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Ginsentology I: Differential Ca^{2+} Signaling Regulations by Ginsenosides in Neuronal and Non-neuronal cells

Jun-Ho Lee and Seung-Yeol Nah#

Ginsentology Research Laboratory and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul Korea, 143-701

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Abstract : One of the various signaling agents in the animal cells is the simple ion called calcium, Ca^{2+}. Ca^{2+} controls almost everything that animals do, including fertilization, secretion, metabolism, muscle contractions, heartbeat, learning, memory stores, and more. To do all of this, Ca^{2+} acts as an intracellular messenger, relaying information within cells to regulate their activity. In contrast, the maintenance of intracellular high Ca^{2+} concentrations caused by various excitatory agents or toxins can lead to the disintegration of cells (necrosis) through the activity of Ca^{2+}-sensitive protein-digesting enzymes. High concentrations of calcium have also been implicated in the more orderly programs of cell death known as apoptosis. Because this simple ion, acts as an agent for cell birth, life and death, to coordinate all of these functions, Ca^{2+} signalings should be regulated precisely and tightly. Recent reports have shown that ginsenosides regulate directly and indirectly intracellular Ca^{2+} level with differential manners between neuronal and non-neuronal cells. This brief review will attempt to survey how ginsenosides differentially regulate intracellular Ca^{2+} signaling mediated by various ion channels and receptor activations in neuronal and non-neuronal cells.

Key word : Panax ginseng; Ginsenosides; Differential Ca^{2+} signaling; Neurons; Non-neuronal cells

INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer, is a well-known representative tonic medicine and has been used for thousands of years. Recent studies have demonstrated that ginseng contains medically active ingredients that possess pharmacologically beneficial effects. Those are ginseng saponins or ginsenosides. They are the main active and unique ingredients of ginseng. Ginsenosides are one of the derivatives of triterpenoid dammarane consisting of thirty carbon atoms. They consist of aglycone and carbohydrates portions. Aglycone is the main backbone of ginsenosides with a hydrophobic four-ring steroid-like structure. The carbohydrates linked to aglycone consist of monomor, dimmer, or tetramer. About 30 different types of ginsenosides have been isolated and identified from the root of *Panax* ginseng. Each type of ginsenoside also has at least three side chains at carbon-3, -6, or -20 and these side chains are free or coupled with sugar containing monomer, dimer, or trimer. These sugar components might provide a specificity of each ginsenoside effect and might affect the solubility of each ginsenoside. Recent studies have shown that ginsenosides regulate various types of ion channels and ligand-gated ion channels.[1)]

This review will show you that there are at least three different sources for cytosolic Ca^{2+} elevations allowing Ca^{2+} signalings to initiate. This review will also show you that ginsenosides regulate three different Ca^{2+} sources and how regulations of ginsenosides-induced three different Ca^{2+} sources are coupled to Ca^{2+} signalings in neuronal and non-neuronal cells. Furthermore, this review will show the possibility that the regulations of ginsenoside-induced Ca^{2+} signalings could be one of main factors for *in vivo* tonic or therapeutic actions of *Panax* ginseng.

Voltage-dependent ion channels and Ca^{2+}

Ca^{2+} signalings depend on intracellular Ca^{2+} level, which is derived either from outside the cell or release of stores within the endoplasmic reticulum (ER). First, the outside source of Ca^{2+} is mainly derived from voltage-dependent Ca^{2+} channels in excitable cells such as neurons or muscle cells. The neurons possess a variety of volt-

To whom correspondence should be addressed.
(Tel) +82-2-450-4154; (Fax) +82-2-450-3037
(E-mail) synah@konkuk.ac.kr

age-dependent Ca^{2+} channels such as L-, N-, P/Q-, R-, or T-types depending on cell types. Muscle cells such as heart, skeletal muscle and smooth muscle mainly contain L-type Ca^{2+} channels. Recent reports show that ginsenosides inhibit Ca^{2+} channels in rat 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 pertussis toxin (PTX)-sensitive GTP-binding proteins reversibly.[2, 3] 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.[4] 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.[5] 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.[6]

