Central Effects of Ginsenosides on the Feeding Behavior and Response to Stress in Rats

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Abstract: To clarify central mechanisms of ginsenosides, changes in ingestive and ambulatory behaviors were investigated in rats after single or continuous infusion into the third cerebroventricle or various hypothalamic loci. Following single infusion into the third cerebroventricle, ginsenoside Rb_1 at doses of 0.05, 0.10 and 0.20 μmol dose-dependently decreased food intake. None of the doses tested affected ambulation. Drinking suppression was only observed at the maximum dose of 0.20 μmol. Equimolar injections into the peritoneum had no effects on ingestive behavior or ambulation. These findings indicated that ginsenoside Rb_1 specifically and centrally inhibited food intake. According to analyses of daily feeding patterns, this feeding suppression was the result of a decrease in meal size, not from changes in the postprandial intermeal interval or eating speed. The suppressed food intake was accompanied by hyperglycemia, leaving plasma insulin unaffected. Unilateral microinjection of 0.01 μmol ginsenoside Rb_1 into the ventromedial hypothalamus specifically decreased food intake, although equimolar injection into the lateral hypothalamic area did not affect food intake. Following continuous infusion of Rg_1 into the third cerebroventricle, the feeding inhibition due to surgical operation was attenuated. Rb_2 administered by the same procedure abolished the toxic effect of toxohormone-L on food intake. Taken together, these findings suggest that ginsenosides as a whole may have pharmacological potency to maintain feeding at a certain physiological level.

Key Words: Ginsenoside Rb_1, Rb_2 & Rg_1, Food Intake, Response to Stress, Decreased Meal Size, Hyperglycemia and Euinsulinemia, Microinjection into the Ventromedial or Lateral Hypothalamus

Certain humoral factors, in particular glucose, free fatty acids and insulin, have been shown to have an important in the central control of food intake[1]. Ginsenosides have been known to decrease serum cholesterol, triglyceride and free fatty acids, and improve hyperglycemia observed in rats[2,3]. These findings led us to investigate the central effects of ginsenosides on ingestive behavior and ambulation, concomitant changes in plasma glucose and insulin[4]. and the response to stress in rats. In addition, we attempted to clarify the involvement of the rat hypothalamic loci[4].

General Procedure

Male Wistar King A rats weighing 280-320g were housed in a soundproof room which was illuminated daily from 0800-2000hr and maintained at a temperature of 21±1℃ and humidity of 55±5%. The rats had free access to standard rat chow (mean pellet weight±SE, 49.8±0.4 mg) and tap water (mean droplet volume±SE, 35.2±0.6 μl) unless otherwise noted. The test chamber(30×25×25 cm) used for assessing ingestive and ambulatory patterns was equipped with a pellet-sensing eatometer[5,6], a photoresistor drinkometer[7]and photo-sensing

counters to measure ambulatory activity[8]. The testing apparatus and procedures for recording behavioral patterns have been described in detail elsewhere[6,7].

For the purpose of infusion into the third cerebroventricle, a rat was implanted chronically with a cannula (29 gauge) under pentobarbital anesthesia (50mg/kg). Under the unanesthetized and unrestrained conditions[7], test solutions (10.0 μl) were infused through the catheter at the rate of 1.0 μl/min[9]. For continuous infusion into the third cerebroventricle, an osmotic minipump (Model 2001, Alza Inc. Ltd.) filled with the test solution was placed underneath the skin and attached to the cannula[7]. The unilateral microinfusion cannula (29 gauge) was chronically implanted into hypothalamic loci and a 1.0 μl volume of test solution was infused at 0.2 μl/min. The coordinates were: A 4.5, L 0.5, H 3.4mm in the ventromedial hypothalamus(VMH) and A 4.5, L 1.5, H -2.8mm in the lateral hypothalamus (LHA)[10]. All test solution infusions were started at 1930 hr, unless otherwise stated.

Blood samples were collected from a chronically indwelling right atrial catheter implanted through the right jugular vein. The surgery and the blood sampling system have been described in detail elsewhere[6]. The rats were fasted on infusion day from 1530hr until completion of the experiment. Infusion of the test solution was started at 1800 hr.

