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Mechanism of Ca^{2+}-activated Cl^- Channel Activation by Ginsenosides in *Xenopus* Oocytes

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Abstract : Relatively little is known about the signaling mechanism of ginseng saponins (ginsenosides), active ingredients of ginseng, in non-neuronal cells. Here, we describe that ginsenosides utilize a common pathway of receptor-mediated signaling pathway in *Xenopus* oocytes: increase in intracellular Ca^{2+} concentration via phospholipase C (PLC) and Ca^{2+} mobilization. Ginsenosides induced a marked and robust activation of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. The effect of ginsenosides was completely reversible, in a dose-dependent manner with EC_{50} of 4.4 μg/ml, and specifically blocked by niflumic acid, an inhibitor of Ca^{2+}-activated Cl^- channel. Intracellular injection of BAPTA abolished the effect of ginsenosides. Intracellular injection of GTPγS also abolished the effect of ginsenosides. The effect of ginsenosides on Ca^{2+}-activated Cl^- currents was greatly reduced by the intracellular injection of heparin, an IP_3 receptor antagonist or the pretreatment of PLC inhibitor. These results indicate that ginsenosides activate endogenous Ca^{2+}-activated Cl^- channels via the activation of PLC and the release of Ca^{2+} from the IP_3-sensitive intracellular store following the initial interaction with membrane component(s) from extracellular side. This signaling pathway of ginsenosides may be one of the action mechanisms for the pharmacological effects of ginseng.

Key word : Ginseng; Ginsenosides; PLC; Ca^{2+}-activated Cl^- channels; *Xenopus* oocytes

INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer, is a well-known folk medicine that has been shown to produce a variety of medicinal effects, both within and out of nervous systems. Recent studies showed that ginseng saponins, which are called ginsenosides, are the main molecular components responsible for the actions of ginseng. Ginsenosides are well characterized and have a four-ring, steroid-like structure with sugar moieties attached and about 30 ginsenosides have been isolated and identified from the root of *Panax* ginseng.[1)]

Several reports demonstrated the action of ginsenosides at the level of single neuronal cell. For example, ginsenosides inhibited voltage-dependent Ca^{2+} channels in sensory neurons via pertussis toxin (PTX)-sensitive G proteins.[2)] Ginsenosides were also found to inhibit voltage-dependent Ca^{2+} channels in rat chromaffin cells.[3)] Ginsenosides attenuate acetylcholine (ACh)-stimulated catecholamine release in bovine chromaffin cells via inhibition of Na^+ influx through nicotinic receptor-gated cation channels.[4,5)]

On the other hand, *in vivo* chronic treatment of ginsenosides induced not only an increase of the incorporation of $^{32}PO_4$ into inositol phospholipids but also stimulated phospholipase C (PLC) activity in mouse liver and brain.[6)] Ginsenosides have also shown to induce an increase of intracellular Ca^{2+} in non-neuronal cells such as macrophages or NIH3T3 cells.[7,8)] However, relatively little is known about the signal transduction mechanism of ginsenosides. Especially, it is still unclear in non-neuronal cells how ginsenosides are coupled with their binding site(s) and effector systems to produce second messengers and to exert their final pharmacological or physiological responses.

Xenopus laevis oocytes have been a useful model system for investigating the machinery of membrane signal transduction. Due to their large size and easy handling, oocytes permit the intracellular injection of various compounds and allow the analysis of the receptor-channel coupling system. Especially, investigations of intermediate steps in signaling

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pathway could be substantially facilitated by direct injection of putative second messengers or agents that interrupt the action of second messenger. Moreover, *Xenopus* oocytes have well-studied endogenous Ca^{2+}-activated Cl^- channels.[9,10] The activation of these channels are dependent on both intracellular Ca^{2+} and transmembrane voltages.[11] They have been used to understand or to monitor Ca^{2+} release-activating receptors, since these channels generate membrane current in responses to various stimuli that elevate intracellular free Ca^{2+} levels.[12-14] For example, ACh activates endogenous muscarinic receptors, which activate PLC, that increases the amount of inositol 1,4,5-triphosphate (IP_3), thus leading to intracellular Ca^{2+} mobilization and activation of Ca^{2+}-activated Cl^- channels.[12,15,16]

In the present study, we examined the effects of ginsenosides on Ca^{2+}-activated Cl^- channels in native *Xenopus* oocytes. We found that ginsenosides induced the activation of Ca^{2+}-activated Cl^- channels via receptor-mediated activation of PLC and the release of Ca^{2+} from IP_3-sensitive intracellular Ca^{2+} store. Our results indicate that ginsenosides utilize a well-defined signaling pathway to increase the concentration of intracellular Ca^{2+} in non-neuronal single cell.

