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A comparison of the delayed outward potassium current between the nucleus ambiguus and hippocampus: sensitivity to paeonol

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ABSTRACT

Whole-cell patch-clamp recordings investigated the electrophysiological effects of 2′-hydroxy-4′-methoxyacetophenone (paeonol), one of the major components of Moutan Cortex, in hippocampal CA1 neurons and nucleus ambiguus (NA) neurons from neonatal rats as well as in lung epithelial H1355 cells expressing Kv2.1 or Kv1.2. Extracellular application of paeonol at 100 µM did not significantly affect the spontaneous action potential frequency, whereas paeonol at 300 µM increased the frequency of spontaneous action potentials in hippocampal CA1 neurons. Paeonol (300 µM) significantly decreased the tetraethylammonium-sensitive outward current in hippocampal CA1 neurons, but had no effect upon the fast-inactivating potassium current (I_A). Extracellular application of paeonol at 300 µM did not affect action potentials or the delayed outward currents in NA neurons. Paeonol (100 µM) reduced the Kv2.1 current in H1355 cells, but not the Kv1.2 current. The inhibitor of Kv2, guangxitoxin-1E, reduced the delayed outward potassium currents in hippocampal neurons, but had only minimal effects in NA neurons. We demonstrated that paeonol decreased the delayed outward current and increased excitability in hippocampal CA1 neurons, whereas these effects were not observed in NA neurons. These effects may be associated with the inhibitory effects on Kv2.1 currents.

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1. Introduction

In traditional Chinese medicine, Moutan Cortex, the root bark of *Paeonia suffruticosa* Andrews, has long been used for its anticoagulant, anti-inflammatory, analgesic and sedative activities, and as a remedy for cardiovascular, extravasated blood, stagnated blood and female genital diseases (Hirai et al., 1983; Kubo et al., 1979; Li, 1988; Okubo et al., 2000). A major component of Moutan Cortex, 2′-hydroxy-4′-methoxyacetophenone (paeonol), has been reported to have analgesic, antipyretic and antibacterial properties as well as anti-inflammatory, antioxidant activities and antiplatelet aggregation effects (Chen et al., 2012; Harada et al., 1972; Hirai et al., 1983; Zhang et al., 1999).

Recent evidence from a rat model of Alzheimer's disease (AD) indicated that paeonol was able to protect against many alterations that arise from administration of Aβ1–42, including morphological, biochemical and behavioral changes (Zhou et al., 2011). Inflammation and oxidative stress play important roles in the pathogenesis of neurodegenerative disorders such as AD. It has been suggested that paeonol may exert neuroprotective effects via the inhibition of microglia-mediated inflammation and blocking of the neuronal damage caused by oxidative stress (Tseng et al., 2012). However, the exact mechanism of paeonol in AD treatment is not clear.

The hippocampus, one of the most studied structures in the human brain, plays an important role in learning and memory (Shu et al., 2014). Spatial navigation and memory are primarily associated with the hippocampus, both in rodents and humans (Bannerman et al., 2014). Damage to the hippocampus can lead to loss of memory and difficulty in establishing new memories (King et al., 2004). In AD, the hippocampus is one of the first regions of the brain to be affected, leading to the confusion and loss of memory so commonly seen in the early stages of the disease (Milner et al., 1998; Padurariu et al., 2012). Clinical evidence has

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confirmed a significant loss of hippocampal neuronal density in patients with AD (Padurariu et al., 2012). Acetylcholinesterase (AChE) inhibitors, such as galantamine and rivastigmine, can delay the breakdown of acetylcholine released into synaptic clefts and so enhance cholinergic neurotransmission. In addition, galantamine has been shown to increase the excitability of hippocampal neurons and decrease the delayed rectifier potassium current in these neurons (Oh et al., 2006); rivastigmine can also decrease the delayed rectifier potassium current in these neurons (Pan et al., 2003a). Furthermore, galantamine potently blocks Kv2.1, but not Kv1.5, channels in HEK293 cells (Zhang et al., 2004). This evidence raised questions as to whether paeonol has similar electrophysiological effects on hippocampal neurons.

The nucleus ambiguous (NA), located in the medulla oblongata, is one of the original nuclei of the vagus nerve, which innervates the soft palate, pharynx, esophagus, larynx, and heart (Hayakawa et al., 1996; Pasaro et al., 1983). Thus, NA neurons play an important role in swallowing, peristalsis, vocalization, and cardiovascular function. Lesions of the NA almost completely abolish the baroreceptor reflex function controlling heart rate(Cheng et al., 2004) and result in dysphagia as well as dysphonia (Ishibashi and Fujishima, 2012; Saito et al., 2003). Many human diseases are associated with abnormal excitable cell function (Goodman, 2008). Thus, compounds that affect the excitability of the NA neurons may result in side effects relating to their functions.

In the present study, we used the whole-cell patch-clamp technique to determine the effects of paeonol on membrane potentials and the delayed outward potassium current in rat hippocampal CA1 neurons and NA neurons.

