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Ginseng and ion channels: Are ginsenosides, active component of *Panax ginseng*, differential modulator of ion channels?

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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 review will introduce recent findings on the ginsenoside-induced differential regulations of ion channel activities and will further expand the possibilities how these ginsenoside-induced ion channel regulations are coupled to biological effects of *Panax ginseng*.

Key words : *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

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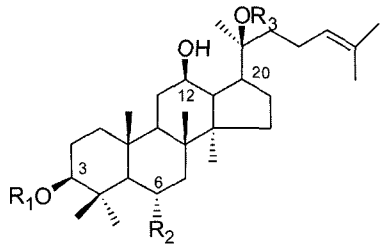

Ginsenosides	R_1	R_2	R_3	PD or PT
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc	PD
Rb_2	-Glc_2-Glc	-H	-Glc_6-Ara(pyr)	PD
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)	PD
Rd	-Glc_2-Glc	-H	-Glc	PD
Re	-H	-O-Glc_2-Rha	-Glc	PT
Rf	-H	-O-Glc_2-Glc	-H	PT
Rg_1	-H	-O-Glc	-Glc	PT
Rg_2	-H	-O-Glc_2-Rha	-H	PT
Rg_3	-Glc_2-Glc	-H	-H	PD

Fig. 1. Structures of the nine representative ginsenosides. They differ at three side chains attached to the common steroid-like ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara(pyr), arabinopyranoside; Rha, rhamnopyranoside, Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

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 (C_m) in rat chromaffin cells.[8] The order of inhibitory potency on 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+} acti-

vates 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 GDPbS 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 a1b1dg (embryonic form) or a1b1de (adult form) subunits.[19] Neuronal form of nicotinic receptors consists of a ($\alpha2$ - $\alpha9$) and b ($\beta2$ - $\beta4$) 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 $\alpha3\beta4$ 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 $\alpha3\beta4$ subunit but not with $\alpha7$ 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 abde or $\alpha3\beta4$ 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 $\alpha3\beta4$ 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 $\alpha3\beta4$, $\alpha3\beta2$, $\alpha4\beta4$, and $\alpha4\beta2$ but not $\alpha7$.[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.

The possible mechanisms on ginsenoside-induced ion channel regulations

Besides the regulation of ligand-gated ion channels by ginsenosides, the previous reports show that ginsenosides inhibit voltage-dependent Ca^{2+} channel or activate Ca^{2+}-activated Cl^- and GIRK channels through indirect ways following interaction with unidentified membrane component (s).[4, 38, 40] Supporting this notion is that extracellular but not intracellular treatment of ginsenosides activated this channel and that ginsenosides utilize PTX-sensitive (i.e. Ca^{2+} channels) or -insensitive (i.e. GIRK and Ca^{2+}-activated Cl^- channel) G proteins, indicating that ginsenosides interact with membrane components. Moreover, pretreatment of PLC inhibitor, antibody against PLCβ3 but not PLCβ1 and PLCβ2, intracellular injection of BAPTA (a free Ca^{2+} chelator), or heparin (an IP_3 receptor antagonist) also blocked the ginsenoside effect on Cl^- channel. These results indicate that ginsenoside activates Cl^- channel via PTX-insensitive Gαq family protein coupled to PLCβ3-IP_3 pathway in *Xenopus* oocytes. Thus, the inhibitory or stimulatory effect of ginsenosides on G protein-coupled effectors, which might be depending on cell types, like ion channels suggests that ginsenosides first interact with membrane component(s) for their ion channel regulations as other well-known ligands on G protein coupled receptors do (Figs. 2 and 3). However, further investigations on a novel membrane component(s) that interact with ginsenoside are required.

Differential effects of ginsenosides on intracellular Ca^{2+} level in neurons and non-neuronal cells may be associated with multiple pharmacological effects of *Panax ginseng*