MATERIALS AND METHODS

1. Materials

Xenopus laevis were obtained from Xenopus I (Ann Arbor, MI). Ginsenosides were obtained form Korea Ginseng and Tobacco Research Institute (Taejon, Korea). BAPTA for chelating intracellular free Ca^{2+}, low molecular weight heparin for blocking the IP_3 receptor, niflumic acid (Ca^{2+}-activated Cl^- channel blocker), and GTPγS were obtained from Sigma. U-73122 (active PLC inhibitor) and U-73343 (inactive PLC inhibitor) were purchased from Calbiochem, and PTX were from List Biological Laboratories.

2. Collection of *Xenopus* oocytes

Xenopus laevis care and handling was in accordance with the highest standards of institutional guidelines. Frogs underwent no more than two surgeries, at an interval of at least 3 weeks. To isolate oocytes, frogs were anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester. Oocytes were separated by treatment with collagenase, agitating for 3 hours in $CaCl_2$-free medium containing 82.5 NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM sodium pyruvate, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Stage V-VI oocytes were collected and were maintained at 16°C with continuous gentle shaking in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.5) supplemented with 0.5 mM theophylline and 50 μg of gentamycin per ml. All solutions were changed every day. All experiments were performed within 2~4 days following isolation of the oocytes.

3. Recording of Ca^{2+}-activated Cl^- currents

A single oocyte was placed in a small Plexiglas net chamber (0.5 m*l*) and was superfused constantly with standard recording solution in the absence or presence of ginsenosides during recording. The microelectrodes were filled with 3 M KCl and had a resistance of 0.2~0.7 MΩ. For most of the electrophysiological experiments, the oocytes were clamped at a holding potentials of -80 mV and 300 ms voltage steps were applied from -60 to +60 mV in 20 mV increments for current and voltage relationship. For time course experiments, outward Cl^- currents were evoked by a series of pulses, 500 ms duration, from -80 mV to +40 mV. Two-electrode voltage-clamp recordings were performed at room temperature with a Geneclamp 500 amplifier (Axon Instruments) or Oocyte Clamp (OC-725C, Warner Instrument) with Digidata 1200B. Linear leak and capacitance currents were corrected with leak subtraction procedure. Standard recording solution contained ND96.

4. Oocyte injection

Oocytes were injected with ginsenosides, BAPTA, and heparin by using a Nanoject Automatic Oocyte Injector (Drummond Scientific, Broomall, PA). The injection pipette was pulled from glass capillary tubing that has been used for recording electrodes and the tip was broken to 20-μm-OD. 23~50 n*l* of ginsenosides, BAPTA, heparin, and GTPγS solution were injected to give a calculated oocyte concentration of ~1 μg/m*l*, 1 mM, 1 μg/m*l*, and 150 μM, respectively.

5. Data analysis

All values are presented as mean±S.E.M. The differences between means of control and treatment data were analyzed using unpaired Students *t* test. A value of $p<0.05$ was considered statistically significant.

RESULTS

1. Effect of ginsenosides on endogenous channel activity in *Xenopus* oocytes

First, we tested the effect of ginsenosides on endog-

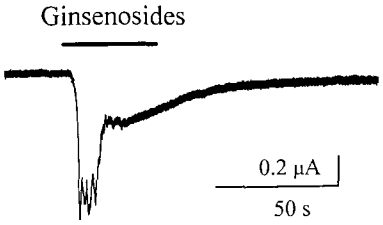

Fig. 1. Ginsenosides-induced current activity in *Xenopus* oocytes. Ginsenosides were applied and removed where indicated at concentration of 30 μg/ml. The resting membrane potential of the oocyte was -35 mV and was voltage-clamped at a holding potential of -70 mV prior to addition of ginsenosides. Tracings are representative of four separate oocytes from four different frogs for each ginsenosides.

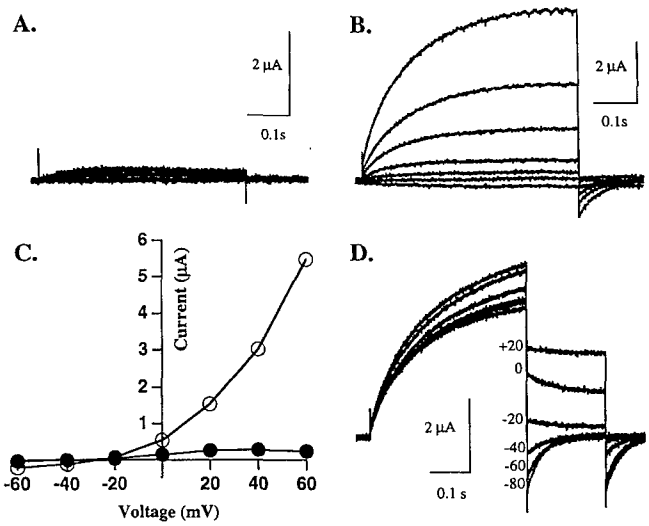

Fig. 2. Activation of a Cl^- channels by ginsenosides in *Xenopus* oocytes. (A) Basal current recorded at potentials ranking from -60 to 60 mV, with 20 mV increments, from a holding potential of -80 mV. (B) On the same oocyte, ginsenosides (10 μg/ml) induced outward currents. (C) Current-voltage relationships of basal (closed circles) and ginsenosides-induced (open circles) currents. Currents were measured as an average of points at the end of the 500 ms depolarization pulse. (D) Tail currents induced by ginsenosides (30 μg/ml) were determined by activating the current at 20 mV for 300 ms followed by voltage steps from 20 to -80 mV, with -20 mV increments. Note that the reversal potential of the tail current is between -20 and -40 mV.

