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Research article

Ginsenoside Rg1 modulates medial prefrontal cortical firing and suppresses the hippocampo-medial prefrontal cortical long-term potentiation

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ABSTRACT

Background: *Panax ginseng* is one of the most commonly used medicinal herbs worldwide for a variety of therapeutic properties including neurocognitive effects. Ginsenoside Rg1 is one of the most abundant active chemical constituents of this herb with known neuroprotective, anxiolytic, and cognition improving effects.
Methods: We investigated the effects of Rg1 on the medial prefrontal cortex (mPFC), a key brain region involved in cognition, information processing, working memory, and decision making. In this study, the effects of systemic administration of Rg1 (1 mg/kg, 3 mg/kg, or 10 mg/kg) on (1) spontaneous firing of the medial prefrontal cortical neurons and (2) long-term potentiation (LTP) in the hippocampal–medial prefrontal cortical (HP–mPFC) pathway were investigated in male Sprague–Dawley rats.
Results: The spontaneous neuronal activity of approximately 50% the recorded pyramidal cells in the mPFC was suppressed by Rg1. In addition, Rg1 attenuated LTP in the HP–mPFC pathway. These effects were not dose-dependent.
Conclusion: This report suggests that acute treatment of Rg1 impairs LTP in the HP–mPFC pathway, perhaps by suppressing the firing of a subset of mPFC neurons that may contribute to the neurocognitive effects of Rg1.

1. Introduction

The neuropyschopharmacology of *Panax ginseng*, one of the most famous traditional herbs, has been extensively explored by both preclinical and clinical studies. *P. ginseng* and its pharmacologically active constituents, ginsenosides, have found their use in various neuropsychiatric and neurodegenerative conditions such as depression, ischemic stroke, Alzheimer's disease, and Parkinson's disease [1–3]. One of the most abundant constituents among these ginsenosides is Rg1 [4], which is structurally classified under the panaxitriol group [2]. Many preclinical studies delineate the neuroprotective and procognitive effects of Rg1 in various animal models. Behavioral investigations in mice showed that Rg1 enhances spatial memory in naïve [5] and Tg-mAPP overexpressing mice [6] and cognitive performance of senescence-accelerated mouse prone 8 (SAMP8), a model of Alzheimer's disease [7]. Furthermore, Rg1 treatment ameliorates learning and memory impairments, induced by morphine [8], chronic restraint stress [9], scopolamine [10,11], and beta-amyloid peptide (25–35) [12]. In rats, Rg1 was shown to reverse the cognitive impairments ensuing

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electrical injury of the hippocampus [13], bilateral fimbria fornix transection [14], ovariectomy followed by D-galactose treatment [15] and lipopolysaccharide-induced neuroinflammation [16]. It is noteworthy that the aforesaid reports substantiated the procognitive behavioral effects with data on anatomical, electrophysiological, protein, and neurotransmitter level changes in the rodent brain.

This *in vivo* electrophysiological investigation will draw attention to the effects of Rg1 focusing on the changes in the medial prefrontal cortex (mPFC). The mPFC is bilateral brain loci that receives neuronal projection from different parts of the brain [17–19]. The mPFC integrates complex information from various brain regions such as cortex, hippocampus (HP), midbrain, and brainstem to maintain and modulate emotion, cognition, and reward processing. Long-term potentiation (LTP) in the HP–mPFC pathway is a reliable model to study pharmacological and behavioral manipulations that could influence the aforesaid processes [20–22]. The electrophysiological studies on Rg1 that were published to date focused on its modulatory effects on cognitive behavior mediated by the hippocampus. To mention a few, systemic administration of Rg1 increased the synaptic plasticity in the perforant path–dentate gyrus of conscious rats [23], and central administration Rg1 or its metabolites (Rh1 or Ppt) increased hippocampal excitability in unconscious rats [11,23,24]. Rg1 induced LTP in the hippocampus mediated by calcium dependent *N*-methyl-D-aspartate (NMDA) receptor [24] and reversed the chronic morphine-induced impairment of LTP in the CA1-Schaffer collateral [8]. Ginseng dose-dependently reversed the deficits in T-shaped water maze performance (errors) due to prefrontal cortical lesioning in rats [25]. Although this study did not specifically examine the effects of Rg1, it stands as a good representation to accentuate the role of prefrontal cortex underlying the effects of ginsenosides Rg1 and Rb1, taken together. It is noteworthy that another ginsenoside (Re) with reported procognitive effects, belonging to the same group of ginsenoside, dose-dependently increases the extracellular levels of acetylcholine and dopamine in the hippocampus and mPFC with the effect being prominent in the former structure [26]. The present study, first of its kind, has been designed to examine the effects of acute treatment of Rg1 on the changes in the firing rate of mPFC neurons and LTP in the HP–mPFC pathway in unconscious rats.

