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NO mediates ginsenoside Rg_1-induced long-term potentiation in anesthetized rats[1]

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KEY WORDS ginseng; saponins; long-term potentiation; synaptic transmission; nitric oxide; indazoles; hippocampus; dentate gyrus; arginine

ABSTRACT

AIM: To investigate the effect of ginsenoside Rg_1 on synaptic transmission in anesthetized rats and the effect of NOS inhibitor, 7-nitroindazole (7-NI), on long-term potentiation (LTP) induced by Rg_1. **METHODS**: Extracellular recording technique was used to record the population spike (PS) in the dentate gyrus (DG) of anesthetized rats. Drug or vehicle injections were delivered via a cannula in the lateral cerebral ventricle. **RESULTS**: Rg_1 (10 and 100 nmol/L) enhanced the basic synaptic transmission and the magnitude of LTP induced by high frequency stimulation (HFS). Selective nNOS inhibitor 7-NI (5 nmol) icv could inhibit the induction of perforant path-dentate gyrus LTP elicited by Rg_1 ($P < 0.05$), and *L*-arginine 250 g/L ip prevented the action of 7-nitroindazole ($P < 0.05$). **CONCLUSION**: Rg_1-accelerated synaptic transmission and nitric oxide produced by nNOS played a role in the induction of PP-DG LTP in anesthetized rats.

INTRODUCTION

Ginsenoside saponins are known to be the active principles of ginseng. In the previous studies, Rg_1 could improve acquisition, consolidation, and retrieval of memory impaired by amnestic agents[1-3]. Synapses are essential structures in the nervous system and are considered as the neurobiological basis of learning and memory. To investigate further the action of ginsenoside Rg_1 on the central nervous system, crude ginseng saponins-evoked potential in the dentate gyrus of anesthetized rats was detected[4]. On the basis of the previous experience, we studied the effect of Rg_1 on synaptic transmission in the hippocampal dentate gyrus of anesthetized rats and its possible mechanism of action.

MATERIALS AND METHODS

Rats Male Sprague-Dawley rats (200 g ± 20 g, Grade Ⅱ, Certificate № SCXK 11-00-0006), from the Center of Experimental Animals, Chinese Academy of Medical Sciences were fed lab chow and water *ad lib* and housed under a 12-h light/dark cycle.

Drugs and drug delivery Rg_1, supplied by the Department of Basal Organic Chemistry, Norman Bethune Medical University was dissolved with 0.9 % NaCl solution; 7-nitroindazole (7-NI, Sigma, USA) was dissolved in Me_2SO (0.1 mol/L) and diluted to the required concentration with 0.9 % NaCl solution. Drug or vehicle injections were delivered via a cannula inserted through the outer guide cannule that was located in the lateral cerebral ventricle, following the measurement of basline for 30 min from the dentate gyrus (DG) of the same hemisphere, and the cannule was left in place for 5 min after each injection.

Drug doses were calculated on the basis that drugs theoretically achieved the brain concentrations required, assuming the brain volume to be approximately 2 mL[5]. The final brain concentration of Rg_1 10, 100 nmol/L and 7-NI 1.5 μmol/L were used. The vehicle or 7- NI was injected into the lateral cerebral ventricle 15 min before the use of Rg_1, *L*-arginine 250 mg/kg ip was given 10 min before the application of 7-NI.

Electrophysiologic recording[6] Recording of evoked potential was made as described in our previous study[6,7]. Briefly, male SD rats were anaesthetized with urethane carbamate (1.5 g/kg, ip) and fixed in a

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stereotaxic frame. A bipolar stimulation eletrode was stereotaxically placed in the left entorhinal cortex to stimulate the perforant path (PP) and the evoked potential was extracellularly recorded from the granule cell layer of ipsilateral dentate gyrus. Electrodes were slowly lowered to a depth of 2.5 mm, and the final depths were adjusted until maximal extracellular population spike were obtained. A single test stimulus (200 μs) was applied at intervals of 30 s and the stimulus intensity was set at a level when a population spike of 33 % of the maximum was evoked. The theta-like stimulation-induced LTP consisted of 10 bursts of 5 pulses (100 Hz, stimulus duration: 200 μs, interburst interval: 200 ms), and the brief tetanic stimulation was applied at the same intensity through the same stimulation electrode as used for test stimulation.