As mentioned above, ginsenosides showed the tendency of decreasing Ca^{2+} influx by inhibiting voltage-dependent Ca^{2+} and Na^+ channels and by inhibiting Ca^{2+} permeable ligand-gated ion channels such as nicotinic acetylcholine receptor or NMDA receptor.[9, 23, 35] The negative couplings on these channels by ginsenosides may not favor the accumulation of intracellular free Ca^{2+} in neuronal cells. In contrast, in non-neuronal cells such as macrophages or NIH3T3 cells, treatment of ginsenosides mobilizes intracellular Ca^{2+}.[36, 37] Furthermore, Choi *et al* (2001) demonstrated that ginsenoside-induced mobilization of cytosolic free Ca^{2+} is mediated via IP_3 receptor in endoplasmic reticulum in *Xenopus* oocytes.[4, 38] Moreover, Jeong *et al.* (2004) demonstrated that ginsenosides induce store-operated Ca^{2+} entry (SOCE) following intracellular depletion in *Xenopus* oocytes, whereas ginsenosides inhibit ACh-mediated SOCE in cultured hippocampal neurons.[39] Thus, these ginsenoside-induced differential regulations of intracellular free Ca^{2+} level via Ca^{2+} entry

from outside or Ca^{2+} release from endoplasmic reticulum between neuronal and non-neuronal cells might induce quite a different cellular responses; i.e. in nervous systems the presence of ginsenosides might induce an inhibition of extracellular Ca^{2+}-influx or agonist-induced cytosolic Ca^{2+}-release, resulting in an attenuations of exocytosis or excitability caused by depolarization, activations of voltage-dependent ion channels, or activations of ligand-induced ion channels. These actions of ginsenosides in nervous system may exert their beneficial effects protecting nervous system damages caused by over-stimulations or excitotoxins.[34] In contrast, in non-neuronal cells the presence of ginsenosides induces cytosolic free Ca^{2+} up-regulation via mobilization from endoplasmic reticulum or SOCE pathway, resulting in activations of a variety of cellular functions that are mainly dependent on Ca^{2+}; i.e. activations of various Ca^{2+}-dependent protein kinases and enzymes related with gene transcription. These differential actions of ginsenosides might finally affect biological activities depending on types of cells or organs.[40]

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 concentra-

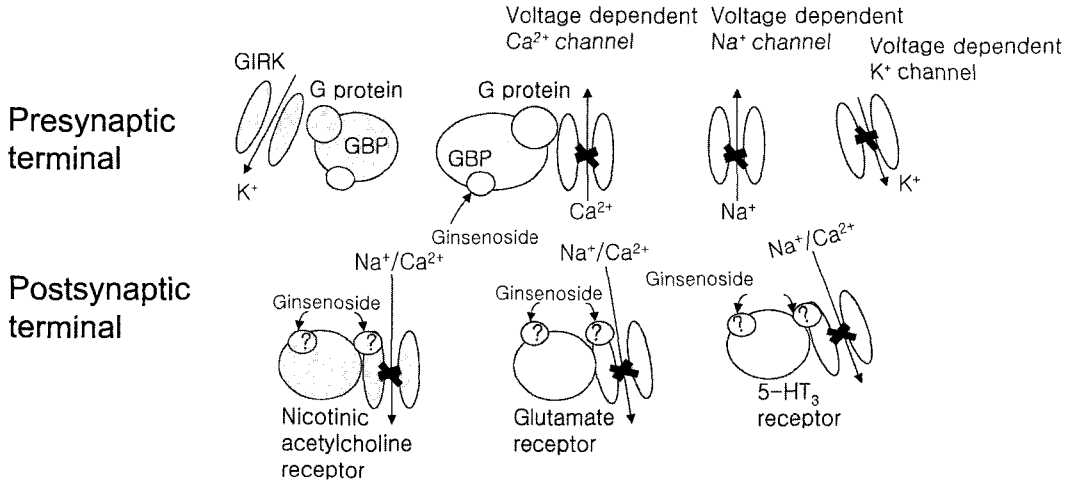

Fig. 2. The hypothetical scheme on site(s) of ginsenoside action in neuronal cells. Ginsenosides might interact with ginsenoside-binding protein (GBP), other receptors, or various ion channels in both presynaptic and postsynaptic terminals. In presynaptic terminals, ginsenosides inhibit voltage-dependent Ca^{2+} channel via PTX-sensitive G proteins, suggesting that ginsenosides might inhibit Ca^{2+} channels following activation of a novel GBP coupled PTX-sensitive G protein (3). Ginsenosides also inhibit voltage-dependent K^+ (Kv1.4) and Na^+ channels (brain type) but it is not yet known whether or not G protein is also involved in the regulatory mode of Na^+ by ginsenosides (17, 18). In presynaptic terminals, ginsenosides activate GIRK channels via PTX-insensitive G proteins (16). However, the evidences that GBP exists in both presynaptic and postsynaptic sites were not yet obtained. Ginsenosides also inhibit stimulatory ligand-gated receptor channel activities such as nicotinic acetylcholine receptor, glutamate receptor, and 5-HT_3 receptor in postsynaptic terminals. There is direct evidences that ginsenosides inhibited NMDA-induced ionic currents and recent reports also showed that ginsenosides attenuate glutamate-induced cell damages in neuronal and glia cells (29, 30). However, the exact binding site(s) or regulatory mode of ginsenosides in voltage-dependent ion channels and stimulatory ligand-gated ion channels requires more investigations. For an easy explanation, the possible ginsenoside interaction site(s) with ligand-gated ion channels, ginsenoside binding site(s), and voltage-dependent ion channels are drawn separately.

