technologies, USA) supplemented with 10% fetal bovine serum (Gibco, Life Technologies, USA). Then, the cells were gently triturated using a series of fire-polished Pasteur pipettes and plated on poly-L-lysine (Sigma-Aldrich China, China)-coated glass coverslips placed in 35 mm dishes (Becton, Dickinson Company, USA). The dishes were maintained in a humidified atmosphere of 5% CO_2 at 37 °C for 2 h prior to the measurements.

2.2. Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings were performed on small-diameter(<30 μm) TG neurons.The external solution for the $I_{K,A}$ current recordings contains 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 70 mM tetraethylammonium (Sigma-Aldrich China, China), 70 mM choline-Cl, 10 mM D-glucose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 0.1 mM $CdCl_2$ (pH = 7.4). For the $I_{K,V}$ recordings, the solution contains 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3 mM 4-aminopyridine (Sigma-Aldrich China, China), 137 mM choline-Cl, 10 mM D-glucose, 10 mM HEPES and 0.1 mM $CdCl_2$ (pH = 7.4). The external solution volume in the dishes was adjusted to 2 mL. Patch-clamp pipettes were pulled from borosilicate glass and filled with the internal solution composed of 120 mM potassium methanesulphonate, 20 mM KCl, 7.5 mM HEPES and 2 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) (pH = 7.3) for both the $I_{K,A}$ and $I_{K,V}$ recordings. The whole cell recordings were conducted using an Axopatch 200 B patch-clamp amplifier (Axon Instruments, USA), and the output was digitized with the Digidata 1440A converter (Axon Instruments, USA). Both the capacitance and series resistance were well compensated. All data were acquired using Clampex 10.0 software (Axon Instruments, USA). All recordings above were performed at a conditioned temperature of 25–26 °C.

Activation and inactivation currents of Kv subtypes were generated using various stimulus protocols. After achieving a gigaohm(GΩ) seal between the cell membrane and patch pipette, neurons were initially held at −80 mV followed by hyperpolarization to −100 mV for 250 ms as a conditioning prepulse potential. The inward K^+ activation currents were elicited using 250 ms pulses stepping from −80 mV to +60 mV in 10 mV increments. The inactivation properties of the $I_{K,A}$ and $I_{K,V}$ were analyzed using another stimulus protocol. The neurons held at −80 mV were subjected to a series of 250 ms pre-pulses stepping from −120 mV to +50 mV followed by a 250 ms test pulse depolarizing to +50 mV.

2.3. PGE_2 and CGA delivery

In order to observe the effect of PGE_2 (Sigma, USA) on Kv channels, coverslips attached with dissociated TGNs were washed 3 times and maintained in external solution without PGE_2 in the 35 mm dish for 5 min to replace the DMEM/F12 medium. Then, whole-cell patch-clamp was carried out in a control condition. After above recoding, the external solution with 20 μM PGE_2 was added to the dish via gravity through a bath perfusion apparatus integrated into the patch-clamp system. Meanwhile, the external solution without PGE_2 in the dish was sucked out through a duct. Bath solution perfusion was maintained at a rate of 1 mL/min. The following patch-clamp recordings were initiated 2 min after the perfusion began.

In the CGA (Sigma, USA) experiment, the dissociated TGNs growing coverslips were washed 3 times and maintained in external solution with 20 μM PGE_2 in the 35 mm dish for 5 min to substitute for the DMEM/F12 medium. Then whole-cell patch-clamp was performed to analyze the effect of PGE_2 on Kv channels in a formal condition. After recording, the external solution containing 0.2 mM CGA and 20 μM PGE_2 was delivered to the dish through the bath perfusion apparatus. At the same time, the external solution in the dish was sucked out. Bath solution perfusion was maintained at a rate of 1 mL/min for 2 min. Then further recordings were carried out under PGE_2 pretreated condition.

2.4. Data analysis

For $I_{K,A}$ and $I_{K,V}$, current densities were obtained by dividing the peak currents with their own whole-cell capacitances. The channel conductance (G) at various membrane potentials was calculated by the equation: $G = I/(V_m - V_{rev})$. Normalized activation curves were plotted as G/G_{max} against the voltage commands. The curves of all groups were fitted to a Boltzmann equation: $G/G_{max} = 1 - 1/(1 + exp[(V_m - V_{1/2})/k])$. In above equations, I represents the current density, V_m represents the voltage command,