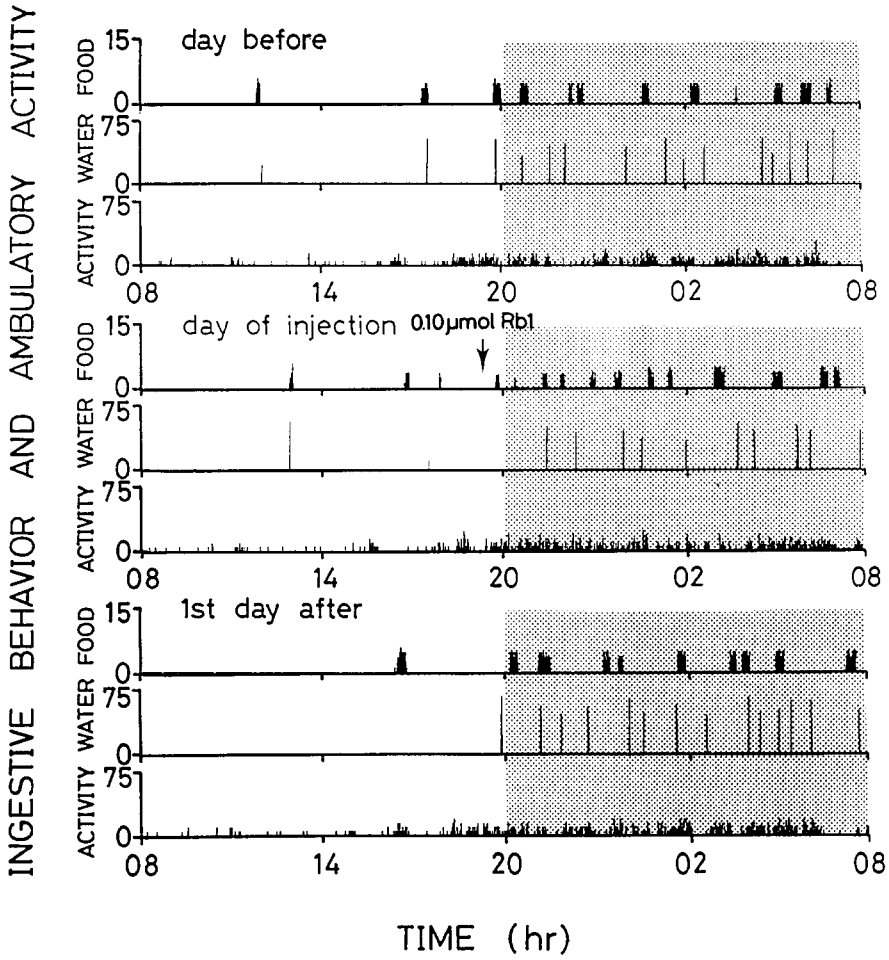
Table I. *Effects of ginsenoside Rb_1 on ingestion and ambulation after infusion into the third cerebroventricle or the peritoneum*[4].

Dose (μmol)	Day Before	1st Day After	2nd Day After
Food Intake (pellets)			
Rb_1			
0.05 (icv)	509.0±15.3	472.6±15.6*	510.3±21.8
0.10 (icv)	527.4±21.8	424.0±25.8*	563.0±18.9
0.10 (ip)	507.7±32.3	507.3±28.9	492.3±41.6
0.20 (icv)	548.6±31.6	369.1±40.0*	478.4±22.1
Saline			
1.5(icv)	531.1±16.4	557.1±21.1	536.7±22.9
Water Intake (droplets)			
Rb_1			
0.05(icv)	905.3±40.4	813.0±31.6	966.3±83.3
0.10(icv)	924.8±38.8	759.6±66.6	997.8±43.9
0.10(ip)	966.7±26.2	906.3±29.6	910.0±69.1
0.20(icv)	889.3±41.5	615.0±34.1*	878.6±59.7
Saline			
1.5(icv)	964.0±47.9	979.7±72.5	972.9±65.8
Ambulation(activijy units×10)			
Rb_1			
0.05(icv)	872.0±78.3	849.3±60.3	940.3±92.3
0.10(icv)	919.8±42.9	863.2±47.5	1022.2±83.3
0.10(ip)	961.7±59.3	913.0±69.1	1090.3±28.8
0.20(icv)	999.3±64.8	758.3±66.4	1061.6±66.8
Saline			
1.5(icv)	892.4±29.4	870.0±55.0	913.0±72.7

icv, intra-third cerebroventricle infusion. ip, intraperitoneal injection. * = $p<0.05$, compared to the corresponding initial levels.

