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Voltage-dependent inhibition of brain Na^+ channels by American ginseng

Dong Liu [a,1], Bei Li [a], Yi Liu [b], Anoja S. Attele [c], John W. Kyle [a], Chun-Su Yuan [c,d,*]

[a] *Department of Neurobiology, Pharmacology and Physiology, The Pritzker School of Medicine, University of Chicago, Chicago, IL, USA*

[b] *Division of Biology, ICAgen, Inc., Durham, NC, USA*

[c] *Tang Center for Herbal Medicine Research, and Department of Anesthesia and Critical Care, The Pritzker School of Medicine, University of Chicago, Chicago, IL, USA*

[d] *Committee on Clinical Pharmacology, The Pritzker School of Medicine, University of Chicago, Chicago, IL, USA*

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Abstract

American ginseng (*Panax quinquefolius*) is a major species of ginseng that has many pharmacological effects. Studies have demonstrated that constituents of ginseng have neuroprotective effects during ischemia. Neuronal damage during ischemic episodes has been associated with abnormal Na^+ fluxes. Drugs that block voltage-dependent Na^+ channels provide cytoprotection during cerebral ischemia. We thus hypothesized that American ginseng may block Na^+ channels. In this study, effects of an American ginseng aqueous extract was evaluated in tsA201 cells transfected with cDNA expressing α subunits of the $Brain_{2a}$ Na^+ channel using the whole-cell patch clamp technique. We found that American ginseng extract tonically and reversibly blocked the channel in a concentration- and voltage-dependent manner. It shifted the voltage-dependence of inactivation by 14 mV (3 mg/ml) in the hyperpolarizing direction and delayed recovery from inactivation, whereas activation of the channel was unaffected. Ginsenoside Rb_1, a major constituent of the American ginseng extract, produced similar effects. The data were compared with the actions of lidocaine, a Na^+ channel blocker. Our results suggest that Na^+ channel block by American ginseng extract and Rb_1 was primarily due to interaction with the inactive state of the channel. Inhibition of the Na^+ channel activity by American ginseng extract may contribute to its neuroprotective effect during ischemia. © 2001 Published by Elsevier Science B.V.

Keywords: American ginseng; Ginsenoside Rb_1; Lidocaine; tsA201 cell; $Brain_{2a}$ α subunits; Patch clamp; Na^+ current

1. Introduction

Ginseng (*Panax ginseng*, C.A. Meyer) is a plant root with many active components, and there is evidence that ginseng possesses a number of pharmacological effects (Lee, 1992; Gillis, 1997). American ginseng (*Panax quinquefolius)* is a major species of ginseng that has been commonly used for its therapeutic effects (Huang, 1999). Many pharmacological actions of ginseng are attributed to its ginsenosides, a diverse group of steroidal saponins (for review, see Attele et al., 1999).

Past investigations suggest that ginsenosides have neuroprotective activity. Data from in vitro studies show that ginsenoside Rb_1 can rescue hippocampal neurons from lethal ischemic damage (Lim et al., 1997), and delay neuronal death from transient forebrain ischemia (Wen et al., 1996). A growing body of data suggests that agents which possess anti-ischemic, anti-convulsant, and other neuroprotective effects may act by inhibiting voltage-gated Na^+ channels (Erdo et al., 1996; Taylor and Meldrum, 1995). Thus, it was reasonable to expect that American ginseng would also inhibit brain voltage-gated Na^+ channels.

Voltage gated Na^+ channels are pore-forming membrane proteins that have long been known to underlie axonal and somatic action potentials. They are also involved in actively propagating information within the dendritic tree of pyramidal neurones (Stuart and Sakmann,

* Corresponding author. Department of Anesthesia and Critical Care, The University of Chicago Medical Center, 5841 S. Maryland Avenue, MC 4028, Chicago, IL 60637, USA. Tel.: +1-773-702-1916; fax: +1-773-834-0601.

E-mail address: cyuan@midway.uchicago.edu (C.-S. Yuan).

[1] Present address: Division of Biology, ICAgen, Inc., Durham, NC, USA.

