Effect of Ginseng Saponin on Hypothalamus-Pituitary-Adrenal Axis under Stress in Mice

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ABSTRACT

Ginseng total saponins (GTS) injected intracerebroventricularly (i.c.v.) at doses from 0.1-1 μg inhibited the i.c.v. injection stress-induced plasma corticosterone levels in mice. The inhibitory action of GTS was blocked by co-administered N^G-nitro-L-arginine methyl ester (L-NAME; 1.5 μg, i.c.v.), an inhibitor of nitric oxide synthase (NOS). Of the ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, 20(S)-Rg_3, and 20(R)-Rg_3 injected i.c.v. at doses from 0.01 to 0.3μg(or 1 μg), 20(S)-Rg_3 and Rc significantly inhibited the o.c.v. injection stress-induced plasma corticosterone levels. The inhibitory actions of 20(S)-Rg_3 and Rc were blocked by co-administered L-NAME(1.5 μg, i.c.v.). These results suggest that GTS, 20(S)-Rg_3 and Rc may inhibit the i.c.v. injection stress-induced hypothalamo-pituitary-adrenal response by inducing NO production in the brain.

Key Words :Plasma corticosterone; Ginsenosides; 20(S)-Rg_3 ; Rc; Nitric oxide; Intracere broventricular injection stress; Mice

Introduction

Nitric oxide (NO) is an important molecule which is involved in the regulation of a vast array of biological functions[1,2]. Recent studies suggest that NO may be involved in the regulation of hypothalamic-pituitary-adrenal (HPA) axis. No synthase (NOS) was shown to be present in hypothalamus, median eminence and pituitary[3-5]. *In vivo* inhibition of NO production by N^G-Nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, increases the HPA response to various stimuli, including interleukin-1β, vasopressin, and oxytocin[6,7] or acute local inflammation[8], suggesting that NO may play a suppressive role in the responses of HPA axis[7].

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae) is a traditional medicine in Korea, China, and Japan, and it has become popular in Western countries. The major active ingredients of ginseng have been demonstrated to be a group of ginsenosides isolated and purified from ginseng

saponin fraction (ginseng total saponins; GTS) and whose chemical structures have been established[9-11]. Recently, accumulating evidence suggests that ginsenosides increase NO production in the various tissues. Ginsenosides induce relaxation of pulmonary artery[12] and corpus cavernosum[13], which is blocked by L-NAME. Additionally, ginsenosides increase NO production from endothelium of blood vessels[12], non- adrenergic, non-cholinergic nerves[14] and kidney[15]. Furthermore, several reports suggest that ginseng saponins are effective in inhibition of various stress-induced phenomena[16-18].

Recently, we proposed the intracerebroventricular (i.c.v.) injection trauma in mice as a stress model, which can be utilized in assessing the central effect of substances on the stress- induced activation of HPA axis[19]. In the present study, the potential modulatory effect of ginsenosides on the i.c.v. injection stress-induced HPA system and the involvement of NO therein were investigated in mice.

Materials and Methods

Animals and materials: Male ICR mice weighing 25-30 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22±1℃ with an alternating 12 hour light-dark cycle. Food and water were available ad libitum. Ginseng total saponins (GTS) and the ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 were obtained from the Korea Ginseng and Tobacco Research Institute (Taejeon, Korea). 20(S)-Rg_3, and 20(R)-Rg_3 were isolated from ginseng total saponins. GTS was composed of Rb_1(16.6%), Rb_2(5.4%), Rc(4.35%), Rd(1.2%), Re(2.5%), Rf(0.55%), Rg_1(2.5%) and Rg_3(0.1%). GTS and Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 were dissolved in a sterile saline just before use and was administered i.c.v. at doses from 0.01 to 1 μg. 20(S)-Rg_3, and 20(R)-Rg_3 were dissolved in concrete dimethylsulfoxide (DMSO) and diluted to 2% DMSO just before use and was administered i.c.v. at doses from 0.01 to 0.3 μg. Control animals received vehicle (saline or saline containing 2% DMSO). 2% DMSO did not affect the plasma corticosterone levels in our preliminary experiments. L-NAME was purchased from Research Biomedicals International (Natick, MA, USA) and dissolved in normal saline solution (0.9%NaCl). The dose of L-NAME represents the salt.