enous channel activity in *Xenopus* oocytes. As shown in Fig. 1, the addition of ginsenosides to the bathing solution induced two components of inward currents in previously quiescent oocyte held at -70 mV. The first phase was a large inward current with slight oscillations or fluctuations typically starting at 5~20 seconds after the application of ginsenosides and two or more transient peaks were usually observed. The second phase was a sustained inward current following the first phase and maintained until ginsenosides were removed. The current activity evoked by ginsenosides was fully reversed within 10~20 seconds after the termination of treatment in this cell but in certain batch of oocytes the sustained inward currents lasted for several minutes even after washing out ginsenosides (data not shown).

2. Characterizations of ginsenosides-activated endogenous channels in *Xenopus* oocytes

To identify the type of channel(s) activated following ginsenosides treatment, we have investigated the current-voltage (I-V) relationship of the currents using conventional two-electrode voltage clamp. In the absence of ginsenosides, there was a basal level of background current at -60 mV and small outward current in the range of 0.1 to 0.4 μA at +60 mV in defolliculated native oocytes (Fig. 2A). The addition of ginsenosides into bathing solution elicited a slight increase in inward current at -40 mV and -60 mV. At more positive than -20 mV, ginsenosides evoked a large and voltage-dependent increase in outward currents (Fig. 2B). Typically, several microamperes of currents were recorded at +60 mV after treatment of 10 μg/ml ginsenosides. As shown in Fig. 2C for current-voltage relationship on the endogenous currents, these endogenous control currents were small and voltage-dependent with the reversal potential about -30 mV. The addition of ginsenosides to bathing solution induced marked increase in outward currents with no significant change in reversal potential. Tail currents elicited in the presence of ginsenosides more directly suggest that ginsenosides activate Cl^- channels and increase Cl^- conductance in oocytes, since the direction of currents were reversed near -30 mV closed to the calculated reversal potential of Cl^- (Fig. 2D). In addition, we used niflumic acid (NFA), a reversible blocker of Ca^{2+}-activated Cl^- channel, to confirm whether ginsenosides induce the activation of endogenous Ca^{2+}-activated Cl^- channels in native oocytes.[17] As shown in Fig. 3, pretreatment of 100 μM NFA slightly attenuated the basal current and ginsenosides failed to evoke current increase in the presence of NFA. After washing out NFA, reapplication of ginsenosides elicited a large increase in outward currents, indicating that ginsenosides activate endogenous Ca^{2+}-activated Cl^- channels in oocytes and that the effect of NFA is reversible. Thus, the kinetics of

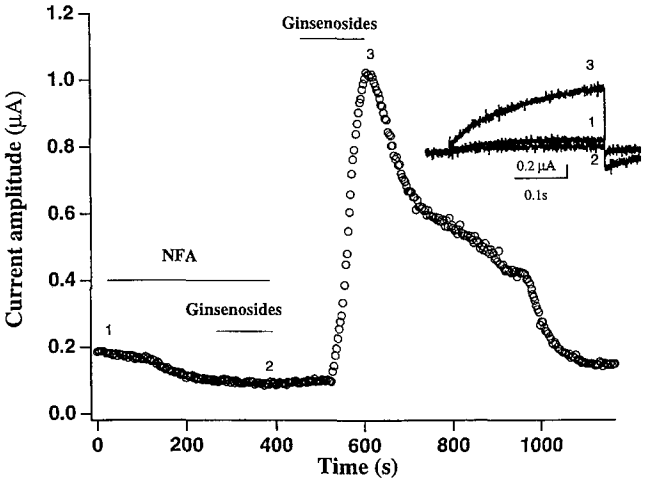

Fig. 3. Ginsenosides increase a Cl^- permeability. The current amplitude recorded at 40 mV from holding potential of -80 mV every 5 s was illustrated as a function of time. Bars represent continuous applications of Ca^{2+}-activated Cl^- channel blocker, 100 μM niflumic acid (NFA), or 10 μg/m*l* ginsenosides alone or together. *Inset*; ginsenosides-induced current traces recorded in the presence and absence of NFA were superimposed. Time points were indicated on the graph as 1, 2 and 3. Tracings are representative of six separate oocytes from three different frogs