Effects of Ginsenoside Rb_1 after Infusion into the Third Cerebroventricle[4)]

Effects of ginsenoside Rb_1 at doses of 0.05, 0.10 and 0.20 μmol, and 1.5 μmol saline on feeding, drinking and ambulatory behavior after infusion into the third cerebroventricle or the peritoneum are shown in Table I. Rb_1 at all doses tested decreased linearly food intake ($p<0.05$ in each) with dose dependence($Y=643.5-101.41\log x$, $r=0.41$, $p<0.05$) on the first day following the intra-third cerebroventricular infusion. The maximum dose of 0.20 μmol reduced the water intake ($p<0.05$), but the other smaller doses had no effect. Ambulation was not affected by any doses tested. All of the behavioral changes due to Rb_1 returned to the initial level on the second day after infusion. Thus, the dose of 0.10 μmol was the optimum dose to induce feeding suppression. Equimolar injection of Rb_1 into the peritoneum did not affect feeding, drinking or ambulatory behavior. These results indicated that the suppressive effect on feeding was selectively produced by the specific action of Rb_1, and not by the secondary action due to the drinking reduction. In



Patterns of typical eating, drinking and ambulatory activity for one rat before and after infusion of 0.10 μmol Rb_1 into the third cerebroventricle[4)]. Shading represents the dark time. On the injection day, feeding suppression accompanied the decrease in meal size for at least the first 6hr after injection. Drinking and ambulation were not affected.

Table II. *Changes in plasma glucose and insulin after infusion of 0.10 μmol ginsenoside Rb_1 into the third cerebroventricle*[4]

Time Course (min)	Glucose (mg/dl)		Insulin (μU/ml)	
	Rb_1[a]	Saline	Rb_1	Saline
0	118.3±4.8	117.8±4.1	28.2±4.0	28.3±6.3
10	132.8±4.2	117.0±4.4	25.5±5.8	21.9±3.6
20	140.8±3.4	118.8±7.4	24.9±2.3	26.7±7.2
40	128.8±3.4	118.5±5.4	19.6±1.9	17.2±4.4

[a]Analysis of variance with replication (ANOVA) revealed Rb_1-induced hyperglycemia to be significant ($F[3, 24] = 4.0$, $p < 0.05$).

addition, it can be said that the effect was not mediated peripherally, since equimolar peripheral injection had no effect on ingestion or ambulation.

According to the analyses of daily behavioral patterns as represented in Fig.1, the suppression of feeding was found to be the result of a decrease in meal size during the first 6hr after infusion of 0.10 μmol Rb_1 ($p < 0.05$, compared to the corresponding pretreatment meals), rather than from changes in the postprandial intermeal interval or eating speed(meal size devided by meal duration). Meal size can be decreased by the activation of VMH glucoreceptor neurons, since electrical stimulation of VMH glucoreceptor neurons has been shown to terminate ongoing feeding[1,11]. In contrast, LHA neuronal activity has been reported to be closely associated with the onset of feeding[11]. Thus, augmented neuronal activity of the VMH leads to decrease meal size, and sustained inactivation of LHA activity suppresses meal onset and prolongs the postprandial intermeal interval[9,11,12]. Therefore, the present findings suggest that the main action of Rb_1 may be at least partially produced by the activation of VMH rather than by the inactivation of LHA.