In addition to Ca^{2+} channel inhibition by ginsenosides, Kim *et al.* (1998) also showed that ginsenosides attenuate the stimulated membrane capacitance increase (C_m) in rat chromaffin cells.[4] 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). More recently, Lee *et al.* (2006), using heterologous gene expression system on five different subtypes of voltage-dependent Ca^{2+} channels in *Xenopus laevis* oocytes, studied which subtypes of Ca^{2+} channels are more affected by ginsenosides and showed that ginseng total saponins (GTS) induced voltage-dependent, dose-dependent and reversible inhibition of the five channel subtypes, with particularly strong inhibition of the T-type. Of the various ginsenosides, Rb_1, Rc, Re, Rf, Rg_1, Rg_3, and Rh_2, ginsenoside Rg_3 also inhibited all five channel subtypes and ginsenoside Rh_2 had most effect on the L- and R-type Ca^{2+} channels. Compound K (CK), a protopanaxadiol ginsenoside metabolite, strongly inhibited only the T-type of Ca^{2+} channel, whereas M4, a protopanaxatriol ginsenoside metabolite, had almost no effect on any of the channels. Rg_3, Rh_2, and CK shifted the steady-state activation curves but not the inactivation curves in the depolarizing direction in the N- and P/Q-types. Thus, it appears that Rg_3, Rh_2 and CK are the major inhibitors of Ca^{2+} channels in *Panax* ginseng, and that ginsenosides show some Ca^{2+} channel subtype selectivity in their channel activity.[7] On the other hand, activation of voltage-dependent Na^+ channels are also indirectly involved in intracellular Ca^{2+} signalings, since Ca^{2+} channels open following depolarization caused by voltage-dependent Na^+ channel activations. Thus, it is possible that the regulations of voltage-dependent Na^+ channel activities by ginsenosides are indirectly coupled to Ca^{2+} signaling regulations. In fact, Jeong *et al.* (2004) and Lee *et al.* (2006) showed that ginsenoside Rg_3 inhibits neuronal Na^+ channels expressed in *Xenopus laevis* oocytes.[8] and also showed the possibility that ginsenoside Rg_3 is a main candidate for neuronal Na^+ channel regulations.[9] These results showed that ginsenosides directly regulate Ca^{2+} signalings by inhibiting extracellular Ca^{2+} influx via Ca^{2+} channels or by depolarization in neurons.

Ligand-gated ion channels and Ca^{2+}

Besides voltage-dependent Ca^{2+} channel activations and depolarization induced by voltage-dependent Na^+ channel activations, Ca^{2+} can enter into cells indirectly via various excitatory ligand-gated ion channel activations. Thus, second sources outside the cell of Ca^{2+} are via receptor-mediated Ca^{2+} influxes in response to various excitatory neurotransmitters. The way producing cytosolic Ca^{2+} elevation is excitatory ligand-gated ion channel activations. Some subtypes of neuronal nicotinic acetylcholine receptor (nAChR) channels are permeable to Na^+ as well as Ca^{2+}. In the central and peripheral nervous systems, nAChRs are involved in the regulation of the processes such as transmitter release, cell excitability and neuronal integration, which are crucial for network operations and influence physiological functions such us arousal, sleep, fatigue, anxiety, the central processing of pain, food intake and a number of cognitive functions.[10] Furthermore, it is becoming evident that the perturbation of nAChRs-mediated neurotransmission can lead to various diseases involving nAChR dysfunction during development, adulthood and aging.[10]

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 $\alpha3\beta4$ nAChR.[11,12] Furthermore, ginsenosides also inhibited acetylcholine-induced inward currents in oocytes expressing nAChR $\alpha_1\beta_1\delta\varepsilon$ or $\alpha3\beta4$ subunit but not with $\alpha7$ subunit, showing the possibility that ginsenosides regulate nAChR channel with differential manner.[13]

The inhibition of acetylcholine-induced inward current by ginsenosides in oocytes expressing nAChR αβδε or α3β4 subunit was reversible, voltage-independent, and non-competitive manner but ginsenosides themselves had no effect on basal currents in oocytes expressing nAChR αβδε or α3β4 subunit. Interestingly, it appears that ginsenosides such as Re, Rf, Rg_1, or Rg_2 was more potent than ginsenosides such as Rb_1, Rb_2, Rc, Rd for the inhibition on acetylcholine-induced inward current.[13] 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 nAChRs such as α3β4, α3β2, α4β4, and α4β2 but not α7. [14] Although we have observed that ginsenosides regulate various subtypes of nAChR channel activities, it might require more investigations how ginsenosides-induced regulations of nAChR channel activity in central and peripheral nervous systems is relevant with ginsenosides-mediated physiological or pharmacological effects in neuronal cholinergic systems. But one thing is evident that Na^+ permeable nAChR activations are coupled to depolarization of neurons and subsequent activation of voltage-dependent Ca^{2+} channels. Thus, it seems that ginsenosides affect intracellular Ca^{2+} signalings, which are coupled to catecholamine releases, through the ginsenoside-mediated nAChR regulations.

