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Does ginsenoside act as a ligand as other drugs do?

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Abstract: The last two decades have shown a marked expansion in publications of diverse effects of *Panax ginseng*. Ginsenosides, as active ingredients of *Panax ginseng*, are saponins found in only ginseng. Recently, a line of evidences shows that ginsenosides regulate various types of ion channel activity such as Ca^{2+}, K^+, Na^+, Cl^-, or ligand gated ion channels (i.e. 5-HT_3, nicotinic acetylcholine, or NMDA receptor) in neuronal, non-neuronal cells, and heterologously expressed cells. Ginsenosides inhibit voltage-dependent Ca^{2+}, K^+, and Na^+ channels, whereas ginsenosides activate Ca^{2+}-activated Cl^- and Ca^{2+}-activated K^+ channels. Ginsenosides also inhibit excitatory ligand-gated ion channels such as 5-HT_3, nicotinic acetylcholine, and NMDA receptors. This presentation will introduce recent findings on the ginsenoside-induced differential regulations of ion channel activities as a ligand as other drugs do.

Keywords: *Panax ginseng*; ginsenoside; ion channel; ligand-gated ion channel; differential regulation

Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer, has been used as a representative tonic for two thousand years in the Far East countries like Korea, China, and Japan. Now, ginseng is one of the most famous and precious herbal medicines consumed in around the world.[1] Although ginseng exhibits multiple pharmacological actions *in vitro* or *in vivo* studies such as antistress, antihypertension, antioxidant, or neuroprotection, its mechanisms on various efficacies are still elusive. Recent accumulating evidences show that ginsenosides are the main active ingredient of ginseng (Fig. 1). Ginsenoside is one of the derivatives of triterpenoid dammarane consisting of thirty carbon atoms. Each ginsenoside has a common hydrophobic four ring steroid-like structure with sugar moieties attached. About 30 different types of ginsenosides have been isolated and identified from the root of *Panax ginseng*. They are mainly classified into protopanaxadiol (PD) and protopanaxatriol (PT) ginsenosides according to the position of different carbohydrate moieties at the C-3 and C-6 position.[2] Each type of ginsenoside also has at least three side chains at the C-3, C-6, or C-20 position and these side chains are free or coupled with sugar containing monomer, dimer, or trimer. These sugar components might provide a specificity of cellular effects of each ginsenoside.[3-5] However, ginsenosides still appear to be hydrophobic compounds, since these individual ginsenosides are not water-soluble.

As mentioned above, ginsenosides produce diverse pharmacological effects *in vivo* or *in vitro*. This review will mainly focus on ion channel regulations by ginsenosides, since recent reports show that ginsenosides regulate various types of ion channels, which of them are activated or inhibited in neurons or in non-neuronal cells in the presence of ginsenosides. Therefore, this article will cover some recent observations on ginsenoside-induced ion channel regulations and will also speculate the possible biological effects mediated via ginsenoside-induced ion channel regulation.

Effects of ginsenosides on voltage-dependent Ca^{2+} channels

Ca^{2+} is an important regulator for many neuronal functions, including exocytosis and excitability. Voltage-dependent Ca^{2+} channels play an important role in control of cytosolic free Ca^{2+}. The neurons possess a variety of voltage-dependent Ca^{2+} channels such as L-, N-, P/Q-, R-, or T-types depending on cell types. However, excessive

