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Ginsenoside Rb_1 Reduces Spontaneous Bursting Activity in Thalamocortical Slices of the Rat

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Abstract : Spontaneous bursting activity was studied in rat thalamocortical slices using extracellular field potential recording to test the potential utilization of ginsenoside Rb_1 in controlling overactivated neural systems. In order to induce bursting activity, slices were perfused with Mg^{2+}-free artificial cerebrospinal fluid (ACSF). Two major types of spontaneous bursting activity, simple thalamocortical burst complexes (sTBCs) and complex thalamocortical burst complexes (cTBCs), were recorded in Mg^{2+}-free ACSF. Ginsenoside Rb_1 selectively suppressed cTBCs. Duration and occurrence rate of cTBCs were reduced by 87.3±10.2% and 85.3±14.7% in the presence of 90 μM ginsenoside Rb_1 respectively, while amplitude and intraburst frequency were slightly changed by ginsenoside Rb_1. In contrast, ginsenoside Rb_1 was much less effective in reducing duration and occurrence rate of sTBCs. We also tested effects of ginsenoside Rb_1 on bursting activity in the presence of a $GABA_A$ receptor antagonist, bicuculline methiodide (BMI). Ginsenoside Rb_1 had no effect in suppressing BMI-induced bursting activities. These results suggest that ginsenoside Rb_1 may be useful in controlling seizure-like bursting activity under pathological conditions.

Key words : Ginseng, Ginsenoside Rb_1, Seizure, Thalamocortical Slice, Antiepileptic Activity.

INTRODUCTION

Ginseng root has long been used as an oriental folk medicine to alleviate a variety of diseases. A number of previous studies suggest that ginseng root influences various functions of the central nervous system (CNS) either by enhancing or suppressing neural activity. Among actions of ginseng root in the CNS are an improvement of cognitive ability[1,2], protection against glutamate-induced neural damage[3], and analgesic action.[4] Extensive studies on underlying mechanisms of ginseng effects have been carried out following isolation and purification of ginsenosides, the main effective component of ginseng. More than 30 different types of ginsenosides have been isolated, which have a steroid ring-like structure in common.[5]

It is now recognized that certain steroidal hormones such as progesterone and deoxycorticosterone augment $GABA_A$ receptor-mediated chloride conductance through barbiturate-like modulation of $GABA_A$ receptor-chloride channel complex.[6] While major targets for ginsenoside action are diverse, a possibility has been raised that ginsenosides elicit their inhibitory actions in the CNS through modulation of $GABA_A$ or $GABA_B$ receptor activation.[7] Anticonvulsant actions of ginseng saponin in chemical- or hyperthermiainduced seizure activity have been reported in an animal study.[8] No further study has been followed, however, probably due to the limited availability of a convenient experimental model for seizure activity. Recently, *in vitro* thalamocortical slice preserving thalamocortical connections has been shown to be valuable for the evaluation of the effects of various pharmacological drugs on generalized seizure.[9,10] In the present work, we aimed to test the potential suppressive action of ginsenoside Rb_1 which is one of the most abundant ginsenosides in ginseng saponin on hyperexcitability, using the *in vitro* thalamocortical slice preparation.

MATERIALS AND METHODS

Ginsenoside Rb_1 was provided by Korea Ginseng and Tobacco Institute and bicuculline methiodide (BMI) was purchased from Research Biochemicals Inc. (Natick, MA). After rats (Sprague Dawly; 150-180 g) were decap-

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itated, the brain was rapidly removed and rinsed with cold normal artificial cerebrospinal fluid (ACSF, in mM); NaCl 125.3, KCl3, KH_2PO_4 1.4, $CaCl_2{\cdot}2H_2O$ 1.8, $MgSO_4$ 1.3, $NaHCO_3$ 23 and glucose 10 pregassed for 1 h with 95% O_2/5% CO_2. The thalamocortex was cut on a vibratome according to the angles described by Agmon and Connors.[11] After allowing at least 1 h for recovery, the slices were transferred to a submersion-type chamber and perfused with Mg^{2+}-free ACSF. Field excitatory postsynaptic potentials (fEPSPs) were recorded mostly in layer III-IV of sensory cortical region, using low resistance glass-electrode filled with normal ACSF (1-2MΩ).