As shown in Table II. concomitant changes in humoral factors were observed after infusion of 0.10 μmol Rb_1. The suppressive effect was accompanied by elevated plasma glucose concentration($p < 0.05$), but not by a change in plasma insulin. Stimulation of the VMH induces hyperglycemia due to the activation of

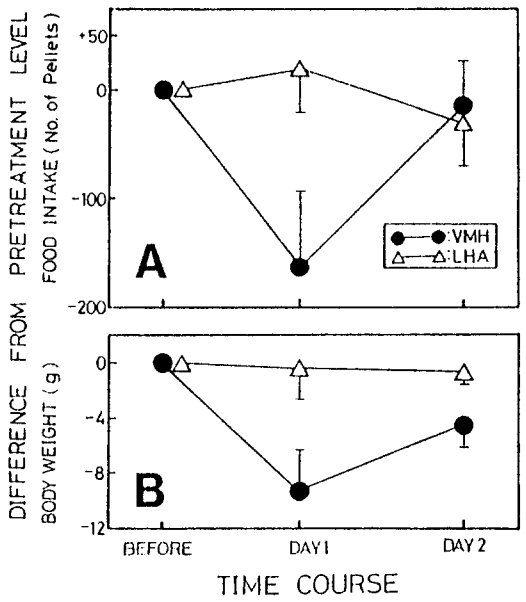


Fig.2. *Changes in differences from initial levels of food intake (A) and body weight (B) following unilateral microinjection of 0.01 μmol ginsenoside Rb_1 into the ventromedial(VMH) or lateral hypothalamus(LHA). Changes in food intake and body weight were significant between these two groups($*p < 0.05$, based on AVOVA).*

afferent sympathetic nerves in the liver[13,14]. but reciprocally inhibits insulin secretion from the pancreas due to the concomitant activation of pancreatic afferent sympathetic nerves[15]. Together with the behavioral results of the present study, it can be concluded that these