2. Material and methods

2.1. Animals

Adult male Sprague–Dawley rats (250–380 g) obtained from InVivos Pte. Ltd. (Singapore) were housed in pairs in the animal housing facility of the National University of Singapore for at least 48 h prior to the start of experiments. All cages were individually ventilated in temperature-controlled (range, 22–24°C) rooms with 12-h cycles of day/night light (07:00–19:00). Animals had free access to food and water. All experimental procedures were conducted in accordance with National Institutes of Health Guide for Care and Use of Animals following the approval by the Institutional Animal Care and Use Committee of the National University of Singapore, Singapore.

2.2. Drugs and chemicals

The 7% w/v solution of chloral hydrate (Sigma Aldrich, St. Louis, MO, USA) and 1 mg/ml, 3 mg/ml, or 10 mg/mL solutions of ginsenoside Rg1 (95%; Nature Standard, Shanghai, China) were prepared in sterile normal saline (B Braun, Bayan Lepas Pulau Pinang, Malaysia). Pentobarbital (Valabarb) was purchased from Jurox Pty Ltd. (Rutherford, NSW, Australia). A 2% w/v solution of Pontamine sky blue (Alfa Aesar, Karlsruhe, Germany) in 2M NaCl (Schedelco, Penang, Malaysia) filled the glass electrode that was used for single unit recording. Solutions of 0.9% w/v sodium chloride (Schedelco) and 4% w/v paraformaldehyde (PFA; Sigma Aldrich) in phosphate buffer (Na_2PO_4 and $NaH_2PO_4 \cdot 2H_2O$; Merck, Darmstadt, Germany) were used for perfusion. The 30% w/v sucrose (Fisher Chemicals, Loughborough, UK) in 10% phosphate buffer saline (1st BASE, Singapore) was used for saturating the harvested brain prior to cryosectioning.

2.3. Surgery

Rats were acclimatized to the electrophysiology procedure room for 30 min, after which they were anesthetized via a single intraperitoneal injection of chloral hydrate (400 mg/kg). Typically, the anesthetized rat was depilated at the head region and mounted on a stereotaxic frame. The body temperature was maintained at 37°C by a homeothermic blanket with rectal temperature probe. The level of anesthesia was maintained by supplemental doses of chloral hydrate administered through the cannulated lateral tail vein. A single sagittal incision on the scalp exposed the bare skull, and burr holes were drilled to target the infralimbic medial prefrontal cortical area (anterior-posterior (AP): 3.3 mm, medial-lateral (ML): ±0.8 mm) for single unit (Fig. 1A) or evoked potential (Fig. 1B) recording, and the ventral hippocampal area (AP: −6.3 mm, ML: ±5.5 mm) for evoked potential stimulation (Fig. 1C), based on the standard coordinates [27].

2.3.1. Extracellular single unit recording of the mPFC neurons

Glass electrodes were pulled from Starbore glass capillaries (Radnoti, Monrovia, CA, USA) using a micropipette puller (PE-21; Narishige Instruments, Tokyo, Japan) and were filled with Pontamine sky blue dye (2% w/v in 2M NaCl). The impedance was adjusted to 20–40 MΩ. The glass electrode was gradually lowered (1–100 μm steps) into the brain via the burr hole on the skull using

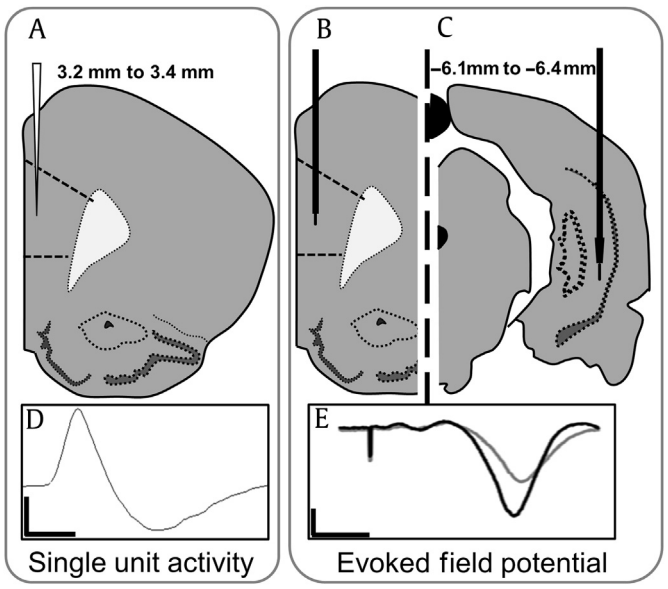

Fig. 1. Schematic representation of sites of intervention at specified distances from bregma. (A) The glass recording electrodes for single unit recording in the medial prefrontal cortex (mPFC). (B) The monopolar electrode in the mPFC for recording evoked field potentials in response to (C) the concentric bipolar stimulating electrode at the CA1/vH. (D) A representative spike from an mPFC neuron (scale bar: 2 mV and 0.5 ms). (E) Representative evoked potential waveforms during baseline (gray) and after high frequency (black) stimulation (scale bar: 0.2 mV and 10 ms).

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