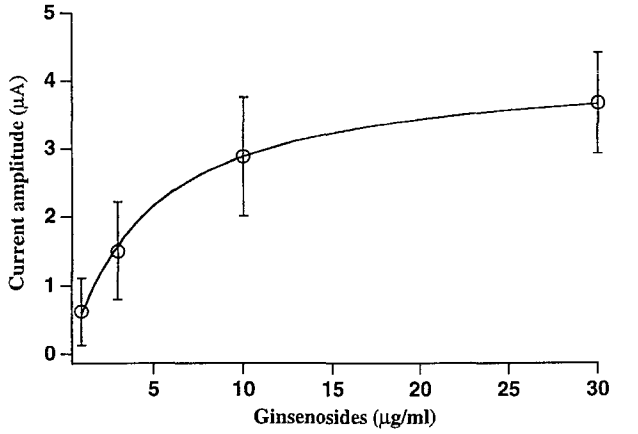

Fig. 4. Dose-dependent effect of ginsenosides on Ca^{2+}-activated Cl^- channels. Outward currents were evoked by voltage pulses to 40 mV from -80 mV holding potential every 5 s. Currents for dose-response curve were measured as an average of points at the end of the 300 ms depolarization pulse. The continuous line shows the curve fitted according to the equation. $I=I_{max}$. [ginsenosides]/([ginsenosides])+EC_{50}), where I_{max}, the maximum current amplitude and EC_{50}, the concentration producing half-maximum activation, are equal to 4.6 μA and 4.4 μg/m*l*, respectively. Symbols and bars represent mean ± S.E.M.

the currents and the sensitivity to NFA demonstrate that ginsenosides induce the activation of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. The activation of endogenous Ca^{2+}-activated Cl^- channels by ginsenosides was dose-dependent and saturable. The half-maximal activation was 4.4 ± 0.5 μg/m*l* and the currents were saturated at 10 μg/m*l* (Fig. 4).

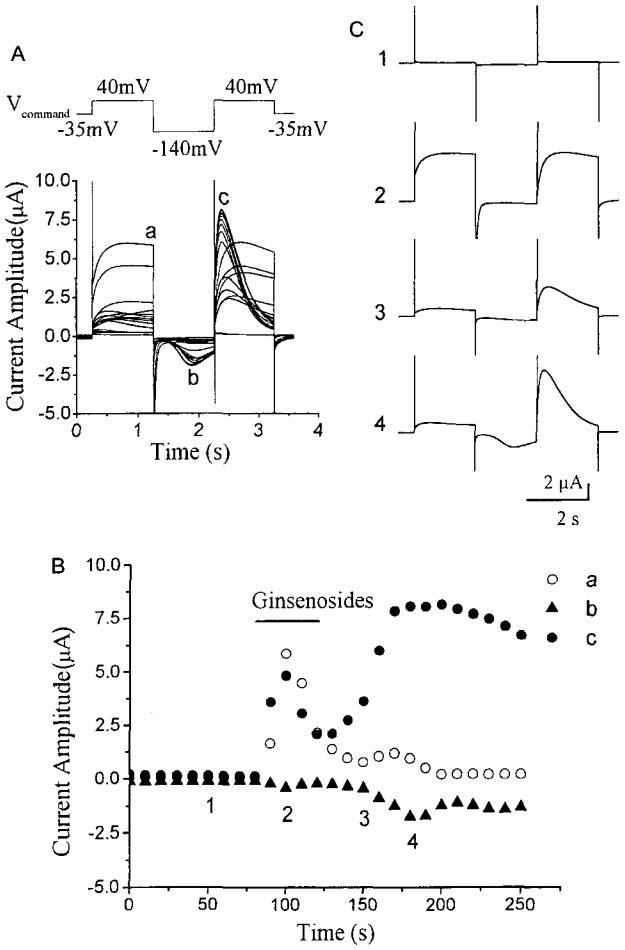

Fig. 5. Effect of ginsenosides on non-inactivating outward Cl^- current ($I_{Cl1\text{-}S}$), slow inward Cl^- current (I_{Cl2}), and transient outward Cl^- current ($I_{Cl1\text{-}T}$). A *Xenopus* oocyte was voltage clamped with two microelectrodes and ginsenosides (30 μg/m*l*) was added into bathing solution following the voltage pulse of every 10 sec. (A) Current amplitudes recorded at every 10 s for 26 trials with a three-step pulse command were overlapped. Peak current values of three Cl^- current components were measured at the points *a*, *b*, and *c* indicated on the figure, respectively. (B) Current amplitudes recorded at the point a, b, and c were illustrated as a function of time. Bar represents continuous application of ginsenosides (30 μg/m*l*). (C) Four representative current traces at time 1, 2, 3, and 4 as indicated on the Figure B are illustrated.