Similarly, 5-HT_3 receptor is a ligand-gated cation channel found in the central and peripheral nervous systems. In the periphery, it is found on autonomic neurons and on neurons of the sensory and enteric nervous systems. In the central nervous system, 5-HT_3 receptors have been localized in the area postrema, nucleus tractus solitarii, nucleus vaudatus, nucleus accumbens, amygdala, hippocampus, entorhinal, frontal, cingulate cortex, and in the dorsal horn ganglia. Further extraneuronal locations include lymphocytes, monocytes, and fetal tissues. 5-HT_3 receptors modulate the release of neurotransmitters and neuropeptides like serotonin itself, dopamine, cholecystokinin, acetylcholine, GABA, and substance P.[15] They have been demonstrated to be involved in sensory transmission, regulation of autonomic functions, integration of the vomiting reflex, pain processing and control of anxiety. While the physiologic functions of 5-HT_3 receptors are discrete and difficult to detect, it plays a key role in certain pathologic situations related to increased serotonin release. Clinical development of 5-HT_3 receptor antagonists revealed a remarkable range of activities. 5-HT_3 receptor antagonists do not modify any aspect of normal behavior in animals or induce pronounced changes of physiological functions in human. Clinical efficacy was shown for various forms of emesis like chemotherapy-induced, radiotherapy-induced, and postoperative emesis, diarrhea-predominant irritable bowel syndrome, anxiety, chronic fatigue syndrome, alcohol abuse, and in pain syndromes such as fibromyalgia and migraine.[15]

Ginsenoside Rg_2 and ginsenoside metabolites also inhibit 5-HT_3 receptor-gated ion currents in *Xenopus* oocytes expressing 5-HT_3 receptors.[16,17] The inhibitory effect by ginsenoside Rg_2 and Rg_3 on 5-HT-induced inward current was also non-competitive and voltage-independent, which is similar manner with that of ginsenoside-induced modulation of nAChRs.[16, 17] Since the 5-HT_3 receptors are mainly associated with the nociceptive processes of visceral pain in both humans and animals and with anticancer agent induced-nausea and vomiting as described above,[18,19] it seems possible that ginsenoside Rg_3-mediated regulation of 5-HT_{3A} receptors might be therapeutically relevant. Although there is no direct evidence that Rg_3 could be used as therapeutic agent for alleviation of 5-HT_{3A} receptor-related clinical symptoms such as vomiting and visceral pain, previous reports have shown that ginsenosides can inhibit 5-HT_{3A} receptor-mediated ion currents,[20] attenuate cisplatin-induced nausea and vomiting behavior in ferrets, and mitigate acetic acid-induced visceral hypersensitivity in rats.[21,22] These findings suggest that ginsenosides- and ginsenoside Rg_3-mediated 5-HT_{3A} receptor inhibitions could be one of the mechanisms underlying the alleviation of 5-HT_{3A} receptor-mediated clinical symptoms *in vivo*. In addition, 5-HT_{3A} receptors that are Na^+ permeable are also coupled to depolarization of neurons and subsequent activation of voltage-dependent Ca^{2+} channels as nAChR activations do. Thus, through the ginsenoside-mediated 5-HT_{3A} receptor regulations it seems indirectly that ginsenosides also affect intracellular Ca^{2+} signalings.

Glutamate and its analogs, major excitatory neurotransmitters in the central nervous system, play an important role in neuronal plasticity and neurotoxicity. NMDA receptor activation by glutamate usually shows Ca^{2+} permeability and non-NMDA receptors such as kainate and AMPA prefer Na^+ rather than Ca^{2+}. The increased intracellular Ca^{2+} in neuronal cells via glutamate receptor (NMDA receptors) activations is thought to be responsible for evoking both neuronal plasticity such as long term potentiation (LTP) and other neuronal activities. On the other hand, the sustained activation of glutamate receptors is one of main neurotoxicity of glutamate.[23] Thus, the activations of non-NMDA receptors also induce depolar-

ization and activate NMDA receptor as well as activation of voltage-dependent Ca^{2+} channels. 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+}.[24] 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.[25] 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.[26] Pretreatment of ginsenosides via intrathecal route attenuated NMDA- or substance P- but not glutamate-induced nociceptive behaviors.[27, 28] And pretreatment of ginsenosides via intraperitoneal route also attenuated cell death of hippocampal neurons induced by kainate.[29] Ginsenosides protect hippocampal damages from either ischemia/refusion- or kainate-induced toxicity in animals.[30-33] Thus, *in vitro* inhibition of NMDA/non-NMDA receptor-mediated intracellular Ca^{2+} signalings by ginsenosides might be a basis of *in vivo* or *in vitro* ginsenosides-induced neuroprotections against various excitatory neurotransmitters or neurotoxins.