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1994). Native Na^+ channels exist as polypeptide multimers of an α subunit and subsidiary β_1 and β_2 subunits. The α subunit is the primary pore-forming subunit of the channel (Goldin, 1994). A single α subunit is thought to comprise an independent voltage-gated channel, and most Na^+ channel function can be accounted for by this subunit (Makielski et al., 1999). Heterologous expression of Na^+ channels enables study of drug binding properties without potentially confounding differences in the environment. tsA201 cells offer particular advantages because they have few endogenous ion currents, and they can readily be transiently transfected with cDNA for Na^+ channels. (Sheets et al., 1996). Investigations on cloned voltage-dependent Na^+ channel α subunits have led to the elucidation of structures responsible for various channel functions such as permeation and gating (Goldin, 1994; Sheets et al., 1996), thus rendering them suitable for addressing the hypothesis.

This study sought to determine the effects of American ginseng and ginsenoside Rb_1 on the kinetics of brain voltage-gated Na^+ channels. Effects of an aqueous extract of American ginseng and Rb_1 were evaluated in tsA201 cells transfected with $Brain_{2a}$ α subunits. The effects of American ginseng on the electrophysiological properties of Na^+ channels were examined and its effects were compared with those of lidocaine, a voltage-dependent Na^+ channel blocker. We characterized the effects of American ginseng extract and Rb_1 on current–voltage relationship, voltage-dependence of activation, and inactivation. In addition, the effects of American ginseng extract on recovery from inactivation, and tonic block of Na^+ channels were evaluated.

2. Materials and methods

2.1. Preparation of plant root extract and analysis

American ginseng was obtained from the Roland Ginseng, LLC (Wausau, WI). The plant roots were soaked in cold water for 2 h, and then cut into small pieces (less than 2 mm in diameter). These pieces were mixed with hot water (approximately 95°C) for 1 h, and then filtered. The filtrates of hot water soluble fraction were evaporated and lyophilized. The dried powders were suspended in bath solution at concentrations described in the text. The suspension was centrifuged for 10 min and the supernatant was used for the experiments (Yuan et al., 1998a).

The constituents of the water extracts were analyzed by high performance liquid chromatography (HPLC). A sample of 0.17 g dried powder was dissolved in 10 ml 90% methanol. Total ginsenosides detected was 10.66%. The results of the analysis were as follows: ginsenoside Rb_1, 8.4 mg; Rb_2, 0.24 mg; Rc, 1.91 mg; Rd, 0.99 mg; Re, 6.07 mg; Rg_1, 0.51 mg. Ginsenoside Rf was below the detection limit. 1 mg/ml American ginseng extract, a concentration used in this study, contained ginsenoside Rb_1 44.5 μM, Rb_2 1.3 μM, Rc 10.4 μM, Rd 6.1 μM, Re 37.7 μM and Rg_1 3.7 μM. These ginsenosides belong to a family of steroids named steroidal saponins (for review, see Attele et al., 1999). Fig. 1 shows the chemical structure of ginsenoside Rb_1.

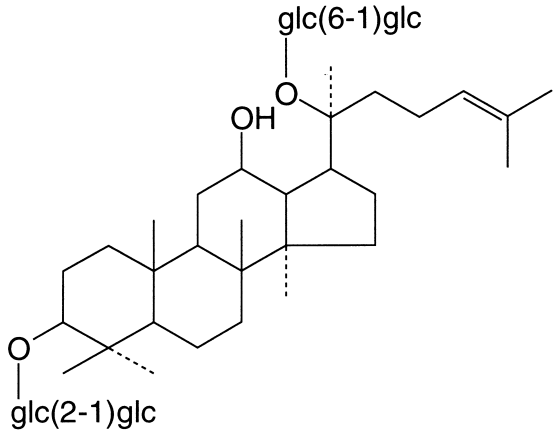

Fig. 1. Structure of ginsenoside Rb_1.

2.2. Transfection of tsA201 cells

tsA201 cells (kindly provided by Dr. J.W. Kyle, University of Chicago) were maintained in culture in Dulbecco's modified Eagle's medium (DMEM) with high glucose and L-glutamine (GIBCO, Gaithersburg, MD) at 37°C with 5% CO_2 in 60 mm culture dishes until the cells were 50–80% confluent. Detailed descriptions of the method employed can be found in the literature (Mishell and Mishell, 1980; Jayme and Blackman, 1985; Sheets et al., 1996). Then cells were washed with OPTI-MEM 1 reduced serum (GIBCO BRL), and transfected with 5 μg of plasmid PzemRVsp6-$Brain_{2a}$ using Lipofectamine (GIBCO BRL). Cells were incubated at 37°C with 5% CO_2 for 18 h, and tested for the recording of Na^+ current.