2. Materials and methods

2.1. Brain slice preparations

The effects of paeonol on action potentials and ionic currents were studied in neonatal rat hippocampal CA1 neurons and NA neurons, using the whole-cell patch clamp method as reported previously (Lin et al., 2015). The experimental procedures were approved by the China Medical University Institutional Animal Care and Use Committee, in accordance with the Chinese Taipei Society of Laboratory Animal Sciences guidelines on the care and use of laboratory animals. In brief, hippocampal slices were obtained from 5- to 15-day-old Sprague Dawley rats. After rats were anaesthetized with ether and decapitated, the brains were removed. A block of tissue containing the hippocampus was separated from the brain and the hindbrains containing the NA were removed. The blocks were glued to the cutting chamber of a tissue slicer with cyanoacrylate glue. The chamber was filled with ice-cold Krebs solution of the following composition (in mM): 127 NaCl, 1.9 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$ and 10 glucose, saturated with 95% O_2 and 5% CO_2. Osmolarity was 310–320 mOsm. Coronal hippocampal slices of 300 μm and NA slices of 250 μm from the compact part of the medulla oblongata were prepared by a tissue slicer (DTK-1000, Dosaka, Kyoto, Japan). The slices were transferred to an incubation chamber in Krebs solution and were incubated for at least 1 h at room temperature under continuous oxygenation.

After incubation, the slice was placed in a glass-bottomed recording chamber (volume < 0.5 ml) fixed to a microscope stage. The slice was immobilized by pressing onto the bottom of the recording chamber with a purpose-designed fabricated grid of nylon threads attached to a U-shaped platinum frame. Paeonol and other compounds were applied by perfusion. Neurons were viewed using an upright microscope with a water-immersion objective lens. Conventional patch pipettes were made from standard-wall glass capillaries. Pipettes were pulled from thin-walled fiber-filled borosilicate glasses (WPI, OD 2.0 mm, World Precision Instruments, Inc., Sarasota, FL) and filled with a solution of the following composition (in mM): 130 K^+ gluconate, 1 $MgCl_2$, 2 $CaCl_2$, 4 K_2ATP, 10 EGTA and 10 HEPES (osmolarity was 295–305 mOsm), with an input resistance of 4–6 MΩ. Whole-cell currents and membrane potentials were recorded with a Multiclamp 700B amplifier, low-pass filtered at 2 kHz and acquired using a PC and pCLAMP software (version 10.2, Axon Instruments) for later analysis. Cells were located and patched under visual control. A gigaohm seal (>10 GΩ) was established in the cell-attached mode prior to perforation of the patch membrane for whole-cell recording.

2.2. Measurement of ionic currents in hippocampal neurons

2.2.1. I_A currents

The transient outward potassium current (I_A) was separated from the total potassium current. Currents were recorded during step depolarizations from -120 mV to voltages between -50 and $+50$ mV, with and without 100 ms prepulse to -40 mV. I_A was isolated by point-by-point subtraction as the current sensitive to the inactivating prepulse that spared delayed currents (Vasilyev and Barish, 2003).

2.2.2. Delayed outward currents

Delayed outward currents were recorded under control conditions. In the voltage protocol illustrated, the neuron was held at -70 mV. After a conditioning hyperpolarization to -120 mV, a prepulse to -40 mV eliminated I_A and the remaining currents were recorded during 360-ms-long steps to voltages between -50 and $+50$ mV in 10 mV increments (Wu and Barish, 1999). The amplitude of the delayed outward current was measured at 360 ms after voltage stepping.

2.3. Cell culture and transfection

Lung epithelial H1355 cells were cultured at 37 °C in 5% CO_2 in Roswell Park Memorial Institute 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and penicillin-streptomycin (100 units/ml, 100 μg/ml) (Invitrogen, Carlsbad, CA). For heterologous expression of Kv2.1 channels, pcDNA3-Kv2.1 (kindly provided by Prof. H. Gaisano, University of Toronto) and Pegfp (as a marker, provided by Clontech, Palo Alto, CA) were transiently transfected into H1355 cells using TurboFect (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions.

2.4. Electrophysiological recording in lung epithelial H1355 cells

Electrophysiological experiments were performed as previously reported (Chen et al., 2013; Leung et al., 2003). H1355 cells were voltage-clamped in the whole-cell configuration. Thin-walled borosilicate glass tubes (Sutter Instrument, Novato, CA) were pulled with a micropipette puller (P-87, Sutter Instrument), then heat-polished with a microforge (Narishige Instruments, Inc., Sarasota, FL). The pipettes were filled with intracellular solution containing (mM) 140 KCl, 1 $MgCl_2$, 1 EGTA, 10 HEPES, and 5 MgATP (osmolarity was 300–310 mOsm), with a typical resistance of 4–7 MΩ. The bath solution contained (mM) 140 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES (pH 7.4 adjusted with NaOH; osmolarity was 300–310 mOsm). The neuron was held at -70 mV and the currents were recorded during 500-ms-long steps to voltages between -70 and $+70$ mV in 10 mV increments. The currents were recorded using an EPC-10 amplifier with Pulse 8.60 acquisition software and analyzed by Pulsefit 8.60 software (HEKA Electronik,

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