3. Effect of ginsenosides on Ca^{2+}-activated Cl^- channel subtypes

Since it has been suggested that there may be more than one type of Ca^{2+}-activated Cl^- channels that are different from their pharmacology and kinetics,[18-20] we tested the possible effect of ginsenosides on Ca^{2+}-activated Cl^- channel subtypes. For this experiment we used a three-step voltage command that can elicit three different Ca^{2+}-activated Cl^- currents following IP_3 injection into *Xenopus* oocytes.[20] We found that the application of ginsenosides into bathing solution induced three kinds of Ca^{2+}-activated Cl^- currents such as the sustained outward I_{Cl1} (or $I_{Cl1\text{-}S}$), transient outward I_{Cl1} (or $I_{Cl1\text{-}T}$) at the beginning and at the end of voltage step, and inward current I_{Cl2} (Fig. 5A). In sequence, the sustained outward currents first appeared and diminished spontaneously with time. Instead, the inward currents started to appear and at the same time the transient outward currents also started to appear at the end of voltage step (Fig. 5B and 5C).

4. Ginsenosides induce the activation of Ca^{2+}-activated Cl^- channels by acting on extracellular site(s)

As mentioned already, since ginsenosides have steroid-like structure with sugars attached, it is not yet clear whether the activation of Ca^{2+}-activated Cl^- channels by ginsenosides in oocytes is mediated by extracellular or intracellular binding site(s). Hence, ginsenosides were either applied in the bathing medium or microinjected into the oocytes. As shown in Fig. 6, following initial induction of Ca^{2+}-activated Cl^- current by extracellular application of ginsenosides, the injection of ginsenosides into the same oocyte did not produce the usual Ca^{2+}-activated Cl^- current. However, the reapplication of ginsenosides into the extracellular medium again induced the activation of Ca^{2+}-activated Cl^- channels as much as the first treatment. These results indicate that ginsenosides elicit Cl^- currents via extracellular site(s) located on the surface of oocytes.

5. Effect of BAPTA or heparin on ginsenosides-induced Ca^{2+}-activated Cl^- currents

To investigate the signaling mechanism involved in the ginsenosides-induced activation of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes, we first tested the possible involvement of external Ca^{2+} on Ca^{2+}-activated Cl^- currents. It was found that ginsenosides-induced Ca^{2+}-activated Cl^- currents were similar both in the presence and absence of extracellular Ca^{2+} (data not shown), suggesting that the activation of Ca^{2+}-activated Cl^- channels by gin-

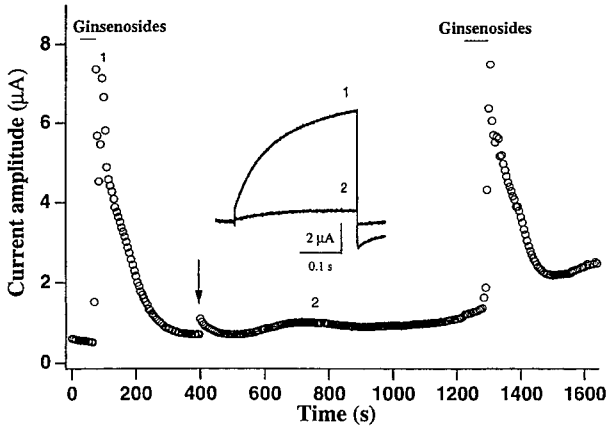

Fig. 6. Ginsenosides induce a Cl^- permeability by acting on the extracellular site of the plasma membrane. The current amplitude recorded at 40 mV from holding potential of -80 mV every 5 s is illustrated as a function of time. Bars represent continuous applications of ginsenosides (10 μg/m*l*); the arrow indicates the injection of 23 n*l* of ginsenosides (1 μg/m*l* final concentration) into oocytes. *Inset*; the illustration of superimposed current traces were obtained in the presence of ginsenosides either applied to the bath or injected into oocyte at times 1 and 2 as indicated in the Figure. Tracings are representative of six separate oocytes from three different frogs.

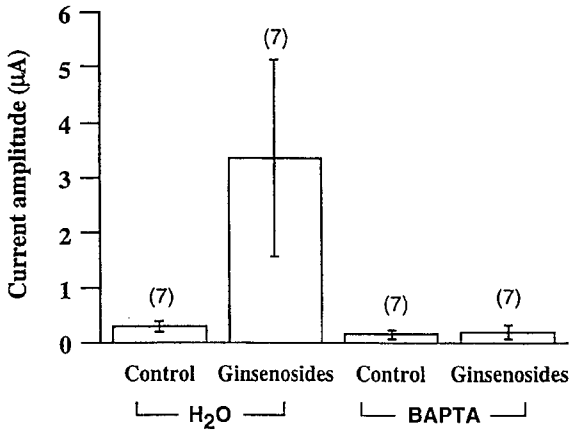

Fig. 7. The activation of Cl^- channels by ginsenosides is intraoocye Ca^{2+} dependent. Histograms of current amplitudes measured from oocytes injected with 23 n*l* of either H_2O or BAPTA (1 mM final) prior to recording. After H_2O or BAPTA injection oocytes were incubated for 20 min. Chloride currents before H_2O and BAPTA injection were 0.29 ± 0.10 μA and 0.15 ± 0.10 μA, respectively. Chloride currents evoked by ginsenosides (30 μg/m*l*) were 3.35 ± 1.80 μA after H_2O injection, whereas chloride currents evoked by ginsenosides (30 μg/m*l*) were 0.19 ± 0.14 μA after BAPTA injection. Current amplitudes are represented as mean ± S.E.M (n=number of oocytes).