2.3. Electrophysiological solutions

Pipette solution contained (in mM) 120 CsF, 20 CsCl, 5 EGTA and 10 HEPES, with pH adjusted to 7.3 with CsOH. Bath solution contained (in mM) 140 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, with pH adjusted to 7.4 with NaOH (Benzinger et al., 1999).

2.4. Recording techniques

Recording protocols were generated using Clampex 6.0.4 (Axon Instruments, Foster City, CA) running on a Pentium-based microcomputer and were imposed through a 12-bit DAC controlling Axopatch-ID amplifier. Recording of the Na^+ currents was made in the whole-cell patch clamp configuration at 22°C with typical pipette resis-

tances of 0.8–1.5 MΩ;. Currents were 4-pole Bessel filtered at 5 kHz and digitized at 12-bit resolution at 100 kHz. Because the binding of lidocaine is state-dependent, with significantly higher affinity for inactivated states than for the resting states, we held the cells at a sufficient negative holding potential (−150 mV) to maintain complete channel availability during the experiments. Our experiments, therefore, probed the interactions of American ginseng extract and lidocaine primarily with the closed conformation of the channels. Specific protocols are given as insets in the figures or in the figure legends.

2.5. Data analysis

Analysis and fitting were performed with the programs 9GRB and DAH running under Matlab 5.0 (The Mathworks, Natick, MA). The effect of leak current and uncompensated capacitance transients was accessed at the end of the experiments by blocking the Na^+ current with a high concentration of tetrodotoxin (150 nM). The resulting peak current was <5–10% of that in the absence of tetrodotoxin and was not corrected in the results. Where appropriate, Student's *t*-tests were used with $P < 0.05$ considered statistically significant.

3. Results

3.1. American ginseng extract partially blocked I_{Na}

Sodium channel current (I_{Na}) was recorded by whole-cell patch clamp from tsA201 cells transiently transfected with cDNA expressing $Brain_{2a}$ α subunits. I_{Na} was recorded by depolarization to potentials between −90 and 40 mV from a holding potential of −150 mV. Shown in Fig. 2 are representative families of I_{Na} responses in control (Fig. 2A) and in 3 mg/ml American ginseng (Fig. 2B). As summarized in Fig. 2C, American ginseng extract caused a dose-dependent reduction in peak I_{Na} at more depolarized voltages (from −40 to 40 mV). During exposure to American ginseng extract (1 mg/ml and 3 mg/ml), maximal peak I_{Na} was reduced by 7% and 14%, respectively. Lidocaine (1 mM) caused a 31% reduction in maximal peak I_{Na}. At both concentrations of American ginseng, peak current–voltage relationship was shifted by 5 mV in the depolarizing direction. Lidocaine (1 mM) caused a 10-mV depolarizing shift in the peak I_{Na}. American ginseng had no significant effect on the kinetics of current decay.

3.2. American ginseng extract produced a tonic block

Lidocaine produces a tonic block of the Na^+ channels at low stimulation frequencies and use-dependent block at high frequencies. In our model, the investigation of use-de-