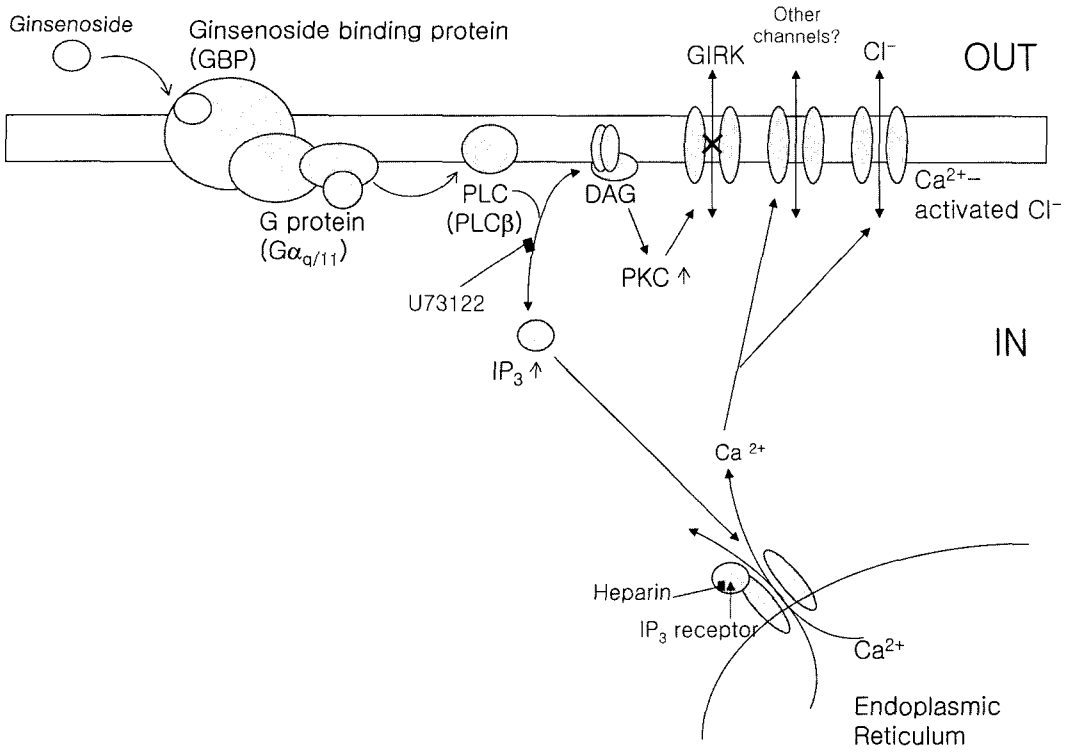

Fig. 3. The hypothetical scheme on site(s) of ginsenoside action in *Xenopus* oocytes. The signal transduction pathway of ginsenosides in *Xenopus* oocytes has been investigated in detail. This figure shows that the extracellular treatment of ginsensides but not intracellular injection elicited Ca^{2+}-activated Cl^- channel activation. Ginsenosides activated Ca^{2+}-activated Cl^- channel in voltage-dependent manner and niflumic acid (a Cl^- channel blocker) blocked the ginsenoside effect on Cl^- channel. The effect of ginsenoside on Cl^- channel was mediated via PTX-insensitive G proteins, blocked by PLC inhibitor (U73122), IP_3 receptor antagonist (heparin) (4, 38). The mobilization of intracellular Ca^{2+} by ginsenosides might activate other many intracellular events that are dependent on Ca^{2+} (40). The mobilization of intracellular Ca^{2+} might also activate Ca^{2+}-activated K^+ in blood vessels. In blood vessel smooth muscle, ginsenosides activate Ca^{2+}-activated K^+ following the mobilization of intracellular Ca^{2+} (13).

tion 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 [ʤinsenŋtáləʤi] (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 [ʤinsenŋtáləʤist]" 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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