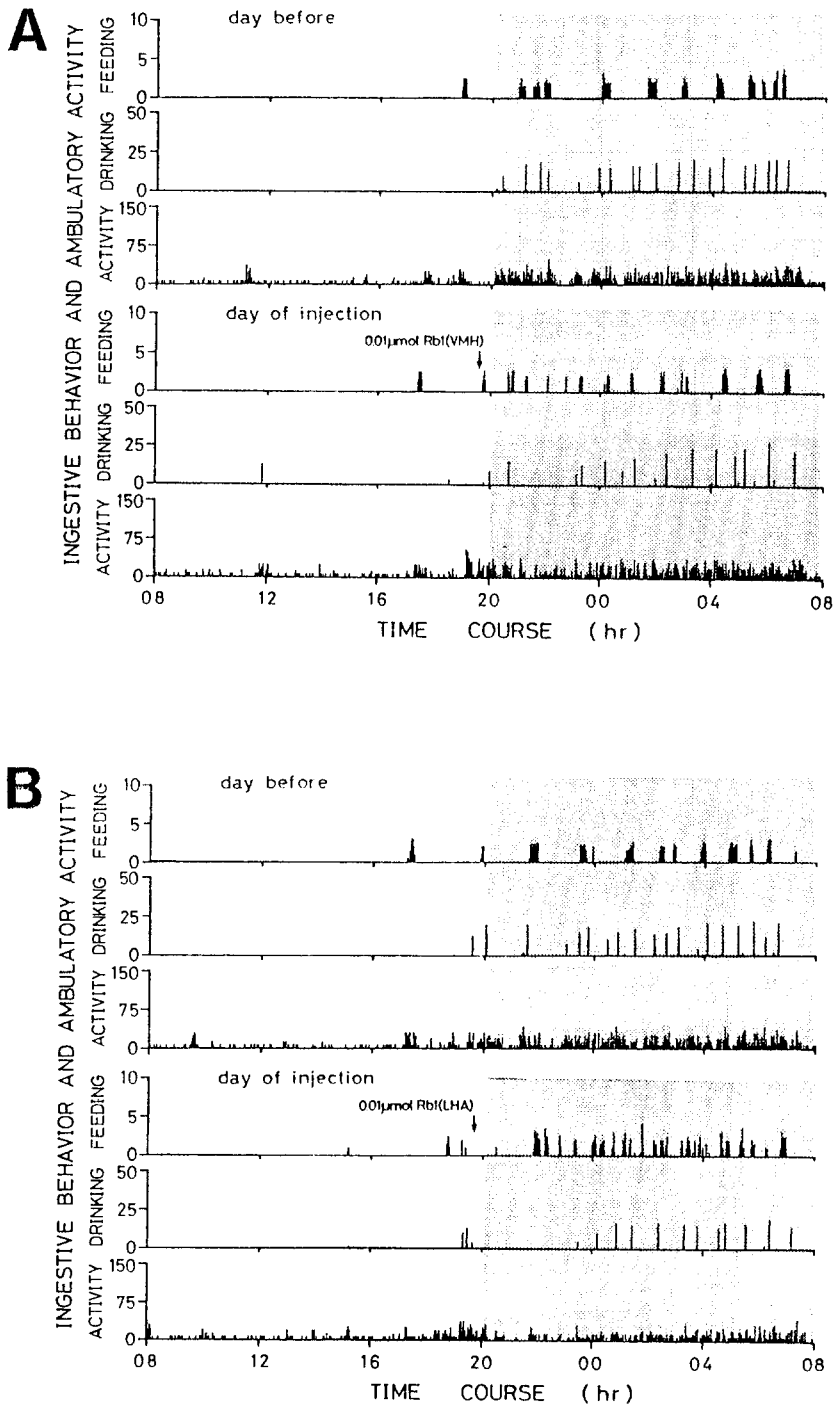


Fig.3. *Eating, drinking and ambulatory patterns of one rat before and after unilateral microinjection of 0.01 μmol ginsenoside Rb_1 into the ventromedial(VMH) (A) or lateral hypothalamus(LHA) (B). Shading, period of dark time. Infusion into the VMH suppressed feeding by reducing the meal size, but drinking and ambulation were not affected*[4].

humoral changes induced by Rb_1 may be derived from activation of the VMH.

Identification of the Active Site for Rb_1-Induced Feeding Suppression[4]

To identify the effective injection sites of Rb_1-induced feeding suppression, 0.01 μmol Rb_1 was microinjected unilaterally into the VMH and the LHA. Fig.2 shows the difference between the initial level of food intake (A) or body weight (B) and that after microinjection. The food intake was decreased ($p<0.05$) on the first day after Rb_1 infusion into the VMH, but it returned to the initial level by the second day. Weight loss continued to the second day ($p<0.05$ on both the first and second days). When Rb_1 was microinjected into the LHA, neither food intake nor body weight was affected(Fig.2A & B). Figs.3A and B show the typical ingestive and ambulatory patterns of one rat after microinjection of 0.01 μmol Rb_1 into the VMH and the LHA, respectively. With microinjection into the VMH, meal patterns showed feeding reduction as a result of a decrease in meal size. However, the patterns of drinking (initial level, 776.7±100.2 droplets) and ambulation (initial level, 7846.6±179.2 units) were not affected(Fig. 3A). Microinjection of Rb_1 into the LHA also affected meal patterns with meals divided, but the total food consumption was not affected. In contrast to injection into the VMH, patterns of drinking and ambulation were suppressed after microinjection into the LHA(Fig.3B) (water intake: 978.7±58.4[initial], 733.7 ± 18.0 [post-treatment], $p<0.05$; ambulatory activity: 8023.4±116.4[initial], 5730.0 ±891.4[post-treatment], $p<0.05$).

These results verify the active site of Rb_1-induced feeding suppression to be in the VMH. In addition, the pharmacological action of Rb_1 *via* these two regions seems to be functionally different; that *via* the LHA is not involved in feeding modulation, but is involved in other functions, such as water intake and ambulatory activity[16]

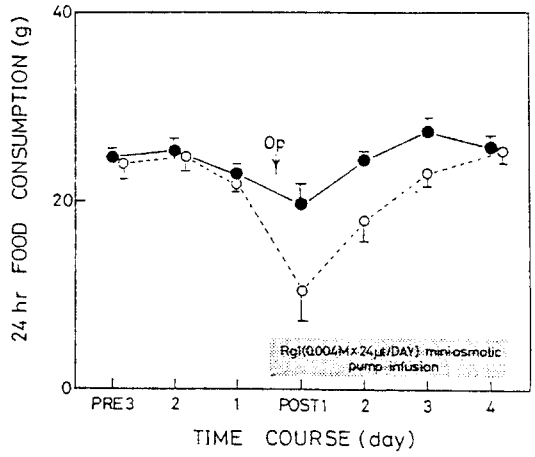


Fig.4. *Effects of continuous infusion of ginsenoside Rg_1 on feeding suppression due to surgery stress. Op, surgical operation. In this figure and Fig.5, ginsenosides were continuously infused into the third cerebroventricle using an osmotic minipump(Alza, Inc. Ltd., 0.004 M×24 μl/day). Rg_1 attenuated the stress-induced feeding suppression. The effect was statistically significant($p<0.05$, based on ANOVA)*

