ADRENOCORTICOTROPIN AND CORTICOSTERONE SECRETION BY GINSENG SAPONIN

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Abundant evidence has now accumulated indicating that ginseng can increase resistance to a wide variety of stresses: physical, chemical and biological stresses.[1-3] This suggested that ginseng somehow potentiates the function of the adrenal cortex and increases plasma corticosterone concentration. However it is still uncertain whether the anti-stress action of ginseng is mediated via the adrenal cortex or the pituitary-adrenocortical system

In attempting to clear the situation, it appears necessary to determine the plasma adrenocorticotropin (ACTH) response and the corticosterone response to ginseng, or to determine the changes in rate of their synthesis and secretion, and also to identify the active principle for this action.

We had previously reported in the 1st International Ginseng Symposium that purified ginseng saponin increased adrenocortical cyclic AMP in intact rats, but not in hypophysectomized rats.[4,5] The finding clearly suggested that synthesis and secretion of ACTH and corticosteroids were stimulated by ginseng saponin. We also presented a perliminary result in the symposium that ginseng saponin did increase plasma 11-hydroxycorticoid concentration which was determined by the fluorometric method.

In the present paper, we determined plasma ACTH by the radioimmunoassay method and plasma corticosterone by the competitive protein binding method of Murphy.[6] As for the ginseng principle, purified ginseng saponin mixture, fraction 5 or 6, and purified ginsenoside Rb_1, Rb_2, Rc, Rd and Re[7] were used. Rats were sacrificed by decapitation at various times after the treatment with ginseng saponin or pyrogen-free saline. Then plasma specimen was prepared from the

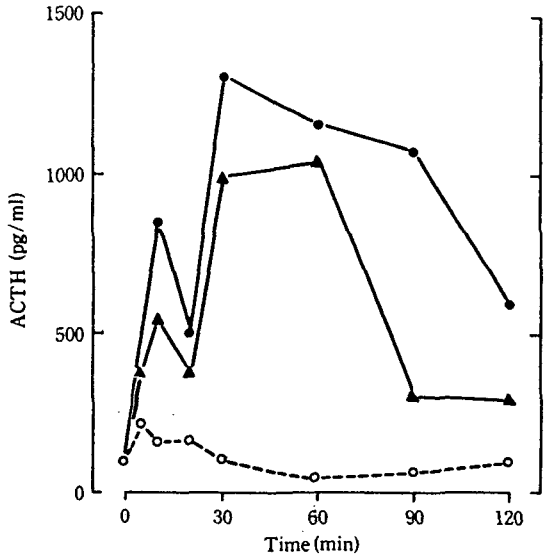

Fig. 1. Effect of ginseng saponin on plasma ACTH. Rats were received fraction 5 or 6 (7 mg/100g, i.p.) or saline (0.5 m*l*). ACTH was determined with pooled plasma of 3 or 4 rats in duplicate. Values at 0 time are for plasma from untreated 6 rats. Animals were sacrificed between 09:00 and 10:00 hr. ●, ▲ Fraction 5, 6; ○ saline-treated control.

Table 1. Effect of ginseng saponin on plasma ACTH and corticosterone.

Time after treatment (min)	ACTH (pg/ml) Saline* treated	Saponin** treated	P	Corticosterone (μg/dl) Saline* treated	Saponin** treated	P
0***	93 ± 21	—	—	4.6 ± 2.1	—	—
30	96 ± 34	1250 ± 50	<0.001	12 ± 8	48 ± 7	<0.02
60	48 ± 6	1430 ± 280	<0.01	0.9 ± 0.6	52 ± 5	<0.001
90	133 ± 27	750 ± 110	<0.01	6.8 ± 3.2	51 ± 5	<0.01

Animals were sacrificed between 09:00 and 10:00 hr. Data were expressed as means ± S.E. for 3 rats.
* 0.5 ml of saline; ** fraction 5, 7 mg/100 g, i.p.; *** taken from Fig. 1, 6 non-treated rats.

trunk blood and used for the determinations.

Fig. 1 shows the kinetic pattern of plasma ACTH concentration, which was assayed in duplicate with the pooled plasma of each group of rats. Plasma ACTH markedly increased 5, 10 and 30 min after the treatment with fraction 5 or 6 saponin in doses of 7 mg/100 g of body weight. Plasma ACTH concentration remained high for 30 or 60 min and then rapidly decreased. In saline-treated control rats, the increase in ACTH was small and transient.

Plasma ACTH and corticosterone of individual rat were determined in duplicate. Table 1 shows that the increase of both ACTH and corticosterone by ginseng saponin was statistically significant vs. saline-injected control 30, 60 and 90 min after the treatment. The increase of plasma ACTH was almost parallel to that of plasma corticosterone.

Fig. 2 shows that plasma corticosterone concentration increased linearly, and reached a maximum level 30 min after the treatment. Saline injection also induced a small rise in plasma corticosterone at 5 min which was followed by a fall to the resting level.