cytosolic free Ca^{2+} induces cell damages and finally cell death.[6] Recent reports show that ginsenosides inhibit Ca^{2+} channels in sensory neurons. Among various ginsenosides such as ginsenosides Rb_1, Rc, Re, Rf, and Rg_1, ginsenoside Rf was more potent for the inhibition of Ca^{2+} channels and inhibits N-type and other high-threshold Ca^{2+} channel via PTX-sensitive G proteins reversibly.[3, 7] On the other hand, Kim *et al* (1998) demonstrated that ginsenosides inhibit Ca^{2+} channels in rat chromaffin cells, which are one of the representative neurosecretory cells in catecholamine releases under various stress situations.[8] The order of inhibitory potency on Ca^{2+} channel in rat chromaffin cells was ginsenoside Rc > Re > Rf > Rg_1 > Rb_1. Ginsenosides also showed a selectivity in Ca^{2+} channel regulation by inhibiting N-, P-, Q/R- but not L-type Ca^{2+} channel in bovine chromaffin cells.[9] Recently, Rhim *et al.*, (2002) showed that ginsenoside Rg_3 more potently inhibits L-, N-, and P-types of Ca^{2+} channels than other ginsenosides tested in rat sensory neurons.[5] In addition to Ca^{2+} channel inhibition by ginsenosides, Kim *et al* (1998) also showed that ginsenosides attenuated the stimulated membrane capacitance increase (Δ C_m) in rat chromaffin cells.[8] The order of inhibitory potency on Δ C_m was ginsenoside Rf > Rc > Re > Rg_1 > Rb_1. Thus, the attenuation of Ca^{2+} channel and membrane capacitance by ginsenosides suggests that ginsenosides might be closely involved in the regulation of neurotransmitter releases from nerve terminal(s).

Effects of ginsenosides on various K^+ channels

There are many kinds of K^+ channels in living cells. The representative K^+ channels are voltage-dependent K^+ channel, Ca^{2+}-activated K^+ channel, ATP-sensitive K^+ channel, and G protein coupled inwardly rectifying K^+ (GIRK) channel in neuronal or non-neuronal systems.[10] Most of K^+ channels are involved in regulation of repolarization or duration of depolarization in excitable cells or in relaxation of smooth muscle by allowing the efflux of K^+ ion from cytosol. It is well-known that ginsenosides relax blood vessels and other smooth muscles but the mechanism was not clearly demonstrated.[11] Recent report shows that ginseng total saponins and ginsenoside Rg_3 activate Ca^{2+}-activated K^+ and ATP-sensitive K^+ channel in rabbit coronary artery smooth muscle cells.[12, 13] Li *et al* (2001) demonstrated that the activation of Ca^{2+}-activated K^+ channels by ginsenosides in vascular smooth muscle cells were mediated

by mobilization of intracellular free Ca^{2+} following ginsenoside treatment.[14] These results show the possibility that treatment of ginsenosides might stimulate membrane components for intracellular Ca^{2+} mobilization cascades and the mobilized Ca^{2+} activates Ca^{2+}-activated K^+ channels, which in turn mediate repolarization of smooth muscle cells from depolarization induced by various endogenous or exogenous stimuli.

On the other hand, GIRK channel is known to regulate the firing rate, membrane potential, and neurotransmitter responses, resulting in postsynaptic hyperpolarization in brain. In the brain, GIRK channel is mainly expressed in the olfactory bulb, hippocampus, dentate gyrus, and cortex. In heart, acetylcholine released from vagus nerve binds m2 muscarinic receptors in heart and activates GIRK channel, resulting in the slowing of the heart rate.[15] Recent study showed that ginsenoside Rf activates GIRK channel when GIRK channel genes were co-expressed in *Xenopus* oocytes with rat brain mRNA. Other ginsenosides such as Rb_1 and Rg_1 slightly activate this channel. Ginsenoside Rf-induced GIRK current enhancement was blocked by Ba^{2+}, a K^+ channel blocker. Intracellular injection of GDPβS but not pretreatment of PTX attenuated ginsenoside Rf-induced GIRK current.[16] These results showed a possibility that ginsenoside Rf first interacts with unidentified ginsenoside Rf-binding protein in brain and the activation of unidentified ginsenoside Rf-binding protein could be coupled to GIRK channel. Thus, the activation of Ca^{2+}-activated K^+ channels through intracellular Ca^{2+} mobilization or the activation of GIRK channel by ginsenosides might provide another evidence that ginsenosides are involved in regulation of excitability of excitable cells. In contrast, Jeong *et al* (2004) showed that ginsenoside Rg_3 inhibits voltage-dependent K^+ channel (Kv1.4) expressed in *Xenopus laevis* oocytes.[17]