●—● : Rb_1, ○—○ : Saline

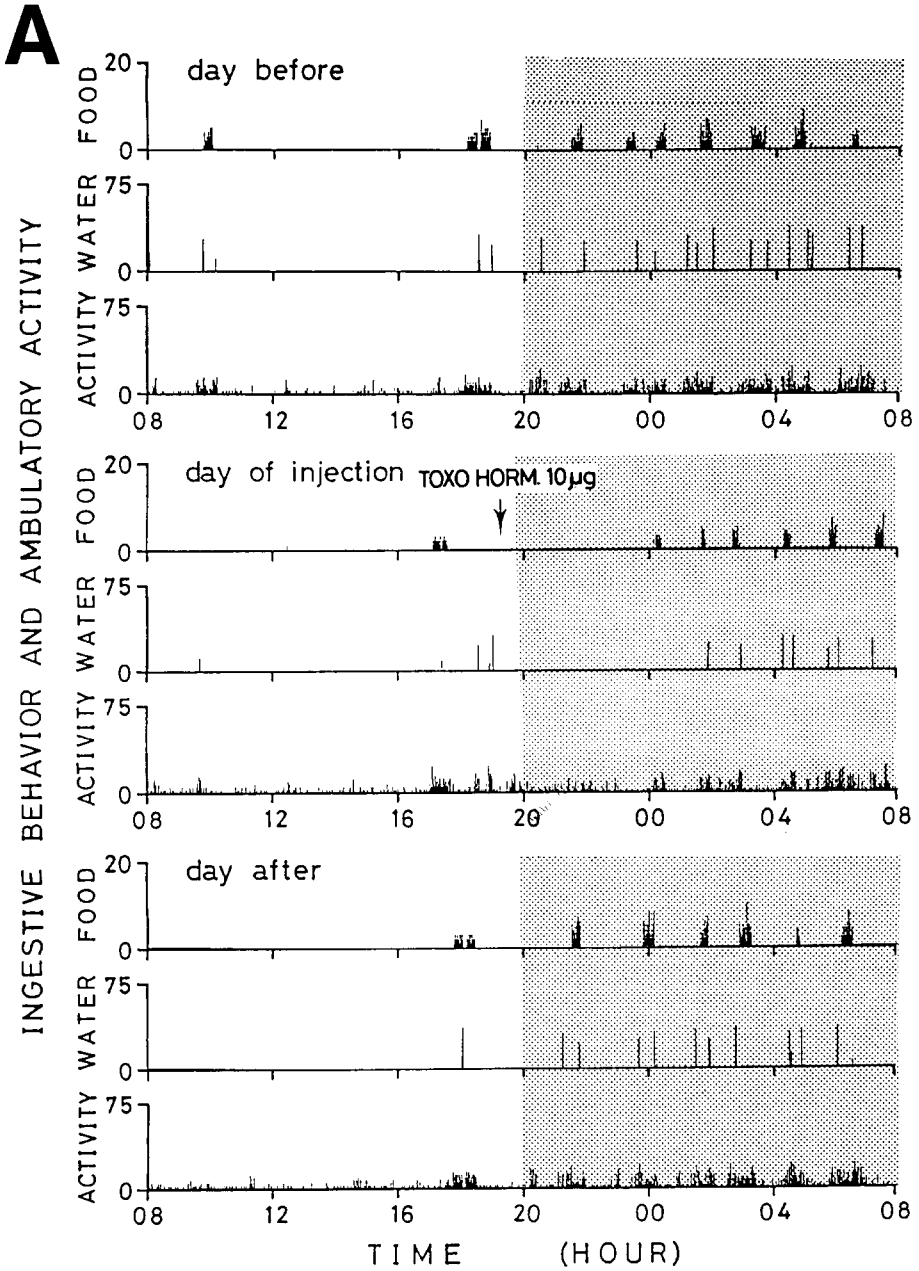
Effects of Continuous Infusion of Ginsenoside Rb_2 or Rg_1 on the Response to Stress

Surgery, such as implantation of a catheter with an attached osmotic minipump into the third cerebroventricle, usually causes a reduction in food intake and body weight[7]. Fig.4 shows the effect of Rg_1 on response to physical stress elicited by surgery. Continuous infusion of Rg_1(0.004M×24 μl/day) markedly attenuated feeding suppression due to surgical operation.