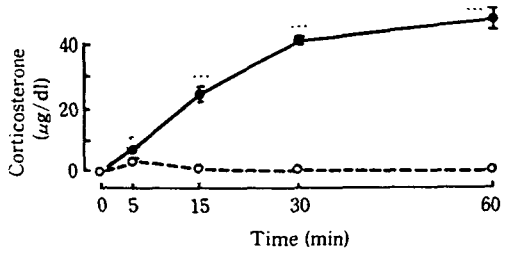

Fig. 2. Effect of intraperitoneal administration of ginseng saponin on plasma corticosterone. ● fraction 6 (7 mg/100 g); ○ saline (0.5 ml); *$p < 0.05$; ***$p < 0.001$.

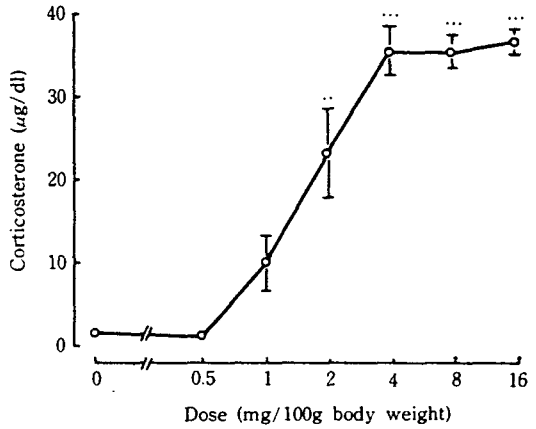

Fig. 3. Dose-response relation between graded doses of fraction 6 and plasma corticosterone in "gentled" rats. Animals were sacrificed between 09:00 and 10:00 hr, 30 min after the treatment. Data were expressed as means ± S.E. for 6 rats.
** $p < 0.01$, ***$p < 0.001$.

Fig. 3 shows a dose-response relation between ginseng saponin and plasma corticosterone concentration 30 min after the treatment. Ginseng saponin induced an increase of plasma corticosterone in a dose-dependent manner in doses of 0.5 mg to 4 mg/100 g of body weight. Ginseng saponin in doses of 4 mg and over seemed to show a maximum response in plasma corticosterone, and the half maximum was obtained in doses of about 1.5 mg/100 g. When 3.5 or 7 mg/100 g of ginseng saponin were administered, the maximum level of plasma corticosterone was kept for about 0.5 and 1.5 hr, respectively. So a large dose of ginseng saponin seemed to lengthen the duration of the maximum level of plasma corticosterone, and to increase the total amount of secreted corticosterone. Thus ginseng probably acted primarily on the hypothalamus to secrete ACTH, and the ACTH acted secondarily on the adrenal cortex to secrete

Table 2. Effect of dexamethasone pretreatment on ginseng saponin-induced increase in plasma corticosterone.

Pretreatment	Corticosterone (μg/dl)		P
	Saline treated	Saponin treated	
Saline	5.6 ± 1.2	51 ± 8	<0.001
Dexamethasone	0.2 ± 0.1	10 ± 4	<0.05
P	<0.01	<0.01	—

Dexamethasone (35 μg/100 g, i.p.) or saline (0.5 ml) was administered to rats 2.5 hr before the second treatment. As the second treatment, fraction 6 (7 mg/100 g, i.p.) or saline (0.5 ml) was administered 30 min before decapitation. Animals were sacrificed between 09:00 and 10:00 hr. Data were expressed as means ± S.E. for 5 rats.

Table 3. Effect of isolated ginsenosides on plasma corticosterone.

Treatment (mg/100 g)	No. of rats	Corticosterone (μg/dl)	P
Saline	8	3.9 ± 0.6	—
Rb_1 7	5	41 ± 4	<0.001
3.5	10	19 ± 5	<0.02
Rb_2 7	5	31 ± 6	<0.001
3.5	5	31 ± 9	<0.01
Rc 3.5	4	50 ± 3	<0.001
Rd 3.5	4	38 ± 12	<0.01
Re 3.5	5	16 ± 6	<0.05

Animals were sacrificed 09:00–10:00 hr, 30 min after the treatment. Data were expressed as means ± S.E.

and synthesis corticosteroid.

Dexamethasone blocks ACTH secretion and therefore corticoid secretion, and the site of the blocking action is known to be the hypophysis and hypothalamus. Rats were treated with dexamethasone 2.5 hr before the ginseng administration, and plasma corticosterone was determined 30 min after the ginseng treatment. As shown in Table 2, the saponin-induced level of plasma corticosterone in dexamethasone-treated rats was clearly depressed from the saponin-induced level of corticosterone without dexamethasone treatment. This result gives further piece of evidence that ginseng saponin acted on the hypophysis and/or hypothalamus primarily, but not on the adrenal cortex directly.