The third way for intracellular Ca^{2+} signalings is from store-operated Ca^{2+} channels, which open when the internal Ca^{2+} stores are depleted, is mainly found in non-neuronal cells. Inositol 1,4,5-trisphosphate (IP_3) is generated by the action of the phospholipase C (PLC) on phosphatidylinositol 4,5-bisphosphate (PIP_2) at the plasma membrane, in response to the action of growth factors, hormones or neurotransmitters at the receptors, which are coupled to $G\alpha_{q/11}$-PLC-IP_3 pathway. IP_3 acts on receptors in the ER, which cause the release of Ca^{2+} from the store. The previous reports showed in *Xenopus laevis* oocytes, one kind of non-neuronal cells, that ginsenosides activate Ca^{2+}-activated Cl^- channels, which are activated via intracellular free Ca^{2+} elevation, through indirect ways following interaction with unidentified membrane component (s).[34, 35] Supporting these results is that extracellular but not intracellular treatment of ginsenosides activated this channel via PTX-insensitive G proteins, indicating that ginsenosides interact with unidentified 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 Ca^{2+}-activated Cl^- channel. These results indicate that ginsenosides utilize Ca^{2+} signalings for the activation of Ca^{2+}-activated Cl^- channel via PTX-insensitive $G\alpha_{q/11}$ family proteins coupled to PLCβ3-IP_3 pathway. Although the previous reports revealed, in *Xenopus* oocytes, that ginsenosides-induced intracellular Ca^{2+} elevations are only coupled to Ca^{2+}-activated Cl^- channel activations, more investigations will show that ginsenosides-induced intracellular Ca^{2+} elevations in non-neuronal cells could also couple to other unidentified signaling pathways.

Interestingly, Jeong *et al.* (2004) demonstrated that ginsenosides induce store-operated Ca^{2+} entry (SOCE) following intracellular depletion in *Xenopus* oocytes, whereas in cultured rat cortical neurons ginsenosides inhibited not only carbachol-stimulated turnover of IP_3, which leads to intracellular $[Ca^{2+}]_i$ mobilization, but also carbachol-induced intracellular Ca^{2+} mobilization.[36] Thus, these ginsenoside-induced differential regulations of intracellular free Ca^{2+} level via Ca^{2+} entry from outside or Ca^{2+} release from ER between neuronal and non-neuronal cells might induce quite different signaling 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.

The possible clinical applications and conclusion

This article has shown that Ca^{2+} signalings are mainly dependent on three different calcium sources and that ginsenosides regulate neuronal Ca^{2+} signalings by direct regulations of voltage-dependent Ca^{2+} channels and NMDA receptors. Ginsenosides also indirectly regulate neuronal Ca^{2+} signalings via regulations of voltage-dependent Na^+ channels and excitatory ligand-gated ion channels such as $5\text{-}HT_3$ and nACh receptors. The clinically applicable beneficial effects of ginsenosides by regulations of Ca^{2+} signalings in central and peripheral nervous systems might be neuroprotections, pain relief, anti-emesis, anti-stress, and irritable bowel syndrome that could be induced by various excitatory neurotransmitters, stress hormones, and neurotoxin insults. Although very little is known about the underlying molecular mechanisms of ginsenoside-mediated diverse efficacies, those efficaies by ginsenosides