Ginsenoside Rb_1 was dissolved in 100% dimethyl sulfoxide and diluted in ACSF to a final dimethyl sulfoxide concentration of 0.05%. Ginsenoside Rb_1 solution was freshly prepared daily and applied into bath medium by addition to perfusing ACSF. A 10-20 min control period of stable activity was recorded, followed by a 10-20 min period of ginsenoside Rb_1 treatment. All data were displayed on a chart recorder and stored on videotapes. Statistical significance was assessed by two-tailed Student's *t* test.

RESULTS

Spontaneous bursting activity began to appear in both cortical and thalamic areas within 10~20 min after perfusion of Mg^{2+}-free ACSF, and remained until Mg^{2+}-free ACSF was replaced with normal ACSF. Though bursting activity varied in duration, interval, and amplitude, two major types could be classified (Fig. 1). The first type had monotonic bursting activity, which oscillated at 3-15 Hz following the triggering paroxysmal depolarizing shift (PDS). The pattern lasted for 1-10 s in each event. The second type consisted of tonic bursting activity and a later phase of periodic clonic activity. The former was named as simple thalamocortical burst complexes (sTBCs), and the latter as complex thalamocortical burst complexes (cTBCs).[12] cTBCs were generally longer in duration (30-60 s), larger in amplitude, and repeated at more delayed intervals than sTBSs.

To test effect of ginsenoside Rb_1 on spontaneous bursting activity, 90 μM of ginsenoside Rb_1 was applied for 10 min to the bath media after 30 min of perfusion with Mg^{2+}-free ACSF. The suppressive action of ginsenoside

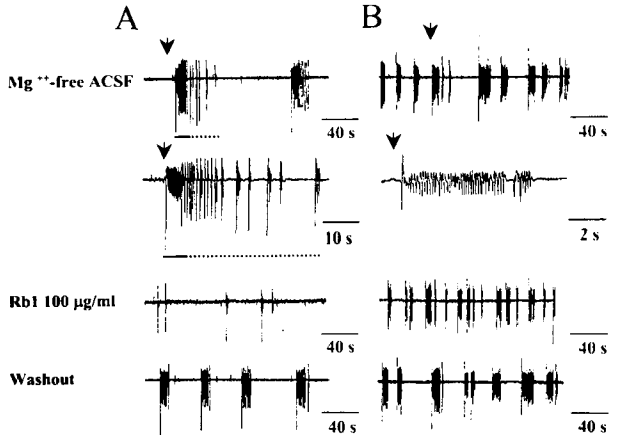

Fig. 1. Suppression of complex thalamocortical burst activity in Mg^{2+}-free ACSF by ginsenoside Rb_1. A. cTBCs consisted of tonic (solid line) and clonic (dotted line) phase activity. Frequency of tonic activity was 5-8 Hz. After treatment of ginsenoside Rb_1 (90 μM), both tonic and clonic activities disappeared almost entirely. Recovery of tonic phase was shown after 10 min of washout. B. sTBCs were resistant to ginsenoside Rb_1. sTBCs were monotonic bursting activities, which had a shorter duration than cTBCs. The duration of sTBCs was only slightly reduced by ginsenoside Rb_1 and fully recovered after 10 min of washout.