Effects of ginsenosides on voltage-dependent Na^+ channel

Activation of voltage-dependent Na^+ channels is directly involved in induction of action potentials of axonal and somatic portion of neurons. They are also involved in actively propagating axonal or dendritic information from one part to another part of neuron. There are two recent reports on the regulation of Na^+ channel by ginsenosides; Liu *et al* (2001) and Jeong *et al* (2004) showed that ginsenosides inhibit neuronal Na^+ channels expressed in tsA201 cell and *Xenopus laevis* oocytes.[17, 18] Liu *et al* (2001) used much higher concentrations of ginseng extract and ginsenoside Rb_1 than those

used in other channel regulation to inhibit Na^+ channel, whereas Jeong *et al* (2004) showed that ginsenoside Rg_3 was much more potent than ginsenosides tested and also showed the possibility that ginsenoside Rg_3 is a main candidate for neuronal Na^+ channel regulation.[17]

Effects of ginsenosides on excitatory ligand-gated ion channels

Nicotinic acetylcholine receptor is one of most extensively investigated receptors among various ligand-gated ion channels (LGIC). The activation of this receptor channel by acetylcholine allows influx of cations, most of Na^+ ions, into cells through this channel pore. Muscular nicotinic receptor channel consists of $\alpha 1\beta 1\delta\gamma$ (embryonic form) or $\alpha 1\beta 1\delta\varepsilon$ (adult form) subunits.[19] Neuronal form of nicotinic receptors consists of α ($\alpha 2 - \alpha 9$) and β ($\beta 2 - \beta 4$) subunits. α Subunit alone can form functional homomeric receptors or α and β subunits can form functional heteromeric receptors and their distribution is depending on type of organs or regions of nervous systems.[20] Interestingly, recent reports showed that ginsenosides inhibited Na^+ influx into bovine chromaffin cells stimulated by acetylcholine but not high K^+ and finally attenuated the release of catecholamine from chromaffin cells, which contain mainly $\alpha 3\beta 4$ nicotinic acetylcholine receptor.[21, 22] Furthermore, ginsenosides also inhibited acetylcholine-induced inward currents in oocytes expressed with nicotinic receptor $\alpha_1\beta_1\delta\varepsilon$ or $\alpha 3\beta 4$ subunit but not with $\alpha 7$ subunit, showing the possibility that ginsenosides regulate nicotinic acetylcholine receptor channel with differential manner.[23] The inhibition of acetylcholine-induced inward current by ginsenosides in oocytes expressed with nicotinic acetylcholine receptor $\alpha\beta\delta\varepsilon$ or $\alpha 3\beta 4$ subunit was reversible, voltage-independent, and non-competitive manner but ginsenosides themselves had no effect on basal currents in oocytes expressing nicotinic acetylcholine receptor $\alpha\beta\delta\varepsilon$ or $\alpha 3\beta 4$ subunit. Interestingly, it appears that PT ginsenosides such as Re, Rf, Rg_1, or Rg_2 was more potent than PD ginsenosides such as Rb_1, Rb_2, Rc, Rd for the inhibition on acetylcholine-induced inward current.[23] Sala *et al.* (2002) also demonstrated that ginsenoside Rg_2 reduced the peak current and increased the desensitization on acetylcholine-induced inward current in oocytes expressing human neuronal nicotinic acetylcholine receptors such as $\alpha 3\beta 4$, $\alpha 3\beta 2$, $\alpha 4\beta 4$, and $\alpha 4\beta 2$ but not $\alpha 7$.[24]

On the other hand, 5-HT_3 receptor is also one of LGIC superfamily. The activation of this channel also is permeable to Na^+ and K^+ ions and is similar in many ways to nicotinic acetylcholine receptor. 5-HT_3 receptors are sparsely distributed on primary sensory nerve endings in the periphery and widely distributed in the mammalian central nervous system. This receptor is also clinically significant because antagonists of 5-HT_3 receptor have important applications as analgesics, antiemetics, anxiolytics, and antipsychotics.[25] It has recently been reported that ginsenoside Rg_2 and ginsenoside metabolites also inhibit 5-HT_3 receptor-gated ion currents in *Xenopus* oocytes expressing 5-HT_3 receptors.[26, 27] The inhibitory effect by ginsenoside Rg_2 on 5-HT-induced inward current was also non-competitive and voltage-independent, which is similar manner with that of ginsenoside-induced modulation of nicotinic acetylcholine receptor.[26, 27]