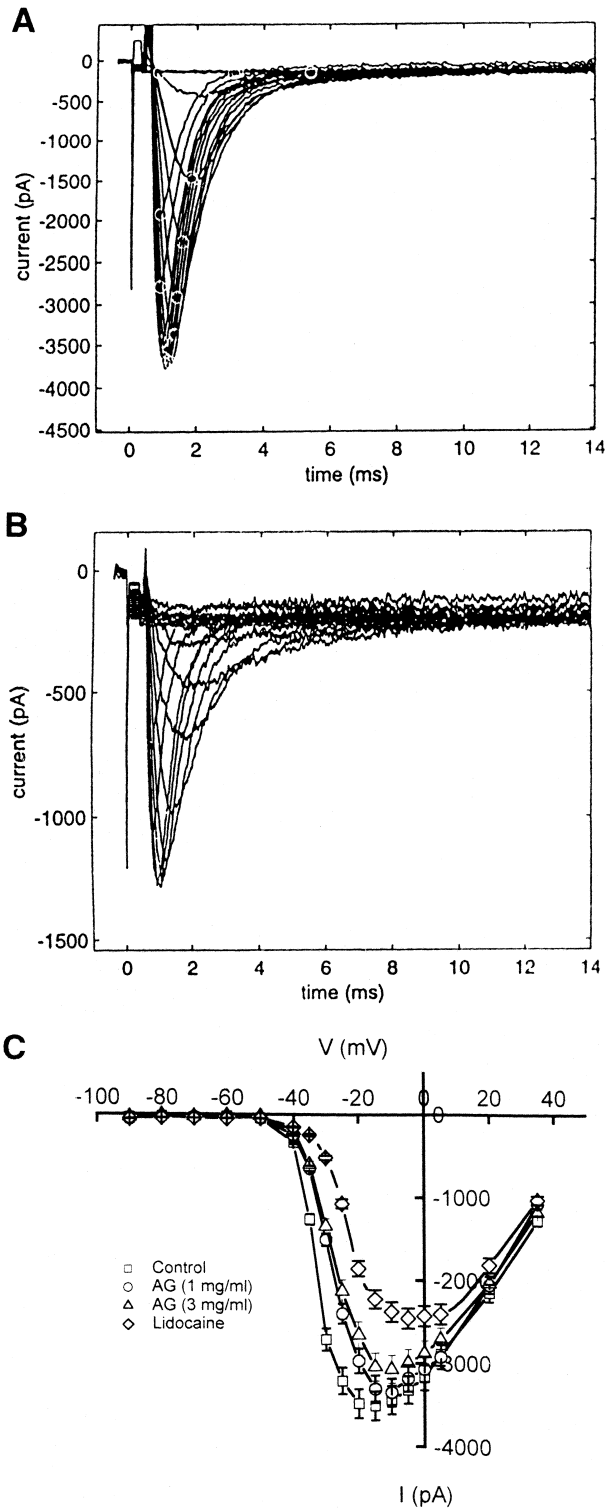

Fig. 2. A family of typical I_{Na} responses recorded from tsA201 cells transfected with cDNA expressing human $Brain_{2a}$ sodium channel α subunit. (A) Control; (B) 3 mg/ml American ginseng extract; (C) average peak current–voltage relationships for control ($n = 10$), American ginseng extract or AG (1 mg/ml, $n = 8$ and 3 mg/ml, $n = 6$), and lidocaine (1 mM, $n = 10$). Currents were recorded during a series of depolarizations to 0 mV for 40 ms from a holding potential of −150 mV. For (A), (B) and (C), the interpulse interval was 1 s. Data are shown as the mean ± S.E.

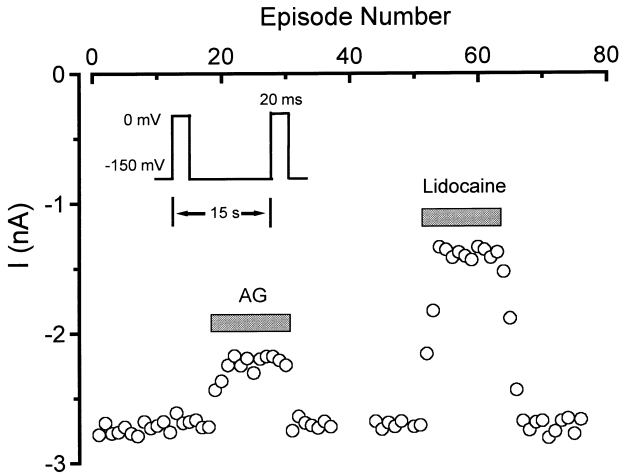

Fig. 3. Tonic block of I_{Na} by American ginseng extract and lidocaine. Open circles represent peak current amplitudes elicited by 20 ms depolarizations to 0 mV from a holding potential of −150 mV (one pulse for every 15 s). American ginseng extract or AG (3 mg/ml) and lidocaine (1 mM) were applied during the periods indicated by the solid bars.

pendent block by subjecting cells to trains of pulses resulted in large fluctuations in amplitude of successive traces. This hindered our studies of use-dependent block, which is measured as an increase of Na^+ current inhibition during successive membrane depolarization at high frequency. Thus, we only measured tonic block. The sensitivity of the resting state to blockage by American ginseng extract and lidocaine was measured by reduction in the peak current amplitude elicited by a brief (20 ms) depolarization to 0 mV from a holding potential of −150 mV (see protocol in Fig. 3). Tonic block by 3 mg/ml American