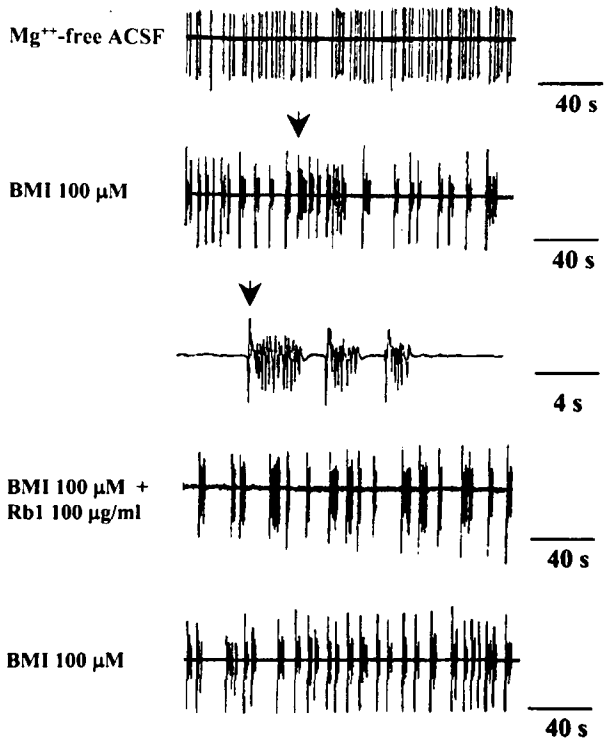

Fig. 2. Effect of 90 μM ginsenoside Rb_1 on BMI-induced tonic bursting activity. Isolated population spikes were most frequently seen in the thalamocortical slice perfused with Mg^{2+}-free ASCF, which could be further transformed into monotonic bursting activity. Bursting activity indicated by arrow oscillated at about 3 Hz. Ginsenoside Rb_1 did not modify this transformed activity.

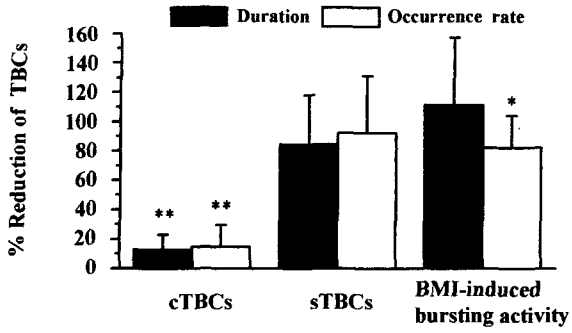

Fig. 3. The histogram showing the selective effect of ginsenoside Rb_1 on various spontaneous bursting activities. Most prominent effect was shown in cTBCs (87.3±10.2% and 85.3±14.7% reduction in duration and frequency (n=9, $^{**}P<0.01$ compared with control period). No significant reduction in sTBCs was shown. In BMI-induced bursting activity, occurrence rate was slightly decreased by 21.7±9.2% (n=7, $^{*}P<0.05$ compared with control period).

Rb_1 was prominent in the cTBCs (Fig. 1A). Duration and occurrence rate of cTBCs were more sensitive to the treatment of ginsenoside Rb_1, being reduced by 87.3±10.2% and 85.3±14.7% on average (9 slices), respectively, whereas peak amplitude and bursting frequency within each cTBCs were not affected by treatment of ginsenoside Rb_1 (Fig. 3). Interestingly, ginsenoside Rb_1 was much less effective in suppressing sTBCs, with little or no effect on its duration and occurrence rate as well as amplitude (7 slices, Fig. 1B).

It has been documented that when neural inhibition is eliminated by a high dose of $GABA_A$ antagonists such as BMI, picrotoxin, and penicillin, neurons generate large, highly synchronized bursts of action potentials.[13] Addition of BMI to Mg^{2+}-free ACSF transformed rapid repetitive interictal-like activity into less frequent, monotonic bursting activity in all slices we tested. The BMI-induced bursting activity resembled sTBCs, with shorter duration than that recorded in Mg^{2+}-free ACSF alone. We also tested effects of ginsenoside Rb_1 on bursting activity in the presence of a $GABA_A$ receptor antagonist, BMI (100 μM). Ginsenoside Rb_1 was ineffective in suppressing BMI-induced bursting activity, further demonstrating its selective effect on cTBCs (Fig. 2). The averaged degree of inhibitory action of ginsenoside Rb_1 on various bursting activity was plotted in Fig. 3.