senosides is not dependent on the presence of extracellular Ca^{2+}. In experiment to confirm whether ginsenosides-activated Cl^- channels in oocytes require the mobilization of intracellular Ca^{2+},[11] we injected BAPTA, a free Ca^{2+} chelator, into oocytes. As shown in Fig. 7, we found that BAPTA itself slightly reduced basal Ca^{2+}-activated Cl^- currents but also found that BAPTA almost completely abolished the effect of ginsenosides on Ca^{2+}-activated Cl^- current induction. These observations strongly suggest that ginsenosides induce an elevation of $[Ca^{2+}]_i$ by mobilizing the Ca^{2+} from the intracellular store.

To verify whether IP_3 was involved in the activation of Ca^{2+}-activated Cl^- channels by ginsenosides, heparin, an IP_3 receptor antagonist, was injected into oocytes.[21] As shown in Fig. 8, the injection of heparin (1 μg/oocyte) into oocytes greatly attenuated ginsenosides-induced Ca^{2+}-activated Cl^- currents, while oocytes injected with distilled water still responded to ginsenosides (Fig. 8). These results strongly suggest that that IP_3 also play a key role in activation of Ca^{2+}-activated Cl^- channels by ginsenosides.

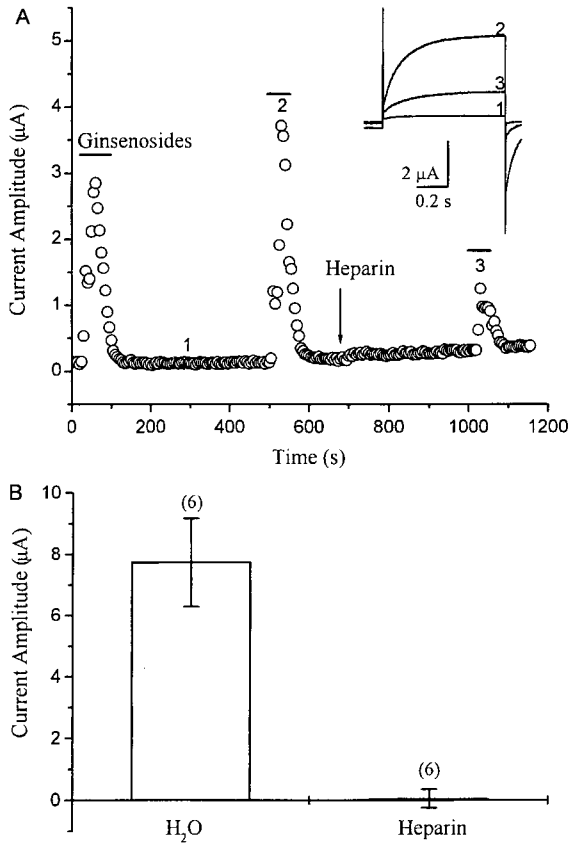

Fig. 8. Intraoocyte injection of heparin attenuates ginsenosides-induced Ca^{2+}-activated Cl^- channel activation. (A) Current amplitude recorded at 40 mV from holding potential of -80 mV every 5 s is illustrated as a function of time. This representative oocyte shows the down-regulation of ginsenosides-induced Ca^{2+}-activated Cl^- currents after injection OF HEPARIN (1 μg/OOCYTE). BARS REPRESENT CONTINUOUS APPLICATIONS of ginsenosides (30 μg/m*l*). *Inset*; the illustration of superimposed current traces was obtained in the presence of ginsenosides before and after injection of heparin at times 1, 2, and 3 as indicated on the graph. (B) Histograms of current amplitudes measured from oocytes injected with 50 n*l* of either H_2O or heparin (1 μg/oocyte) prior to recording and incubated for 20 min. Chloride currents evoked by ginsenosides (100 μg/m*l*) was 7.72 ± 1.44 μA after H_2O injection and was 0.05 ± 0.30 μA after heparin injection. Current amplitudes are represented as mean ± S.E.M (n= number of cells).

6. Effect of PTX or GTPγS on ginsenosides-induced Ca^{2+}-activated Cl^- currents

Previously, we have demonstrated that ginseng root extract and ginsenoside Rf inhibited voltage-dependent Ca^{2+} channels through PTX-sensitive G proteins in sensory neurons.[2,22] Hence, we also tested the effect of PTX on ginsenosides-induced Ca^{2+}-activated Cl^- channel activation. As shown in Table 1, pretreatment of PTX (2 μg/m*l*, 16 h) did not attenuate the action of ginsenosides on Ca^{2+}-activated Cl^- currents. However, the intracellular injection of GTPγS (150 μM final), a non-hydrolyzable GTP analog which induces a persistent activation of G proteins, significantly reduced the effect of ginsenosides on Ca^{2+}-activated Cl^- currents.