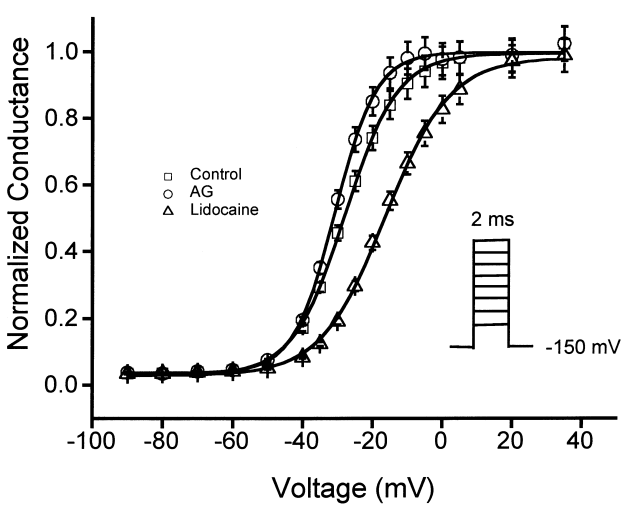

Fig. 4. Steady-state activation of I_{Na}. Currents were elicited by 2 ms depolarizing pulses from a holding potential of −150 mV. Interpulse interval was 1 s. Normalized peak conductances (g/g_{max}) (mean ± S.E.) were fit with a Boltzmann function given by $g/g_{max} = 1 - 1/[1 + \exp(V_t - V_{1/2})/dx]$. For control, American ginseng extract or AG (3 mg/ml) and lidocaine (1 mM), $V_{1/2}$ and dx values were −27.8 ± 1.3 mV and −7.5 ± 0.2 mV ($n = 10$), −30 ± 1.2 mV and −5.9 ± 0.2 mV ($n = 8$) and −16.3 ± 0.3 mV and 9.2 ± 0.3 mV ($n = 8$), respectively.

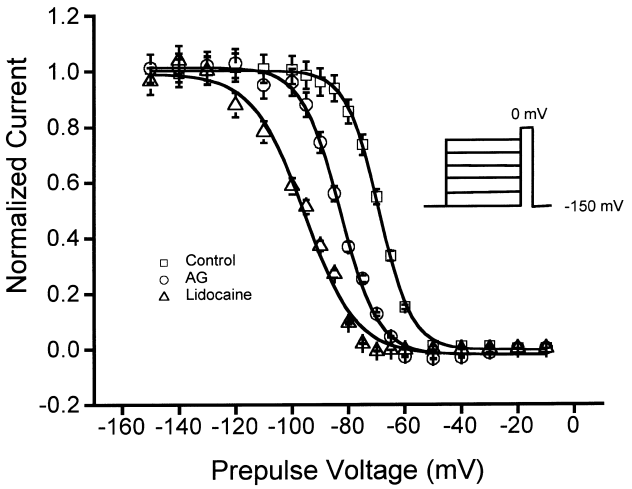

Fig. 5. Steady-state inactivation (SSI) of I_{Na}. A test pulse to 0 mV (2 ms) was given immediately following a 1-s conditioning pulse (−150 to −10 mV in 10 mV increments) from a holding potential of −150 mV. Interpulse interval was 5 s. Normalized currents of steady-state inactivation (I/I_{max}) (mean ± S.E.) are fit with a Boltzmann function given by $I/I_{max} = 1/[1 + \exp(V_t - V_{1/2})/dx]$. For control ($n = 10$), American ginseng extract or AG (3 mg/ml, $n = 8$) and lidocaine (1 mM, $n = 10$), $V_{1/2}$ and dx values were −69.2 ± 0.2 mV and 5.8 ± 0.2 mV, −82.9 ± 0.4 mV and 6.7 ± 0.4 mV, and −95.5 ± 0.9 mV and 9.1 ± 0.9 mV, respectively.

