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Prevention of ginsenoside-induced desensitization of Ca^{2+}-activated Cl^- current by microinjection of inositol hexakisphosphate in *Xenopus laevis* oocytes: involvement of GRK2 and β-arrestin I

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We demonstrated that ginsenosides, the active ingredient of *Panax* ginseng, enhance endogenous Ca^{2+}-activated Cl^- currents via $G\alpha_{q/11}$-phospholipase C-β3 pathway in *Xenopus* oocytes. Moreover, prolonged treatment of ginsenosides induced Cl^- channel desensitization. However, it is not yet determined precisely what is molecular mechanisms are involved in ginsenoside-induced Cl^- channel desensitization. To provide answers to these questions, we investigated the changes in ginsenoside-induced Cl^- channel desensitization after intraoocyte injection of inositol hexakisphosphate ($InsP_6$), which is known to bind β-arrestins and interfere β-arrestins-induced receptor down-regulation, and cRNAs coding β-arrestin I/II and G-protein receptor kinase 2 (GRK2), which is known to phosphorylate G protein-coupled receptors (GPCRs) and attenuates agonist stimulations. When control oocytes were stimulated with ginsenosides, the second, third, and fourth responses to ginsenosides were 69.6 ± 4.1, 9.2 ± 2.3, and 2.6 ± 2.2%, of the first responses, respectively and this desensitization lasts for up to 8 h. Preintraoocyte injection of $InsP_6$ before ginsenoside treatment restored ginsenoside effect to initial response level with concentration, time, and structurally specific manner, in that inositol hexasulfate ($InsS_6$) had no effect. EC_{50} was 13.9 ± 8.7 μM. Injection of cRNA coding β-arrestin I but not β-arrestin II blocked $InsP_6$ effect on prevention of ginsenoside-induced Cl^- channel desensitization. Injection of cRNA coding GRK2 abolished ginsenoside effect enhancing Cl^- current. However, GRK2-caused loss of ginsenoside effect on Cl^- current was prevented by co-injection of GRK2 with GRK2-K220R, a dominant negative mutant of GRK

that lacks kinase activity. Treatment of PMA, a PKC activator, inhibited ginsenoside-induced Cl^- current responses. IC_{50} was 35.6 ± 4.7 nM. Preintraoocyte injection of $InsP_6$ did not inhibit PMA-caused loss of ginsenoside-induced Cl^- current responses. These results indicate that ginsenoside-induced Cl^- channel desensitization is mediated via activation of GRK2 and β-arrestin I and that $InsP_6$ maintains ginsenoside effect on Cl^- channel by preventing arrestin I action in *Xenopus* oocytes.

REFERENCES

Nah, S.Y., (1997) *Kor. J. Ginseng Sci.* **21,** 1-12

Nah, S.Y., and McCleskey, E. W. (1994) *J. Ethnopharmacol.* **42,** 45-51

Choi, S., Rho, S. H., Jung, S. Y., Kim, S. C., and Nah, S. Y. (2001) *British J. Pharmacol.* **132,** 641-648

Palczewski, K., Rispoli, G., and Detwiler, P. B. (1992) *Neuron* **8,** 117-126

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