Inhibition of Intracerebroventricular Injection Stress-Induced Plasma Corticosterone Levels by Intracerebroventricularly Administered Compound K, a Ginseng Saponin Metabolite, in Mice

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Effects of major intestinal metabolites of ginsenosides, including compound K (III-901, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol), compound Y (III-902, 20-O-α-L-arabinopyranosyl (1→6)-β-D-glucopyranosyl)-20(S)-protopanaxadiol), and ginsenoside Mc (III-903, 20-O-α-L-arabinofuranosyl (1→6)-β-D-glucopyranosyl)-20(S)-protopanaxadiol), on acute stress-induced plasma corticosterone levels were studied in mice. Intracerebroventricularly (i.c.v) administered compound K (1 μg) attenuated the i.c.v. injection stress-induced increase in plasma corticosterone level, and this inhibitory effect was not affected by co-administered N\(^{\text{G}}\)-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor. Compound K administered intraperitoneally affected neither the i.c.v. injection stress- nor the immobilization stress-induced increase in plasma corticosterone levels. Compound K and ginsenoside Mc did not affect plasma corticosterone levels induced by the two stress modalities used in this study.

Key words compound K; compound Y; ginsenoside Mc; intracerebroventricular injection stress; immobilization stress; hypotalamo-pituitary-adrenal axis

Ginseng, the root of Panax ginseng C. A. MEYER (Araliaceae), has been used as a folk medicine for thousands of years in East Asian countries and it has recently also become popular in Western countries. The major active ingredients of ginseng have been demonstrated to be ginsenosides (ginseng saponin). It has been reported that ginseng shows anti-stress activities when applied to such stressful conditions as footshock, cold and heat in animals. The hypothalamo-pituitary-adrenal (HPA) axis is one of the most important systems closely related to stress. Previously, we showed that ginseng total saponin and ginsenoside Re administered intracerebroventricularly (i.c.v) attenuate the i.c.v. injection stress-induced increase in plasma corticosterone level.1 In addition, ginseng total saponin and ginsenoside Rc administered intraperitoneally also attenuate the immobilization stress-induced increase in plasma corticosterone levels.

Recent studies have shown that ginsenosides such as Rb\(_1\), Rb\(_2\), Rc, Re and Rg\(_2\) are metabolized by intestinal bacteria to the metabolites compound K, compound Y and ginsenoside Mc in humans as well as in rats. However, there has been no report on the effects of these intestinal metabolites of ginsenosides on the HPA axis. In the present study, we examined the effects of compound K, compound Y, and ginsenoside Mc of ginsenosides on plasma corticosterone levels induced by acute stress-i.c.v. injection stress and immobilization stress.

MATERIALS AND METHODS

Animals Male ICR mice weighing 25—30 g, supplied by Myung-Jin, Inc. (Seoul, Korea), were used for all the experiments. The animals were housed 5 per cage in a room maintained at 22±1°C with an alternating 12-h light–dark cycle. Food and water were available ad libitum.

Drugs Compound K (III-901, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol), compound Y (III-902, 20-O-α-L-arabinopyranosyl (1→6)-β-D-glucopyranosyl)-20(S)-protopanaxadiol), and ginsenoside Mc (III-903, 20-O-α-L-arabinofuranosyl (1→6)-β-D-glucopyranosyl)-20(S)-protopanaxadiol) (Fig. 1) were supplied by Central Research Institute, IL-HWA Co., Ltd. (Guri, Korea). All three substances were dissolved in 100% dimethylsulfoxide (DMSO) and diluted to 2% DMSO just before use. Control animals received saline containing 2% DMSO, which did not affect the plasma corticosterone levels in preliminary experiments. N\(^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME, Sigma Chemical Co., U.S.A.) was dissolved in normal saline solution (0.9% NaCl) and the dose of L-NAME represents the salt. Dexamethasone-Water Soluble (Sigma Chemical Co., U.S.A.) was dissolved in normal saline solution (0.9% NaCl).