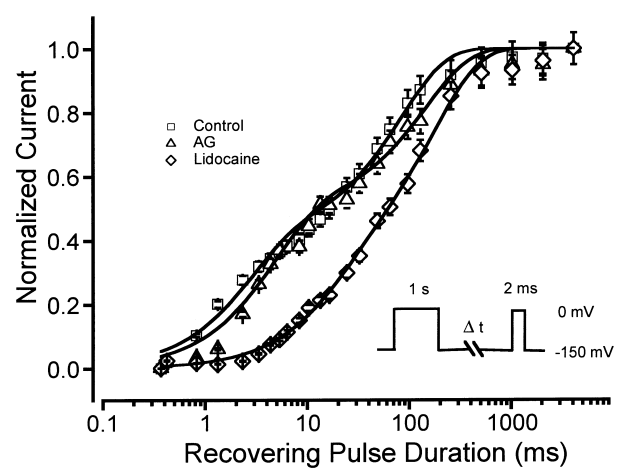

Fig. 6. Time courses of recovery from inactivation studied using a double-pulse protocol: I_{Na} was first completely inactivated by a depolarization pulse to 0 mV for 1 s, followed by a variable-length ($\Delta t = 0.37$ to 4100 ms) hyperpolarizing pulse to −150 mV. Subsequently, recovered peak I_{Na} was assessed by a depolarization to 0 mV for 2 ms. Each set of such pulses was separated by 10-s intervals at −150 mV. Peak I_{Na} elicited by each 2 ms pulse at 0 mV was normalized for each cell to the maximum control current immediately before beginning such a protocol. Data (mean ± S.E., displayed on a semi-log scale) were fit by a double-exponential function. The fast and slow time constants (relative amplitudes) are: 2.8 ± 0.6 ms (42%) and 84.0 ± 9.1 ms (58%) for control ($n = 10$); 4.7 ± 0.9 ms (50%) and 145.1 ± 25.5 ms (50%) for 3 mg/ml American ginseng or AG ($n = 8$); 19.2 ± 4.4 ms (32%) and 180.6 ± 21.4 ms (68%) for 1 mM lidocaine ($n = 10$), respectively.

Table 1
Comparison of American ginseng aqueous extract and ginsenoside Rb_1 on sodium channels in tsA201 cells transfected with human $Brain_{2a}$ sodium channel α subunit

	Control	American ginseng extract (3 mg/ml)	Control	Ginsenoside Rb_1 (150 μM)
Peak current (pA)	−3518 ± 265	−3022 ± 313	−3467 ± 248	−3129 ± 421
Activation				
$V_{1/2}$ (mV)	−27.8 ± 1.3	−30.7 ± 1.2	−29.5 ± 1.4	−30.2 ± 1.2
dx (mV)	7.5 ± 0.2	5.9 ± 0.2	6.8 ± 0.4	5.8 ± 0.3
Inactivation				
$V_{1/2}$ (mV)	−69.2 ± 0.2	−82.9 ± 0.4	−68.7 ± 0.3	−81.5 ± 0.5
dx (mV)	5.8 ± 0.2	6.7 ± 0.4	6.3 ± 0.3	6.8 ± 0.2
n	8	8	5	5

ginseng extract was 25%. Under the same conditions lidocaine (1 mM) produced a 50% block.

3.3. American ginseng extract had no effect on the voltage-dependence of Na^+ channel activation and caused a hyperpolarizing-shift in the voltage-dependence of inactivation

Activation of Na^+ channels was evaluated by a conductance transform of the peak current–voltage relationship. To determine whether American ginseng extract attenuated Na^+ channel conductance in a voltage-dependent manner, the effects of 3 mg/ml American ginseng extract were compared with the control and 1 mM lidocaine (Fig. 4). The curves represent the best fit to the data using a Boltzmann function given by $g/g_{max} = 1 - 1/[1 + \exp(V_t - V_{1/2})/dx]$. Unlike lidocaine (1 mM), there were no significant differences in either the half-maximal activation voltage ($V_{1/2}$), or the slope factor (dx) between control and 3 mg/ml American ginseng extract. Therefore, American ginseng did not alter the voltage-dependence of activation of brain Na^+ channels.

Since many nerve blockers, such as lidocaine, preferentially block Na^+ channels in the inactivated state, we investigated the effects of American ginseng on the voltage dependence of inactivation. The effects of American ginseng extract (3 mg/ml) on the voltage-dependence of inactivation were examined and compared with 1 mM lidocaine using a voltage protocol shown in Fig. 5. The normalized peak currents were plotted against the conditioning prepulse voltage and the data were fitted to a Boltzmann function, given by $I/I_{max} = 1/[1 + \exp(V_t - V_{1/2})/dx]$ to yield a mid point ($V_{1/2}$) and a slope factor (dx). Both American ginseng and lidocaine significantly altered the voltage-dependence of inactivation. American ginseng extract (3 mg/ml) shifted the $V_{1/2}$ of inactivation by approximately 14 mV in the hyperpolarizing direction ($n = 8$, $P < 0.01$). Lidocaine (1 mM) shifted the curve to the left by approximately 26 mV ($n = 10$, $P < 0.01$). The results show that American ginseng, like lidocaine, reduces channel availability by preferentially binding to the inactivated state.