7. Effect of PLC inhibitor on ginsenosides-induced Ca^{2+}-activated Cl^- currents

Since the activation of Cl^- channels by ginsenosides was found to be mediated by IP_3-induced increase in

Table 1. Effect of PTX pretreatment or intracellular injection GTPγS on GTS-induced Ca^{2+}-activated Cl^- currents

	Ca^{2+}-activated Cl^- currents (μA)	
Treatment	Basal	GTS (30 μg ml^{-1})
-PTX	1.25 ± 0.20(6)	6.95 ± 0.71(6)
+PTX[a]	1.16 ± 0.05(6)	6.49 ± 0.26(6)
H_2O	0.22 ± 0.04(16)	2.90 ± 0.52(6)
GTPγS[b]	0.48 ± 0.06(14)	0.84 ± 0.12*(14)

*$p<0.001$ significantly different from H_2O injected oocytes
[a]Current amplitudes were measured after 16 h treatment with PTX (2 μg ml^{-1})
[b]Current amplitudes were measured after 10 min after intracellular injection of H_2O or GTPγS (150 μM). The number of oocytes tested are shown in parenthesis.

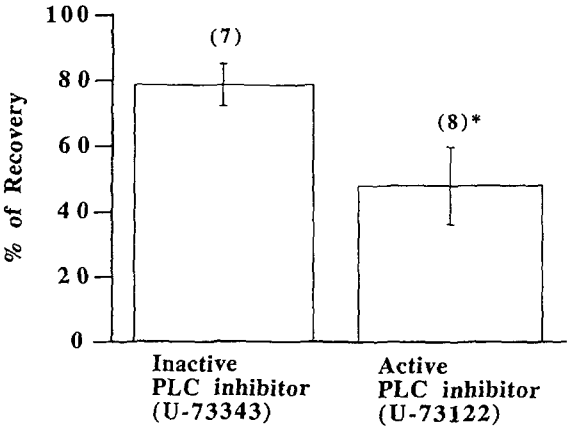

Fig. 9. The activation of Ca^{2+}-activated Cl^- channel by ginsenosides depends on PLC activation. Current amplitudes were first measured in the presence of ginsenosides (30 μg/ml) and oocytes were perfused for 5 min with 1 μM of either the inactive (U73343) or the active (U73122) inhibitor of PLC. Ginsenosides (30 μg/ml) were then co-applied with either of the two compounds. The amplitude of the current induced by that second application of ginsenosides was normalized to the amplitude of the current induced by a first application of ginsenosides prior to the treatment of oocytes with U73343 or U73122. Data are represented as mean ± SEM (n =number of cells). $p<0.05$ compared to U73343 treated cells.

$[Ca^{2+}]_i$ (Fig. 9), it is likely that the initial action of ginsenosides require PLC activation for the production of IP_3. To test this possibility, ginsenosides-induced Cl^- current was measured in the absence and presence of U-73122 and U-73343, an active PLC inhibitor and its inactive analog, respectively.[23] As shown in Fig. 9, ginsenosides evoked Ca^{2+}-activated Cl^- currents by 48±11.7% in the presence of U-73122 compared to those evoked by first ginsenosides treatment alone. However, using the same method as above ginsenosides evoked Ca^{2+}-activated Cl^- currents by 78.6±6.4% in the presence of U-73343 compared to those evoked by first ginsenosides treatment alone. These results show that PLC inhibitor partially blocked the effect of ginsenosides on Ca^{2+}-activated Cl^- channel activation in *Xenopus* oocytes.

DISCUSSION

Ginseng is usually mild and subtle in its efficacy compared to other medicines. Ginsenosides are known to represent a variety of physiological or pharmacological effects of ginseng in non-neuronal cells.[1] However, the underlying mechanism of ginseng efficacy and the cellular basis of ginsenosides action are not fully understood. Here, we demonstrate that ginsenosides utilize a well-known signal pathway of the G protein coupled PLC activation and IP_3 mediated intracellular Ca^{2+} release to activate Ca^{2+}-activated Cl^- channels in native *Xenopus* oocytes.

The activation of Ca^{2+}-activated Cl^- channels by ginsenosides is evident, since the current reversal was near to -30 mV, which is suggestive for chloride as a charge carrier (Fig. 2C). In addition, niflumic acid, the commonly used Ca^{2+}-activated Cl^- channel blocker, suppressed ginsenosides-induced Ca^{2+}-activated Cl^- channel activation (Fig. 3). Ginsenosides increased Ca^{2+}-activated Cl^- currents in a dose-dependent manner (Fig. 4) and the effect was reversible in all concentrations tested. The activation of Ca^{2+}-activated Cl^- channels by ginsenosides was voltage-dependent (Fig. 2B). The currents activated by ginsenosides are dependent on intracellular Ca^{2+}, since chelation of intraoocyte $[Ca^{2+}]_i$ with BAPTA abolished the effect of ginsenosides (Fig. 7). In this study we also showed that PLC activation and IP_3 generation are involved in the activation of Ca^{2+}-activated Cl^- channels by ginsenosides, since PLC inhibitor and heparin attenuated the effect of ginsenosides, respectively (Figs. 8 and 9).