Several ginsenosides, whose sugar moieties are different from each other, were administered to rats intraperitoneally, and the plasma corticosterone was determined 30 min after the treatment. Table 3 shows that all ginsenosides Rb_1, Rb_2, Rc, Rd and Re significantly increased plasma corticosterone in doses of 3.5 mg/100 g. So the stimulating activity of ginseng saponins may be mainly due to their sapogenin moiety, protopanaxadiol or protopanaxatriol. Among these, ginsenoside Rb_2 and Rd seemed more effective than Rb_1 and Re. Glycyrrhizin and saikosaponin-c whose genin are different from those of ginsenosides were inactive in doses of 10 mg/100 g. Therefore the stimulatory activity of ginseng saponins was not an attribute of triterpenoidal saponin.

It is known that epinephrine, insulin or histamine injection stimulates corticosteroid secretion. So we determined plasma glucose and immunoreactive insulin as well as corticosterone. Fig. 4 shows that ginseng tended to increase plasma glucose and to decrease plasma immunoreactive insulin but these responses were not significant statistically vs. those in saline-treated control. Thus ginseng-induced corticosterone secretion seemed not to involve significant release of epinephrine and insulin.

Fig. 5 shows that histamine-induced corticosterone secretion was accompanied by a hyperglycemia, and histamine-induced responses

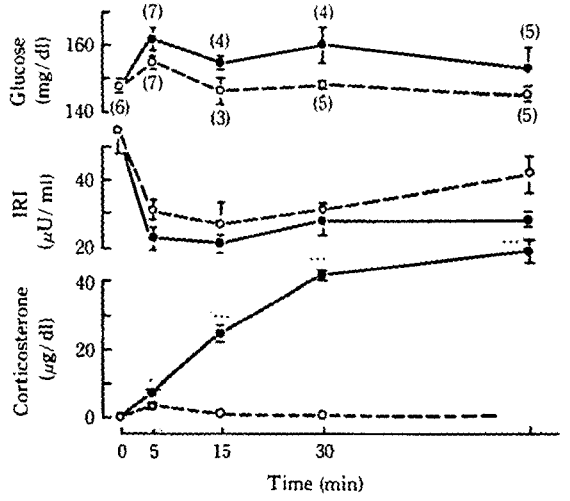

Fig. 4. Effect of intraperitoneal administration of ginseng saponin on plasma corticosterone, glucose and IRI. Means ± SE. Numbers in parentheses. ● Ginseng saponin (7 mg/100 g); ○ saline (0.5 ml); * $p < 0.05$; *** $p < 0.001$.

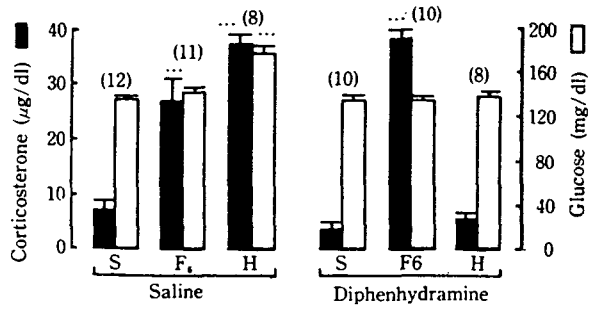

Fig. 5. Effect diphenydramine on ginseng saponin and histamine-induced increase in corticosterone and glucose. Diphenhydramine (2.5 mg/100 g) or saline (0.1 m*l*) was administered intraperitoneally 45 min before the second treatment. Ginseng saponin (F6, 7 mg/100g), histamine (H, 0.5 mg/100g) or saline (S, 0.5 m*l*) was administered intraperitoneally 30 min before decapitation. Means ± SE, numbers in parentheses. ***$p < 0.001$.

were blocked by H_1-receptor antagonist, diphenhydramine, but ginseng saponin-induced responses were not. This implies that ginseng-induced corticosterone secretion did not involve histamine release.

Some triterpenoidal saponins, for example, escin and saikosaponin- a and- d stimulated the pituitary-adrenocortical system. But their stimulation was accompanied by a transient but marked hyperglycemia and a transient hypoinsulinemia.[8]

Now we may summarise our results.[9,10] The first. Ginseng stimulates hypothalamohypophysial system to secrete ACTH. The second. The ginseng-induced ACTH acts on the adrenal cortex to increase cyclic AMP. The third. The increased cyclic AMP induces secretion and synthesis of corticosteroid, which results in an increase of plasma corticosterone. The fourth. The active principle of ginseng for the action is ginseng saponin. The fifth Ginseng saponin is a kind of stressful agent which has some different features in the stimulation of the hypothalamohypophysial-adrenocortical system from insulin-induced acute hypoglycemia, epinephrine, histamine and other saponins.

The present results support the findings by Petkov and Staneva, and proved their suggestion. Some of the known biochemical and pharmacological actions of ginseng saponin as well as ginseng extract may be ascribed partly to the actions of ACTH and corticosteroid secreted by ginseng saponin. Kaku et al.[11] reported that the repeated administration of ginsenoside Rf, Re or Rd caused facilitation of the conditioned avoidance response. Retkov[12] established that an extract of ginseng improved the indices for learning and memory retention in rats. On the other hand, it has been reported recently that ACTH and the related peptides enhance the retention of learned response in rats. Thus, we may suggest that the effect of ginseng saponin and extract may be due to the increase in ACTH secretion.

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