Intracerebroventricular (i.c.v.) injection stress and adminstration of drugs: To assess the effect of ginsenosides on the stress-induced activation of HPA axis, we used an i.c.v. injection-induced traumatic stimulus as a stress model in mice[19]. In this model, the effects of the drugs injected i.c.v. on the simultaneous i.c.v. injection trauma-induced HPA response can be evaluated. The i.c.v. administration was performed following the method described by Laursen and Belknap[20]. Briefly, the animal was injected at bregma with a 50 μl Hamilton syringe fitted with 26-ga. needle of which the tip was

adjusted to be inserted 2.4 mm deep. The i.c.v. injection volume was 5 μl and injection sites were verified by injecting the same volume of 1% methylene blue and then observing the distribution of the injected drugs or dye in the ventricular space. The dye injected i.c.v. was found to be distributed in the ventricular spaces and ventral surface of the brain and in the upper cervical portion of the spinal cord.

Experimental protocol: The activity of the HPA axis was evaluated by measuring plasma corticosterone levels. The i.c.v. injection was done consistantly between 8-9 A.M. to avoid diurnal variation of corticosterone. For the investigation of the effect of ginsenosides on the stress-induced rise of plasma corticosterone levels, plasma corticosterone levels were measured at 30 min after i.c.v. injection of vehicle or different doses of ginsenosides (0.01-0.3 μg or - 1 μg). In the experiment to determine the involvement of NO in the inhibitory effect of ginsenosides on the HPA axis, L-NAME(1.5 μg) and ginsenosides (0.3 or 1 μg) were co-administered by i.c.v. injection. Plasma corticosterone levels were determined fluorometrically by the method of Glick *et al.*, [21] Statistical analysis was carried out by one- way analysis of variance (ANOVA) with post-hoc test. *P* values less than 0.05 were considered to indicate statistical significance.

Results

Effects of GTS on i.c.v. injection stress-induced corticosterone levels

To determine if centrally administered GTS can affect the i.c.v. injection stress-induced plasma

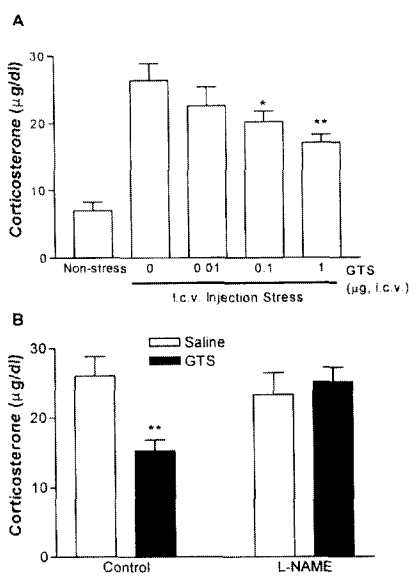

Fig. 1.
(A) Effect of various doses of ginseng total sapomins (GTS) on the intracerebroventricular (i.c.v.) injection stress-induced plasma corticosterone levels at 30min after the injection. The data were means ± s.e.m.(n=16). *P<0.05, **P<0.01, significantly different from saline treated animals (GTS 0 μg). (B) Effects of co-administration of L-NAME (N^G-Nitro-L-arginine methyl ester HCl) on the GTS-induced decrease in i.c.v. injection stress-induced plasma corticosterone levels. L-NAME (1.5μg) was co-injected with either saline(open columns) or GTS(1 μg). Blood samples were obtained 30 min after the injection. The data were means ± s.e.m. (n=10). **P<0.01, significantly different from saline treated control animals.