Ginsenosides exhibited several interesting characteristics in regulating Ca^{2+}-activated Cl^- channels. Firstly, we found that follicular oocytes (stage V to VI) did not respond to ginsenosides but they responded to ginsenosides after defolliculation by collagenase. Secondly, in some oocytes the Cl^- currents evoked by ginsenosides diminished spontaneously after reaching peak amplitude even in the presence of ginsenosides (Figs. 1, 5B and 8A). Thus, the continuous presence of ginsenosides might exhibit a self-desensitization in Ca^{2+}-activated Cl^- channels. Moreover, ginsenosides also showed a cross-desensitization with muscarinic ACh receptor in the activation of Ca^{2+}-activated Cl^- channels (data not shown). These results suggest that ginsenosides were acting on a receptor sharing signaling pathway with other neurotransmitter receptor expressed in oocytes such as muscarinic receptor. However, ginsenosides and ACh act on different receptors, since atropine, a muscarinic receptor antagonist, had no effect on the ginsenosides response (data not shown). Thirdly, pretreatment of PTX (2 μg/ml, 16 hour) did not attenuate the stimulatory effect of ginsenosides on Ca^{2+}-activated Cl^- channels but intracellular injection of GTPγS abolished the effects of ginsenosides on Ca^{2+}-activated Cl^- channels (Table 1). In previous study, we showed that ginseng root extract and ginsenoside Rf regulate Ca^{2+} channels via

PTX-sensitive G proteins in sensory neurons.[2,22] These results suggest that ginsenosides could induce the activation of Ca^{2+}-activated Cl^- channels via PTX-insensitive G proteins in *Xenopus* oocytes.

In *Xenopus* oocytes Ca^{2+}-activated Cl^- channels might play a role as a fertilization signal, since the entry of sperm into oocyte induces the opening of these channels and also produces a transient depolarization to prevent polyspermy.[11] Currently, we do not know exactly what is the pharmacological or physiological role of ginsenosides in activating Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. However, one possibility is that ginsenosides might be involved in oocyte maturation, since ACh, which is also known to activate endogenous Ca^{2+}-activated Cl^- channels, facilitates oocyte maturation when it is co-treated with progesterone.[15] We also observed that the effect of ginsenosides on Ca^{2+}-activated Cl^- channels was progressively reduced following progesterone-treated oocytes as ACh (data not shown). We are now doing further investigation on the effect of ginsenosides on oocyte maturation in the absence or presence of progesterone.

In conclusion, we found that the processes involved in Ca^{2+} mobilization could be one of the explanations of ginsenosides-induced signal transduction pathway(s). We also provide a possibility in *Xenopus* oocytes that ginseng, which is known to exhibit a variety of medicinal efficacies, utilizes the signaling pathway that is common to a variety of neurotransmitters or hormones to exert its pharmacological or physiological action.

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요 약

*Xenopus oocytes*를 이용하여 인삼의 유효 성분으로 알려진 Ginseng total saponin(GTS)의 신호 전달 기작을 two electrode voltage clamp 방법을 이용하여 연구하였다. GTS는 세포 바깥에 처리했을 때 -20 mV보다 더 positive한 voltage에서 커다란 outward current를 유도하였다. 그러나, 세포 안쪽에 GTS를 injection할 경우 아무런 효과가 없는 것으로 나타났다. GTS처리에 의한 outward current 유발 효과는 GTS 투여 용량에 의존적인 것으로 나타났다(EC_{50}: 4.4 μg/ml). GTS의 작용은 Ca^{2+}-activated Cl^- channel blocker인 niflumic acid에 의하여 차단되었다. 칼슘 chelator인 BAPTA와 IP_3 수용체 길항제인 heparin을 세포내 injection에 의하여 차단되었다. 또한 active phospholipase C inhibitor(PLC)인 U-73122를 세포 바깥에 전처리할 경우에도 GTS의 작용이 부분적으로 억제되는 것으로 나타났다. 백일해 독소를 전처리할 경우 GTS의 작용은 억제되지 않은 것으로 나타났으나, GTP analog인 GTPγS를 세포내 injection할 경우 GTS의 작용은 억제되는 것으로 나타났다. 이러한 연구 결과는 GTS가 oocytes 세포막 성분과 상호 작용에 의하여 Ca^{2+}-activated Cl^- channel이 열리도록 하고, 이 과정에 PLC 활성 및 백일해 독소에 민감하지 않은 G 단백질 활성 및 IP_3에 민감한 세포내 Ca^{2+} store로부터 칼슘 방출을 유도하는 것으로 나타났다.

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