The evoked responses were averaged every 5 records, and the mean baseline was obtained by averaging the PS amplitude of 6 time points obtained within 30 min before injection of drug or tetanus.

Statistical analysis The data were expressed as $x \pm s$. The difference between groups was estimated using the t-test.

RESULTS

Effect of Rg_1 on basic synaptic transmission of anesthetized rats Rg_1 (10, 100 nmol/L, icv) was injected into the lateral cerebral ventricle. The mean baseline was obtained by averaging the PS amplitude of 6 time points within 30 min before Rg_1 administration. Fig 1 showed that the amplitude of PS did not change after the vehicle administration over a 60-min recording period. Rg_1 (10 nmol/L, icv) could increase the PS amplitude but could not induce DG LTP in rat hippocampus. For example, the PS amplitude at 10, 30, and 60 min was 115 % ± 15 %, 125 % ± 15 %, and 126 % ± 14 %, respectively ($n = 6$, $P < 0.05$), but the increase in PS amplitude was less than 30 % of baseline. Rg_1 (100 nmol/L, icv) induced PP-DG LTP, and the PS amplitude at 10, 30, and 60 min was 127 % ± 17 %, 166 % ± 19 %, and 173 % ± 20 %, respectively ($n = 6$, $P < 0.01$).

Effect of Rg_1 on LTP induced by high frequency stimulation (HFS) in anesthetized rats Thirty minutes after injection of Rg_1 (10 and 100 nmol/L, icv), tetanic stimulation (100 Hz, 200 μs) was applied, and the mean baseline was obtained by averaging the PS amplitude of 6 time points within 30 min before

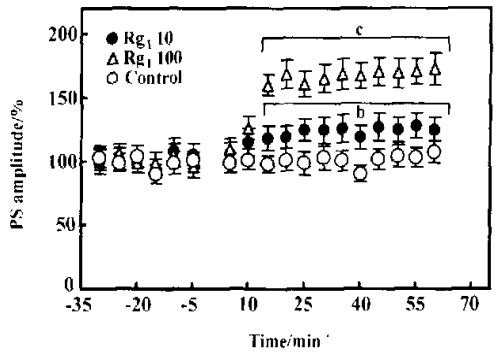

Fig 1. The effect of Rg_1 (10, 100 nmol/L, icv) on evoked potential in dentate gyrus of anesthetized rats. The average amplitude of the population spikes recorded 30 min before Rg_1 injection was defined as 100 %. $n = 6$ observations. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* vehicle-injected group.

Rg_1 administration. As shown in Fig 2, the amplitude of PS did not change markedly after the vehicle administration over a 60-min recording period. The values of PS amplitude were 209 % ± 20 %, 182 % ± 19 %, 180 % ± 19 %, and 178 % ± 17 % at 10, 30, 60, and 90 min respectively after HFS ($n = 6$). The PS amplitude of Rg_1 10 nmol/L treated group was 217 % ± 21 %, 202 % ± 18 %, and 198 % ± 20 % respectively at 10, 30, and 60 min, and that in Rg_1 100 nmol/L-treated group was 249 % ± 27 %, 221 % ± 25 %, and 221 % ± 24 % respectively at 10, 30, and 60 min after HFS application ($n = 6$, $P < 0.01$).