DISCUSSION

In the thalamocortical slice perfused with Mg^{2+}-free ACSF, stable spontaneous bursting activity was developed with distinct characteristics. Coulter and Zhang (1993) reported two major types of spontaneous bursting activity in the thalamocortical slice: simple thalamocortical burst complexes (sTBCs) and complex thalamocortical burst complexes (cTBCs), which were in good agreement with our results. They also proposed that sTBCs and cTBCs correspond to generalized absence (GA) seizure activity and generalized tonic-clonic (GTC) seizure activity, respectively.[9] Neurophysiological studies have demonstrated that nuclueus reticularis thalami (NRT) and thalamocortical network are the important underlying neural substrates for generation of GA.[14,15] In particular, NRT mediated GABAergic feedback onto the thalamic neurons is a critical factor in synchronization of thalamocortical rhythms by activating a low threshold T-type Ca^{2+} current in thalamic neuron.[16] The activation of Ca^{2+} current subsequently triggers a large bursting activity in thalamic nuclei, which activates cortex, and initiates and amplifies thalamocortical oscillatory activity. The thalamus and corticothalamocortical circuitry are also involved in GTC seizure. It is, however, that cTBCs-like discharge can be shown in neocortical slices in which thalamic connections are severed. It is, therefore, suggested that cTBCs, unlike sTBCs, are primarily originated in cortex.

The present study shows that ginsenoside Rb_1 suppresses the spontaneous activity generated by Mg^{2+}-free ACSF in the thalamocortical slices in a selective manner, being more effective in controlling cTBCs. Although a possible involvement of GABAergic system in the suppressive action of ginsenoside Rb_1 has been suggested[17], we could not confirm this possibility because BMI transformed both types of spontaneous activity into monotonic discharges, which were not affected by ginsenoside Rb_1. In general, actions of current antiepileptic drugs (AEDs) are thought to be mainly accomplished through modulation of voltage-dependent ion channels and GABAergic inhibitory neurotransmission. Two well-established examples of AEDs that are related to voltage-dependent channels are phenytoin and ethosuximide acting on voltage-dependent Na^+ channel and Ca^{2+} channel, respectively. AEDs that potentiate GABA-mediated inhibition are benzodiazepines, by enhancing the influx of chloride ion by GABA, and vigabatrin by irreversible inhibition of GABA-transaminase which converts GABA to succinic semialdehyde and glutamate. While felbamate is known to reduce NMDA-induced current, effort to design drugs to specifically target glutamate receptors has not been sat-

isfactory. According to our recent study[18], the inhibitory action of ginsenosides on synaptic transmission is not mediated through benzodiazepine-like enhancement of GABA mediated chloride current. Therefore, alternative explanation related to GABAergic transmission can be the ability of ginsenoside to increase glutamate decarboxylase (GAD), which is a GABA synthesizing enzyme, as demonstrated by Choi et al..[19] However, even if this is the case, the differential effect of ginsenosides remains to be further studied.

Given that the differential effect of antiepileptic drugs on the thalamocortical bursting activity has been reported in the case of phenytoin and carbamazepine, which suppress cTBCs more prominently, a potential action on voltage-dependent channel should be also considered. While phenytoin and carbamazepine showed moderate degrees of inhibitory effect on sTBCs, ginsenoside Rb_1 was only slightly effective, indicating that ginsenoside Rb_1 is more selective on cTBCs and possibly shares common mechamism(s) with phenytoin and carbamazepine. In clinical use, phenytoin and carbamazepine are major drugs in GTC seizures, while ethosuximide is effective in control of GA. And phenytoin and carbamazepine are known to reduce the frequency of sustained firing of action potentials by prolonging the inactivation of voltage-dependent Na^+ channel.[20] Meanwhile, ethosuximide exerts its antiepileptic actions by reduction of low-threshold T-type Ca^{2+} current.[21]

While further studies need to be carried out to elucidate the underlying mechanism(s), this study is the first to demonstrate the potential application of ginsenoside as an antiepileptic drug in *in vitro* brain slice preparation.

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