Glutamate, one of major excitatory neurotransmitter in the central nervous system, plays an important role in neuronal plasticity and neurotoxicity. Glutamate can interact with both NMDA- and non-NMDA receptors, which are also LGIC. The activation of these receptors by glutamate makes permeable to cations such as Ca^{2+}, Na^+ or K^+ ions, although the selectivity of these cations is dependent on receptor subtypes.[28] The increased intracellular Ca^{2+} in neuronal cells is thought to be responsible for evoking both neuronal plasticity such as long term potentiation (LTP) and neurotoxicity of glutamate.[28] In rat cortical cultures, ginsenosides Rb_1 and Rg_3 attenuated glutamate- and NMDA-induced neurotoxicity by inhibiting the overproduction of nitric oxide, formation of malondialdehyde, and influx of Ca^{2+}.[29] In addition, Kim *et al* (2002) showed that in rat hippocampal cultures, ginsenosides and ginsenoside Rg_3 attenuated high K^+-, glutamate-, and NMDA-induced Ca^{2+} influx. Seong *et al* (1995) showed that ginsenosides attenuated glutamate-induced swelling of cultured rat astrocytes.[30] On the other hand, *in vivo* study using anesthetized rats, intracerebroventricular administration of ginsenoside Rb_1 but not Rg_1 significantly inhibited the magnitude of long term potentiation (LTP) induced by strong tetanus in the dentate gyrus, although ginsenoside Rb_1 did not affect the basal synaptic responses evoked by low-frequency test.[31] Pretreatment of ginsenosides via intrathecal route attenuated NMDA- or substance P- but not glutamate-induced nociceptive behaviors.[32, 33] And pretreatment of ginsenosides via intraperitoneal route also attenuated cell death of hippocampal neurons induced by

kainate.[34] These results also indicate that ginsenosides might interact with various excitatory neurotransmitter receptor subtypes for their actions and their interactions with excitatory receptors might be coupled to neuroprotection against excitotoxins in nervous systems.

Future perspective and conclusion

In future, it needs to more studies as follows; first, it might require further identifications for ginsenoside interaction site(s) in ligand-gated and voltage-dependent ion channel proteins to know how they interact or regulate ion channel activity. Second, it might also require further characterization for ginsenoside binding site(s) in ginsenoside-mediated intracellular Ca^{2+} release via $G\alpha_{q/11}$-PLC pathway in non-neuronal cells. Third, it might require the development of specific ginsenoside-derivatives with agonistic or antagonistic properties, since a high concentration of ginsenosides are still needed to elicit ion channel regulations and other cellular effects and there are no specific agent(s) blocking ginsenoside actions.

Although ginseng has been used for over 2000 years as mentioned above, further investigations will be still required for the elucidation on detailed mechanism of multiple actions of ginseng and it is possible for ginseng investigators to propose newly coined words for the solid establishment as one important branch of scientifically independent research fields as follows; "ginsentology [ʤainseŋtáləʤi] (인삼학, 人蔘學)" is made by combination of ginseng + tonic + -logy, which is specific to ginseng and this word comprises all fields on ginseng studies. For example, botanical ginsenotology, which might comprise all studies on ginseng plant itself. Pharmacological ginsenotology might include all studies on pharmacological effects of ginseng in animal systems including human. Further, "ginsentologist [ʤinseŋtáləʤist]" who are also researchers, scientists, specialists, or experts involved in the variety of fields in related with ginseng studies.

In summary, Fig. 2 shows the hypothetical drawings on the possible interaction of ginsenosides with various receptors or ion channels present in presynaptic or postsynaptic sites of the nervous system. Fig. 3 also shows the explanations of the signaling pathway of ginsenosides for the activation of endogenous Ca^{2+}-activated Cl^-

channels in *Xenopus* oocytes. However, the exact regulatory patterns of various types of ion channel activity are not yet clearly understood and these remain to be elucidated in future.

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