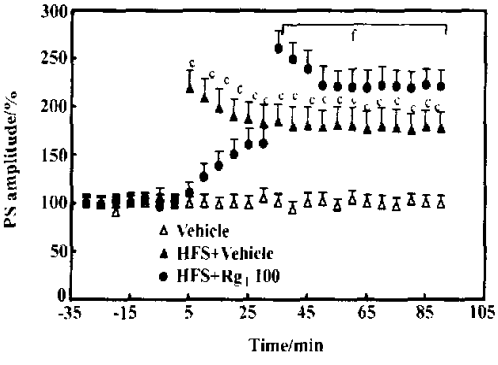

Fig 2. The effect of Rg_1 on perforant path-dentate gyrus long-term potentiation induced by HFS in anesthetized rats. The average amplitude of the population spikes recorded 30 min before Rg_1 injection or HFS application was defined as 100 %. Rg_1 was injected 30 min after HFS application. $n = 6$ observations. $x \pm s$. $^cP < 0.01$ *vs* vehicle-injected group. $^fP < 0.01$ *vs* HFS group.

Effect of 7-NI, a selective inhibitor of nNOS, on LTP induced by Rg_1 As shown in Fig 3, injection of 7-NI did not affect the PS amplitude over the 60-min recording period. As described above, Rg_1 (100 nmol/L) induced LTP in hippocampus, when 7-NI (5 nmol, icv) was injected 15 min before Rg_1, the PS amplitude was decreased to 116 % ± 10 %, 120 % ± 13 %, and 121 % ± 17 % at 10, 30 and 60 min, respectively, showing that 7-NI markedly inhibited the induction of LTP induced by Rg_1 100 nmol/L ($n = 6$, $P < 0.05$).

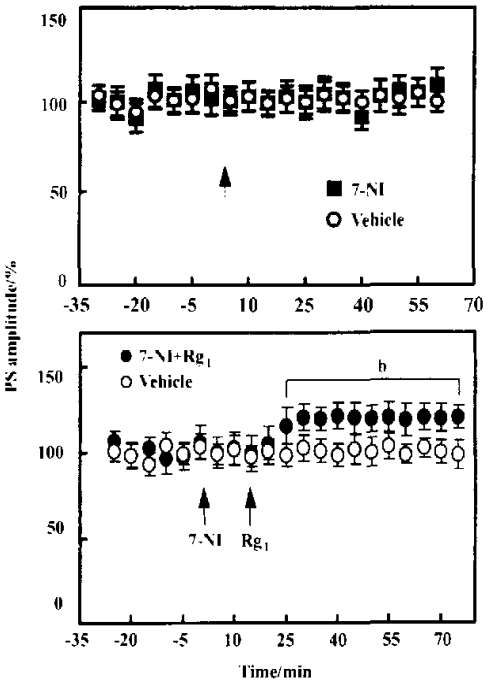

Fig 3. The effect of 7-NI on induction of LTP in dentate gyrus of hippocampus in anesthetized rats. 7-NI (5 nmol, icv) was injected 15 min before Rg_1, the average amplitude of the population spikes recorded 30 min before 7-NI injection was defined as 100 %. $n = 6$ observations. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control group.

Effect of *L*-Arg on the inhibitory effect of 7-NI on Rg_1-induced LTP As shown in Fig 4, *L*-Arg could reverse the action of 7-NI on LTP induced by Rg_1 (100 nmol/L, icv). *L*-Arg (250 mg/kg, ip) was injected 10 min before 7-NI. The PS amplitude of anesthetized rats administered with *L*-Arg and 7-NI was higher than that in rats injected with only 7-NI ($P < 0.05$, $n = 6$). *L*-Arg attenuated the inhibition of 7-NI on LTP induced by Rg_1, although the PS amplitude did not reach the level of the Rg_1 group.

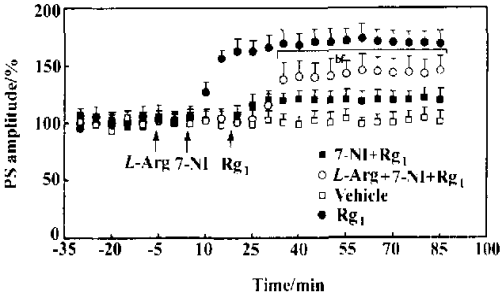

Fig 4. The effect of *L*-Arg on the inhibition of 7-NI on LTP induced by Rg_1 (100 nmol/L). 7-NI (icv) was injected 15 min before Rg_1, and *L*-Arg (ip) was injected 5 min before 7-NI. The average amplitude of the population spikes recorded 30 min before *L*-Arginine injection was defined as 100 %. $n = 6$ observations. $\bar{x} \pm s$. $^bP < 0.05$ *vs* 7-NI + Rg_1 group. $^fP < 0.01$ *vs* Rg_1 group.