Intracerebroventricular (i.c.v) Injection Stress and Administration of Drugs For the i.c.v. injection stress model, we used an i.c.v. injection-induced traumatic stimulus as a stress model in mice. 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. Briefly, the animal was injected at bregma with a 50 μl Hamilton syringe fitted with a 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 injection 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.

Immobilization Stress The immobilization stress proce-
procedure consisted of restraint of each animal for 30 min in a 50 ml Corning tube, with the nose of the mouse at the tip of the tube. Adequate ventilation was provided by means of a hole at the tip of the tube.

**Corticosterone Assay** Four hundred microliters of blood was collected by puncturing the retro-orbital venous plexus. Plasma was separated by centrifugation and stored at −80°C until assayed. Plasma corticosterone levels were determined by the fluorometric determination method.15)

**Experimental Protocol** One day prior to the experiment, the mice caged in groups of five were allowed to be aclimatized to the condition of a quiet laboratory room overnight. I.c.v. or intraperitoneal injections were performed between 9—11 am each day to avoid the diurnal variation of plasma corticosterone levels. Each mouse was bled once and sacrificed. When a mouse was bled from the retro-orbital plexus and killed, other remaining mice were separated to avoid visual and auditory stimulation. To study the effect of compound K, compound Y and ginsenoside Mc on the i.c.v. injection stress-induced rise of plasma corticosterone levels, each mouse was bled once and sacrificed. When a mouse was bled from the retro-orbital plexus and killed, other remaining mice were separated to avoid visual and auditory stimulation. To study the effect of compound K, compound Y and ginsenoside Mc on the i.c.v. injection stress-induced rise of plasma corticosterone levels, plasma corticosterone levels were measured 30 min after an i.c.v. injection of each compound. To determine the involvement of NO in the inhibitory effect of compound K and dexamethasone on the HPA axis, l-NAME (1.5 μg) was co-administered with either compound K (1 μg) or dexamethasone (0.3 μg) by i.c.v. injection.

To examine their effect on immobilization stress-induced plasma corticosterone levels, compound K, compound Y and ginsenoside Mc (1, 2 mg/kg) were injected intraperitoneally 15 min before the 30 min immobilization stress. Immediately after the completion of immobilization stress, blood was collected for the assays of plasma corticosterone levels.

**Statistical Analysis** Statistical analysis was carried out using the Student’s t test or one-way analysis of variance (ANOVA) with post-hoc Bonferroni test. p values less than 0.05 were considered to indicate statistical significance.

**RESULTS**

**Effects of Compound K, Compound Y and Ginsenoside Mc Administered i.c.v. on the i.c.v. Injection Stress-Induced Plasma Corticosterone Level** Compound K (0.01—1 μg, i.c.v.) dose-dependently inhibited the i.c.v. injection stress-induced plasma corticosterone level, and the inhibition reached statistical significance at the dose of 1 μg (Fig. 2). However, compound K administered intraperitoneally (2 mg/kg) 15 min before i.c.v. injection stress did not
i.c.v. significantly inhibited the i.c.v. injection stress-induced pound K, compound Y, and ginsenoside Mc; stress-induced plasma corticosterone levels (control, compound K, compound Y and ginsenoside Mc administered in-duced Increase in Plasma Corticosterone Level

Fig. 3. Effects of L-NAME (N^G-nitro-L-arginine Methyl Ester HCl) on the Compound K- and Dexamethasone-Induced Decrease in i.c.v. Injection Stress-Induced Plasma Corticosterone Level

L-NAME (1.5 μg) was co-injected with compound-K (1 μg) or dexamethasone (0.3 μg). Blood samples were obtained 30 min after the injection. The data were means±S.E.M. (n=8). *p<0.05, significantly different from saline treated animals.

daffect the i.c.v. injection stress-induced plasma corticosterone levels (control, compound K; 30.5±1.9, 33.1±1.9 (μg/dl), respectively, n=7). Compound K administered intraperitoneally (2 mg/kg) also did not affect basal plasma corticosterone levels (control, compound K; 12.7±0.8, 12.1±1.1 (μg/dl), respectively, n=7). Compound Y and ginsenoside Mc injected i.c.v. did not affect the i.c.v. injection stress-induced plasma corticosterone levels at the doses used in this experiment (Fig. 2).