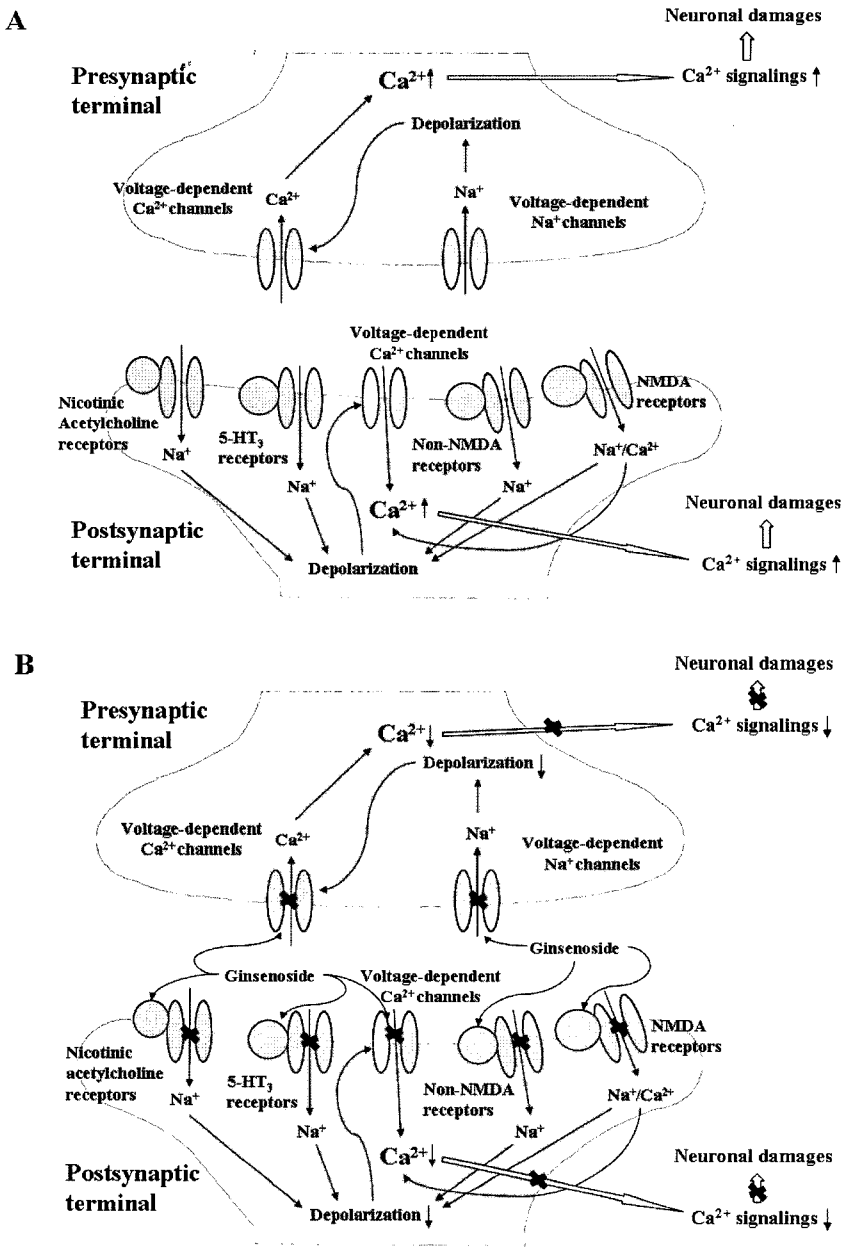

Fig. 1. Ginsenosides-induced regulations of Ca^{2+} signalings. *A*. This schematic drawings show that cytosolic Ca^{2+} levels are elevated via two different sources from pre- and post-synaptic terminals of nervous systems. The elevation of cytosolic Ca^{2+} levels is either achieved via direct activation of voltage-dependent Ca^{2+} channels or depolarization caused by voltage-dependent Na^{+} channel activation at pre-synaptic sites. The second sources of the elevation of cytosolic Ca^{2+} levels are mediated via the activation of 5-HT_3, nACh, and NMDA/non-NMDA receptors at post-synaptic sites. At pre-synaptic sites, the elevations of intracellular free Ca^{2+} play various important roles for synaptic transmissions including neurotransmitter releases and other Ca^{2+}-dependent signalings. At post-synaptic sites the elevations of intracellular free Ca^{2+} also perform various important things such as long-term potentiation and other Ca^{2+}-dependent signalings. But the sustained elevations of cytosolic Ca^{2+} level caused by excitatory neurotransmitters or neurotoxins at both sites could induce persistent activations of Ca^{2+}-dependent signalings, resulting in neuronal damages such as apoptosis or necrosis. *B*. Ginsenosides-induced regulations on ion channels and various ligand-gated in channels attenuate the elevation of cytosolic Ca^{2+} levels mediated by ion channels and ligand-gated ion channels. Thus, the inhibitory effects of ginsenosides on ion channels and ligand-gated ion channels that are directly or indirectly involved in Ca^{2+} signalings could be one of ginsenosides-induced neuroprotective and other beneficial actions in central and peripheral nervous systems.

might derive from the capability of ginsenosides to attenuate cytosolic Ca^{2+} overloads by extracellular Ca^{2+} influx or intracellular Ca^{2+} release caused by the sustained activations of 5-HT_3, nACh, or NMDA/non-NMDA receptors as well as activations of Ca^{2+} or Na^+ channels. In contrast, in non-neuronal cells the presence of ginsenosides induces cytosolic free Ca^{2+} elevation via mobilization from ER 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, ion channels, and enzymes related with gene transcriptions, which might probably be coupled to the processes for cell activations such as immune system activations or cell proliferations.[37] In conclusion, these differential Ca^{2+} signalings induced by ginsenosides between neuronal and non-neuronal cells might be the differential contributions of *Panax* ginseng efficacy in ginseng pharmacology and physiology.

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