DISCUSSION

Elucidation of the physiological basis of learning is among the greatest remaining challenges of the neuroscience. LTP of evoked potentials in the hippocampus is a form of activity-dependent synaptic plasticity and has become widely regarded as a possible physiological substrate for some aspects of learning and memory. In the present studies, ginsenoside Rg_1 improved the basic synaptic transmission and increased the amplitude of LTP induced by 100 Hz HFS. In previous studies, Rg_1 could improve acquisition, consolidation, and retrieval of memory impaired by amnestic agents[1-3]. So Rg_1 provides a linkage between LTP and learning, and gives a clue how endogenously generated LTP mediates its mnemonic functions.

NO is a putative intercellular messenger that has been proposed to be involved in synaptic plasticity, especially in the induction of LTP of excitatory synaptic transmission in the hippocampus and cortex. LTP was strongly inhibited by selective nNOS inhibitor 7-NI (30 mg/kg, ip)[8], and 7-NI could block LTP induced by 100 Hz tetanus stimulation as well[7]. In the present study, 7-NI prevented the induction of LTP induced by Rg_1, and *L*-Arg attenuated the inhibition, while the nonselective NOS inhibitor *L*-NAME did not inhibit LTP induced by Rg_1 in our previous study. Obviously, NO

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synthesized by nNOS was involved in improving synaptic transmission induced by ginsenoside Rg_1. iNOS, nNOS, and eNOS are the three major isoforms of nitric oxide synthase (NOS) expressed in the brain. NO generated by separate isoforms may have different roles and may exhibit even potentially opposing effects. nNOS and eNOS are present in the hippocampus, and mutant mice lacking nNOS and eNOS are observed to have significantly attenuated LTP in hippocampus[9,10]. Our result is in accordance with these reports.

Postsynaptic NO may activate NO-cGMP-PKG signaling pathway, and another potential target for NO is the ADP-ribosyltransferase (ADPRT). Extracellular application of ADPRT inhibitors block tetanus-induced potentiation[11]. Norman has postulated that panax ginseng has a nitric oxide link[12]. In the present study, NO, especially produced by neuronal nitric-oxide synthase contributed to the Rg_1-induced PP-DG LTP. The detailed mechanism by which Rg_1 acts is under progress.

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一氧化氮介导麻醉大鼠人参皂苷 Rg_1 诱发长时程增强[1]

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关键词 人参；皂苷类；长时程增强；突触传递；一氧化氮；吲唑类；海马；齿状回；精氨酸

目的：研究人参皂苷 Rg_1 对麻醉大鼠突触传递效能的影响及作用机制。**方法**：应用细胞外微电极记录技术，记录麻醉大鼠海马齿状回颗粒细胞群体峰电位(PS)。**结果**：Rg_1(10，100 nmol/L)提高麻醉大鼠的基础突触传递，诱导海马齿状回突触传递长时程增强(LTP)，并提高高频刺激所诱导的 LTP。选择性 NOS 抑制剂 7-硝基吲唑(7-NI，5 nmol)侧脑室注射可明显抑制 Rg_1 所诱导的齿状回 LTP，对基础突触传递无明显影响。*L*-Arg (250 mg/kg，ip)可拮抗 7-NI 对 Rg_1 诱导 LTP 的抑制作用($P < 0.05$)。**结论**：Rg_1 显著促进麻醉大鼠的突触传递效能，由神经元型 NOS (nNOS)所产生的 NO 参与了 Rg_1 对齿状回 LTP 的诱导过程。

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