3.4. American ginseng extract slowed recovery from inactivation

We tested whether the differential binding of American ginseng to the inactivated state affected the kinetics of recovery from inactivation. This was measured by a double-pulse protocol illustrated in Fig. 6. The results of recovery from inactivation for American ginseng extract (3 mg/ml) and lidocaine (1 mM) are also shown in the figure. In the control, recovery from inactivation was a double-exponential process, with a fast (τ_1) and a slow (τ_2) time constant. In the presence of American ginseng extract (3 mg/ml, $n = 7$) or lidocaine (1 mM, $n = 10$), both time constants were significantly increased ($P < 0.01$). These results showed that American ginseng also affected the inactivation kinetics.

3.5. Ginsenoside Rb_1 partially blocked I_{Na} and influenced the voltage-dependence of inactivation

Previous studies have shown that ginsenoside Rb_1 has neuroprotective effects during ischemia (Wen et al., 1996; Lim et al., 1997). In our model, Rb_1 caused a significant reduction in peak I_{Na} ($P < 0.05$). As shown in Table 1, during exposure to ginsenoside Rb_1 (150 μM), maximal peak I_{Na} was reduced by 10%, compared to 3 mg/ml American ginseng extract which caused a 14% reduction in maximal peak I_{Na}. Effects of Rb_1 on activation and inactivation were evaluated by the same protocols that were used for testing American ginseng. Rb_1 had no significant effect on activation. However, it shifted the inactivation curve by 13 mV to more hyperpolarized potentials. These results show that Rb_1, like the American ginseng extract, preferentially binds to the inactivated state of the channel.

4. Discussion

In this study, the effects of American ginseng and ginsenoside Rb_1 on brain Na^+ channels were investigated. The pharmacological actions of an aqueous extract of American ginseng on tsA201 cells transfected with $Brain_{2a}$

α subunits were characterized and compared to those of lidocaine. Lidocaine was selected as a positive control for Na^+ channel block because it is clinically used to produce nerve block, and its kinetics on Na^+ channels have been well documented (Bean et al., 1983; Bennet et al., 1995). The HPLC analysis revealed that the major ginsenosides in the extracts were Rb_1, Rc and Re. Our results show that an American ginseng hot water extract as well as ginsenoside Rb_1 caused a voltage-dependent reversible attenuation of the transient current in brain Na^+ channels, and shifted the inactivation curve to more hyperpolarized potentials. This spectrum of activity is similar to that seen with the commonly used Na^+ channel blocker lidocaine.

Voltage-gated Na^+ channels assume a variety of conformational states depending on the transmembrane potential (Hodgkin and Huxley, 1952). At hyperpolarized membrane potentials, they reside in rested, closed conformation. When the membrane potential is depolarized, the channels open briefly and then inactivate. Sodium channels cannot readily reopen from inactivated states, and they require recovery or "repriming" periods at hyperpolarized membrane potentials to regain availability. Local anesthetics such as lidocaine act by partially blocking voltage-dependent Na^+ channels. Our results show that during voltage-dependent attenuation of Na^+ currents by American ginseng extract, the percentage of maximal peak current reduction was approximately a third of that produced by lidocaine.

American ginseng extract also produced a concentration-dependent tonic block of Na^+ currents. Tonic block may result from American ginseng interaction with the resting state of the channel, as has been shown for other Na^+ channel blocking drugs (Hondeghem and Katzung, 1984). The resting state component of tonic block by 3 mg/ml American ginseng extract was significantly less than the block observed for the same concentration when the channel was depolarized with an episodic interval of 15 s. These results show that American ginseng partially blocks the Na^+ channel primarily by interaction with either the open or inactive state of the channel. Previous studies show that lidocaine also displays different binding affinities with each state of the Na^+ channel: closed, open, and inactivated (Strichartz et al., 1987). Hille (1977) proposed a Modulated Receptor Hypothesis for the state-dependent interaction of local anesthetics with the neuronal Na^+ channel. According to this model, the affinity of the drug is greatest for the inactivated state of the channel. The data on American ginseng effect on Na^+ channel inactivation helped to distinguish whether it showed a greater affinity for the open or inactive state of the channel.