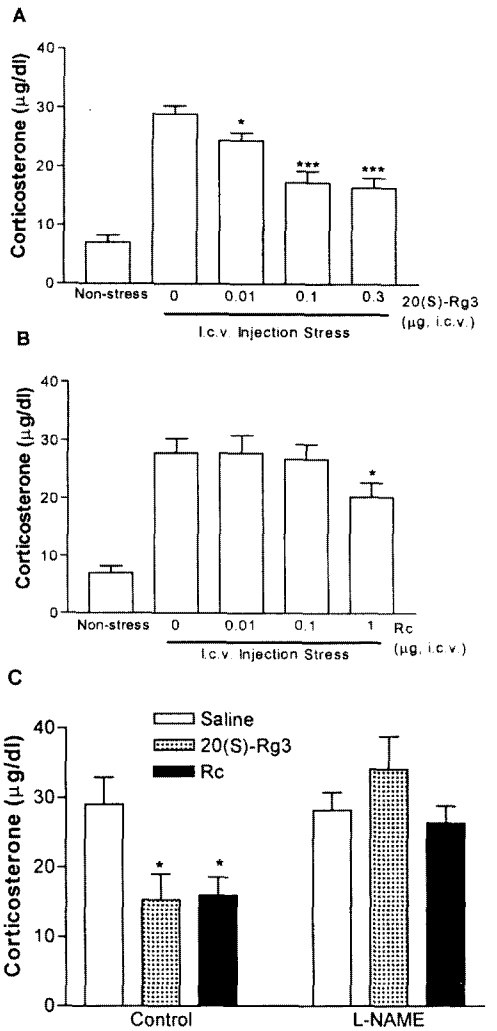

Fig. 2

Effect of various doses of ginsenosided 20(S)-Rg_3 **(A)** and Rc **(B)** on the i.c.v. injection stress-induced plasma corticosterone levels at 30min after the injection. The data were means ± s.e.m. (n=16). *P<0.05, ***P<0.001, significantly different from saline treated amimals (Rg_3 or Rc 0 μg). **(C)** Effects of co-administration of L-NAME (N^G-Nitro- L-arginine methyl ester HCL) on the 20(S)-Rg_3 and Rc-induced decrease in i.c.v. injection stress-induced plasma corticosterone levels. L-NAME (1.5μg) was co-injected with saline (open columns) or 20 (S)-Rg_3 (0.3 μg) or Rc (1 μg). Blood samples were obtained 30 min after the injection. The data were means ± s.e.m.(n=10). *P<0.05, significantly different from saline treated control animals.

corticosterone levels, various doses of GTS (0.01-1 μg) were added to the saline (5 μl)injected i.c.v.

GTS injected i.c.v. at doses of 0.1-1 μg inhibited the i.c.v. injection stress-induced plasma corticosterone levels(Fig. 1A). L-NAME (1.5μg, i.c.v.), an inhibitor of NOS, co-injected i.c.v. with GTS (1 μg) completly blocked the GTS-induced inhibition of i.c.v. injection stress-induced plasma corticosterone levels, while L-NAME(1.5μg, i.c.v.)alone had no effect on plasma corticosterone levels (Fig. 1B).

Effects of individual ginsenosides on i.c.v. injection stress-induced corticosterone levels

To determine the active ginsenosides that can inhibit the stress-induced plasma corticosterone levels, various doses (0.01-0.3 or-1 μg) of ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, 20(S)-Rg_3, and 20(R)-Rg_3 were added to the saline (5 μl)injected i.c.v. 20(S)-Rg_3 (0.01-0.3μg) significantly inhibited the i.c.v. injection stress-induced plasma corticosterone levels in a dose-sependent manner (Fig. 2A), whereas Rc was effective only at the dose of 1 μg (Fig. 2B). The effect of 20(S)-Rg_3 was stereospecific in that 20(R)-Rg_3 was totally inactive in the dose ranges. Ginsenosides Rb_1, Rb_2, Rd, Re, Rf, and Rg_1 were ineffective at the doses used in this experiment. L-NAME(1.5μg, i.c.v.), co-injected i.c.v. with 20(S)-Rg_3 (0.3μg)or Rc(1μg) completely blocked the 20(S)-Rg_3 or Rc-induced inhibition of i.c.v. injection stress-induced plasma corticosterone levels (Fig. 2C).

Discussion

The present study shows that GTS, 20(S)-Rg_3, and Rc administered i.c.v. attenuate the i.c.v. injection stress-induced increase in plasma corticosterone levels, and the effects of these ginsenosides were blocked by L-NAME. These results suggest that i.c.v. administered GTS, 20(S)-Rg_3 and Rc may induce an increase in NO release in the brain, which may inhibit the i.c.v. injection stress-induced HPA response. Recent studies have demonstrated that NO tonically inhibits the vasopressin- or oxytocin-, but not corticotropin releasing factor (CRF)- induced activation of HPA axis. Thus, it can be speculated that ginseng total saponins, 20(s)-Rg_3 and Rc induce NO, which in turn may inhibit the vasopressin-or oxytocin-induced activation of HPA axis. However, the exact anatomical sites, neuronal circuits, and synaptic mechanisms of ginsenosides-induced inhibition of stress-induced HPA response remain to be further clarified.

The inhibitory effect of ginsenosides on the stress-induced plasma corticosterone levels may not be specific to the i.c.v. injection-stress in which administration of drugs was into i.c.v., because intraperitoneal injection of GTS also inhibited immobilization stress-induced plasma corticosterone levels (unpublished observation). Some studies have also reported that ginseng shows inhibitory effects on stress-induced phenomena when applied to stressful conditions such as footshock[16], cold[17,18] and heat[18].