Effects of Co-administered L-NAME on the Compound-K or Dexamethasone-Induced Decrease in the i.c.v. Injection Stress-Induced Plasma Corticosterone Level

L-NAME (1.5 μg, i.c.v.), co-injected with compound K (1 μg, i.c.v.) or dexamethasone (0.3 μg, i.c.v.) did not affect the compound K- or dexamethasone-induced inhibition of i.c.v. injection stress-induced plasma corticosterone levels (Fig. 2), and L-NAME (1.5 μg, i.c.v.) alone had no effect on plasma corticosterone level (Fig. 3).

Effects of Compound K, Compound Y and Ginsenoside Mc Administered i.p. on the Immobilization Stress-Induced Increase in Plasma Corticosterone Level

Compound K, compound Y and ginsenoside Mc administered intraperitoneally (1, 2 mg/kg) did not affect the immobilization stress-induced plasma corticosterone levels (control, compound K, compound Y, and ginsenoside Mc; 46.1±2.6, 45.5±4.6, 48.8±1.2, and 45.5±3.0, respectively, at the dose of 1 mg/kg, n=8; 42.2±3.9, 49.6±4.7, 51.3±2.5, and 41.5±2.1, respectively, at the dose of 2 mg/kg, n=8).

DISCUSSION

The present study showed that i.c.v. administered compound K, one of the major intestinal metabolites of ginsenosides, attenuated i.c.v. injection stress-induced increase in plasma corticosterone levels. Previously we showed that ginsenoside Rc (1 μg) or ginseng saponin (0.1—1 μg) injected i.c.v. significantly inhibited the i.c.v. injection stress-induced plasma corticosterone increase at the similar effective dose of compound K (1 μg). However, the mechanism of compound K action may be different from that of ginsenoside Rc and ginseng total saponin: L-NAME blocked the effect of ginsenoside Rc and ginseng total saponin but not compound K (Fig. 3), suggesting that nitric oxide may not be involved in compound K action. Similarly to the case of compound K, the inhibitory effect of dexamethasone, used as a standard HPA axis inhibitor, was also unaffected by L-NAME (Fig. 3). The exact mechanism of compound K action remains to be investigated.

Although effective when administered i.c.v., compound K injected intraperitoneally (1—2 mg/kg) did not affect the i.c.v. injection stress-induced increase in plasma corticosterone level, suggesting that compound K may not pass the blood-brain barrier, and that its action site is not peripheral but in the brain. Recently we reported that intraperitoneally injected ginseng total saponin or ginsenoside Rc attenuates immobilization stress-induced increase of plasma corticosterone levels. But, compound K injected intraperitoneally did not affect the immobilization stress-induced plasma corticosterone level, further illustrating a difference from ginsenoside Rc and ginseng total saponin. Furthermore, compound K administered intraperitoneally did not affect basal plasma corticosterone levels, while systemic administration of ginseng total saponin and various ginsenosides has been shown to increase these levels.

In conclusion, we found compound K to have HPA axis-modulating activity when administered centrally. However, because only centrally but not systemically administered compound K was effective, the physiological significance of this HPA axis-modulating activity may not be great. Further studies on other ginsenoside metabolites on the HPA axis are needed for more complete understanding of the effects of ginseng saponin and its metabolites on stress.

Acknowledgements This research was supported by research grants from the Korea Research Foundation (1996) and from Hallym University (1999).

REFERENCES