Sodium channel blockers such as lidocaine cause a shift in the voltage-dependence of inactivation (Bean et al., 1983; Bennet et al., 1995). This shift has been explained by the Modulated Receptor Hypothesis as being due to a higher affinity of the drug for the inactive state of the channel (Hille, 1977). We observed that American ginseng also shifted the voltage-dependence of inactivation for Na^+ channels in the hyperpolarizing direction. The hypothesis predicts that recovery of the drug-bound channel from inactivation is slowed and biphasic. Recovery of bound channels is reflected by a slow component, and recovery of unbound channels is reflected by a fast component (Hille, 1977; Hondeghem and Katzung, 1984). Our results confirm this prediction, and show that Na^+ channel recovery in the presence of American ginseng was best fit with double exponential equations, as we and others (Pugsley and Saint, 1995) have observed for lidocaine. Moreover, American ginseng significantly delayed recovery compared to control conditions, similar to the effects of lidocaine. These findings indicate that American ginseng interacts with the inactive state of the brain Na^+ channels. However, whether it preferentially blocks the inactive state cannot be elucidated from our results. In addition, although American ginseng may bind to the open state of the channel, it did not affect the voltage-dependence of activation.

Previous studies have shown that ginseng root and its major active component, ginsenosides, have a neuroprotective function in various pathological conditions. Wen et al. (1996) demonstrated that ginsenoside Rb_1 rescued a significant number of ischemic CA1 pyramidal neurons. It has been suggested that the neuroprotective activity of ginsenoside Rb_1 lies in its ability to scavenge oxygen free radicals (Lim et al., 1997) which are overproduced during brain ischemia. In addition to neuronal damage by oxygen free radicals, aberrant ion fluxes, including abnormal movements of Na^+ and Ca^{2+}, play a role in damaging neurons during hypoxia/ischemia (Choi, 1990; Young, 1987). In both global and focal ischemia, there is a rapid loss of synaptic activity followed by a large negative shift in extracellular DC voltage (Hansen, 1985; Gill et al., 1992), and a loss of ion homeostasis (Hansen and Zeuthen, 1981). There is evidence that voltage-dependent Na^+ channel blockers are neuroprotective in models of global and focal ischemia. In this regard, Stys et al. (1992) demonstrated that procaine and lidocaine provide significant protection from anoxic injury in the optic nerve. Others have shown that Na^+ channel blockers prevent hypoxic damage to mammalian white matter in vitro (Fern et al., 1993). In addition, lidocaine and phenytoin delayed or prevented negative DC shifts from rat hippocampal slices during in vitro ischemia (Weber and Taylor, 1994). It is possible that the attenuation of voltage-gated Na^+ channels by American ginseng that we observed may contribute to its neuroprotective effects. Ginseng and ginsenosides have actions in addition to Na^+ channel blockade, and it might be argued that neuroprotection is caused by other mechanisms. However, the findings that tetrodotoxin is significantly neuroprotective in several models (Tasker et al., 1992; Weber and Taylor, 1994) suggests that Na^+ channel blockade alone may account for neuroprotection in some instances.

We previously reported that American ginseng extract inhibited the activity of brainstem neurons (Yuan et al., 1998a). While an inhibitory effect on brainstem neurons may be brought about by both $GABA_A$ and $GABA_B$ receptor agonists (Yuan et al., 1998b), the mechanism of activity of American ginseng was due to binding $GABA_A$ receptors (Yuan et al., 1998c). The voltage-dependent Na^+ channel blocking activity of American ginseng extract that we reported in the present study may also underlie neuronal inhibition leading to a CNS depressant effect. Takagi et al. (1972) reported that mice injected with a mixture of ginsenosides had a lowered body temperature, reduced spontaneous movement, decreased alertness, and relaxed muscle tone. It is possible that the tranquilizing effect of ginseng extracts could have resulted from a blockage of voltage-gated Na^+ channels leading to a reduction in neuronal excitability.

In summary, in this study we observed that American ginseng extract exhibits concentration- and voltage-dependent Na^+ channel attenuating effects. It interacts with the inactive state of the channel and has no effect on the voltage-dependence of activation. Our results suggest a contribution of this mechanism to the therapeutic efficacy of American ginseng. Finally, our preliminary results with Rb_1 (150 μM) showed that its ability to produce a voltage-dependent attenuation of transient current in brain Na^+ channels was less than that of American ginseng extract (3 mg/ml), which contained Rb_1 (133.5 μM). Whether other components in the American ginseng extract have a synergistic effect remains to be examined in future experiments.

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