Acutely, the i.c.v. injection stress-induced increase in plasma corticosterone level is beneficial to the organism in dealing with the stressful situation by maintaining homeostasis. However, it is well known that excessively high concentrations of plasma corticosterone levels over a prolonged period of time can deteriorate immune functions and damage hippocampal neurons[22-23]. Therefore, it can be suggested that the inhibitory effects of ginsenosides on the stress-induced plasma corticosterone levels may be beneficial in attenuating excessively high rise of plasma corticosterone and thus preventing the harmful effects on the target organs.

20(S)-Rg_3 was much more potent in inhibiting the i.c.v. injection stress-induced HPA response than Rc which was only effective at the dose of 1 μg. These results indicate that 20 (S)-Rg_3 may be one of the responsible ginsenosides for the inhibition of stress-induced plasma corticosterone response by GTS. In our preliminary experiments, 20(S)-Rg_3 was also found to be more potent in endothelium-dependent vasodilatation than any other ginsenosides tested (unpublished observation). Actually, 20(S)-Rg_3 is not present in the natural ginseng root, but is formed during the process of steaming the dried ginseng root (white ginseng) to form the red ginseng. Traditionally red ginseng has been presumed to have more valuable pharmacological effects than white ginseng. Thus, the stress-modulating activity of 20(S)- Rg_3 may be, at least in part, one of the reasons for the purported superiority of red ginseng to white ginseng in some pharmacological modes of action. However, other ginsenosides including Rc may also be involved in the modulatory effects of GTS on stress-induced plasma corticosterone levels.

Conclusion

GTS, 20(S)-Rg_3, and Rc administered i.c.v. attenuated the i.c.v. injection stress-induced increase in plasma corticosterone levels, and the inhibitory actions of these ginsenosides were blocked by L-NAME. Thus, the present study suggests that GTS, 20(S)-Rg_3 and Rc may inhibit the i.c.v. injection stress-induced hypothalamo-pituitary-adrenal response by inducing NO production in the brain. The inhibitory effects of ginsenosides on the stress-induced plasma corticosterone levels may be beneficial in preventing the harmful effects of an excessively high rise of plasma corticosterone on the target organs in stressful cirmcumstances.

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References

1. Moncada S, Palmer R and Higgs E. *Pharmacol Rev* **43**, 109-42 (1991).
2. Nathan C. *FASEB J* **6**, 3051-64 (1992).
3. Sagar S and Ferriero D. *Brain Res* **400**, 348-52 (1987).
4. Vincent S and Kimura H. *Neuroscience* **46**, 755-84 (1992).
5. Bredt D, Hwang P and Snyder S. *Nature* **347**, 768-70 (1990).
6. Rivier C and Shen G. *J Neurosci* **14**, 1985-93 (1994).
7. Rivier C. *Endocrinology* **136**, 3597-603 (1995).
8. Turnbull A and Rivier C. *Endocrinology* **137**, 455-63 (1996).
9. Sanada S, Kondo N, Shoji J *et al. Chem Pharm Bull* **22**(10), 2407-2412 (1974).
10. Sanada S and Shoji J. *Chem Pharm Bull* **26**(6), 1694-1697 (1978).
11. Nagai Y, Tanaka O and Shibata S. *Terahedron* **27**, 881 (1971).
12. Kim H, Chen X and Gillis C. *Biochem Biophys Res Commun* **189**, 670-6 (1992).
13. Chen X and Lee T. *Br J pharmacol* **115**, 15-8 (1995).
14. Peng C, Li Y, Li Y *et al. J Pharm Pharmacol* **47**, 614-7 (1995).
15. Han S and Kim H. *Int J Biochem Cell Biol* **28**, 573-80 (1996).
16. Takahashi M, Tokuyama S and Kaneto H. *Japan J Pharmacol* **59**, 399-404 (1992).
17. Cheng XJ, Liu YL, Lin GF *et al. Acta Pharmacologica Sinica* **8**, 486-489 (1987).
18. Kim C, Kim CC, Kim MS *et al. Lloydia* **33**(1), 43-48 (1970).
19. Kim DH, Jung JS, Song DK *et al. J Pharmacol Toxicol Methods,* in press (1998).
20. Laursen S and Belknap J. *J Pharmacol Methods* **16**, 355-7 (1986).
21. Glick D, Redlich D and Levine S. *Endocrinology* **74**, 653-655 (1964).
22. Kerr D, Campbell L, Applegate M *et al. J Neurosci* **11,** 1316-24 (1991).
23. Sapolsky R, Krey L and McEwen B. *J Neurosci* **5**, 1222-7 (1985).