Toxohormone-L, which was isolated from the ascites fluid from patients with hepatoma[17], has been shown to significantly decrease food and water consumption during the 8 to 10 hr following infusion(10 μg) into the third cerebroventricle, but was not shown to affect the ambulatory activity(Fig.5A)[18]. Con-

tinuous infusion of Rb_2 (0.004 M×24 μl/day) into the third cerebroventricle, however, either attenuated or abolished the supressive effects of toxohormone-L(Fig.5B).

In conclusion, ginsenoside Rb_1 pharmacologically suppressed food intake, at least in part through activation of the VMH. In contrast, ginsenosides Rb_2 and Rg_1 showed antagonistic effects on decreased feeding and weight loss in response to physical stressors.



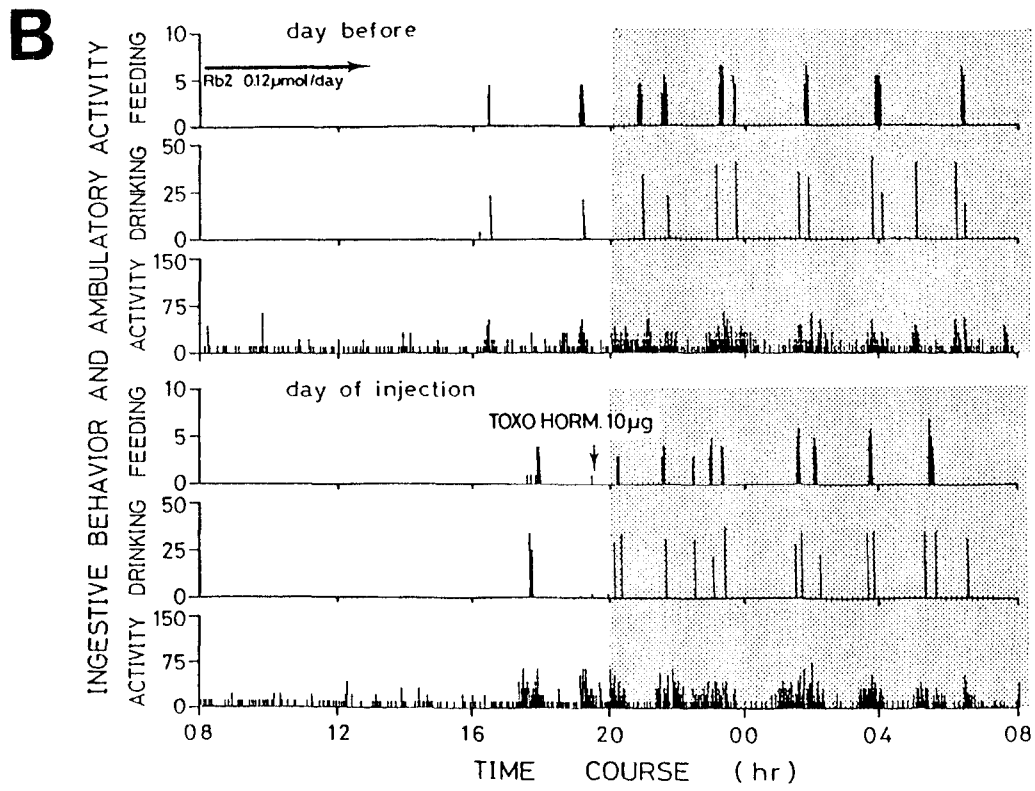


Fig.5. *Antagonistic effects of continuous infusion of ginsenoside Rb_2 on feeding suppression induced by toxohormone-L(TOXOHORM.). Shading, period of dark time. Infusion of 10 μl toxohormone-L into the third cerebroventricle decreased food and water intake (A). Rb_1 attenuated the effect of toxohormone-L (B).*

The present results suggest that ginsenosides as a whole have pharmacological potency to maintain feeding at a certain physiological level.

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