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Hypoglycemic mechanism of ginseng glycopeptide¹

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ABSTRACT

AIM: To study the hypoglycemic mechanism of ginseng glycopeptide (GGP). **METHODS:** After administration of GGP, the levels of insulin, lactate dehydrogenase (LDH), lactic acid (LC), and oxygen consumption, as well as blood glucose (BG) and liver glycogen (LG) were measured. Based on these measurement results, the effects of GGP on insulin secretion and anaerobic/aerobic glycolysis were evaluated. Adenylate cyclase (AC) activity and cAMP level were measured to study the effects of GGP on BG and LG metabolism and to determine whether the effects were through second transmitting message system. Propranolol (β -receptor antagonist) and phentolamine (α -receptor antagonist) were used to investigate whether hypoglycemic activity of GGP was through β - or α -adrenoceptor. [³H]DHA (antagonist of β -adrenoceptor) was used to determine GGP binding affinity to β -adrenoceptor. Citrate synthetase (CTS), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and cytochrome oxidase (CCO) activities were measured to explore GGP effects on aerobic glycolysis in liver mitochondria. Phosphorylase (PP) activity was measured to study GGP effects on liver glycogen metabolism. **RESULTS:** cAMP content and AC activity were increased when BG and LG contents in liver of mice decreased. The decrease in liver glycogen induced by GGP was inhibited by pretreatment with propranolol. Radioligand receptor assay showed that GGP was competing *in vitro* with [³H]DHA to bind to β -adrenoceptor of duck erythrocyte membrane, and IC₅₀ of GGP was 63 nmol/L. GGP inhibited LDH activity at an appropriate dosage, at which contents of BG and LG could be effectively lowered. GGP also stimulated activities of SDH, MDH, CCO, CTS, and PP. **CONCLUSION:** The hypoglycemic activity of GGP may be attributed to the enhancement of aerobic glycolysis through stimulation of β -adrenoceptor and increase of various rate-limiting enzyme activities related to tricarboxylic acid cycle.

INTRODUCTION

In a previous study^[1] we demonstrated that the

ginseng glycopeptides (GGP) from the roots of *Panax ginseng* C A Meyer had a hypoglycemic activity on both normal and hyperglycemic animals. In addition, it was found that the decrease in plasma glucose level caused by GGP was correlated with the decrease in liver glycogen content. Based on the above-mentioned results, we further studied the mechanism of hypoglycemic activity of GGP. In the present study, we investigated whether GGP could affect some biochemical markers

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of carbohydrate metabolism, such as insulin, enzyme activities related to aerobic or anaerobic glycolysis as well as the effects of GGP on receptor to explore its transduction pathway.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 150-160 g, male Kunming strain mice weighing 20-22 g (Grade II, Certificate No 10-5110, Experimental Centre, Changchun College of Traditional Chinese Medicine), and Beijing duck weighing 1-1.5 kg were used. They were maintained at room temperature under alternating 12-h light/dark photoperiod and could access to standard laboratory feed and fresh water *ad libitum*.

Chemicals GGP was isolated from Jilin white ginseng (*Panax ginseng* C A Mayer) by Department of Phytochemistry, Academy of Traditional Chinese Medicine and Materia Medica of Jilin Province. Molecular weight of GGP was 6000. The glycon part of GGP consisted of rhamnose, arabinose, galactose, and glucose and the ratio of these components was 0.46:1.61:1:2.37. The peptide part of GGP consisted of 16 amino acids (Asp, Glu, and Pro, *etc*). GGP was made up into the required concentration with physiological saline just before use. Insulin radioimmunoassay kit and [³H] dihydroalprenolol ([³H]DHA) were obtained from Amersham (HP7 9LL, UK). [³H]cAMP was bought from China Institute of Atomic Energy (Beijing, China).

Methods Plasma insulin level was analyzed as follows. After sampling, plasma was centrifuged at 800×g, 4 °C for 15 min. Insulin level was, then, determined by using the insulin radioimmunoassay kit with human insulin as standard. The level of cAMP in liver tissue was measured by protein binding assay^[2]. The content of lactic acid was determined by colorimetric method^[3]. The activity of lactic dehydrogenase (LDH) in blood and liver tissue was assayed spectrophotometrically^[4]. Adenylate cyclase (AC) activity in liver tissue was assayed by Shima's method^[5]. Cytochrome oxidase (CCO) and succinate dehydrogenase (SDH) activities were measured by Dai's oxygen electrode method^[6]; measurements of malate dehydrogenase (MDH)^[7], citrate synthetase (CTS)^[8], and phosphorylase (PP)^[9] activities were carried out according to the methods in references, respectively. Protein content was determined by Daughaday's method^[10]. Plasma glucose (PG) and liver glycogen (LG) were measured by colorimetric methods^[11,12], respectively. The prepara-

tion and binding assay of β-adrenoceptor in the duck's erythrocyte membrane were carried out following Feng's methods^[13].

Data analysis Data were analyzed and expressed as mean±SD. Statistical differences were evaluated by *t*-test.

RESULTS

Effects of GGP on plasma insulin level in normal mice Forty mice were randomly divided into 5 groups. Group 1 was injected sc saline 5 mL/kg at 60 min before decapitation and other groups were given GGP 200 mg/kg at 10, 20, 30, and 60 min before decapitation. The results showed that the insulin level of control group was (2.5±1.3) mU/L, and levels of Groups 2 to 5 were (5±3), (5.5±2.4), (*P*<0.05 vs control group), (2.0±1.2), and (3.0±0.3) mU/L, respectively. The above-mentioned results showed that the plasma insulin level was raised within only 20 min after administration.

Effects of GGP on blood glucose, liver glycogen and cAMP levels, and liver AC and PP activities Twenty-four mice were randomly divided into 3 groups. Two groups were injected sc GGP 100 and 200 mg/kg, respectively, and the third group was injected saline 5 mL/kg as control. The animals were decapitated and plasma and liver tissue were collected for the measurement of the biochemical parameters 2 h after administration of GGP.

After administration of GGP blood glucose and liver glycogen levels were decreased, while cAMP level slightly increased. Activities of AC and PP in liver tissues were also increased (Tab 1).

Effects of propranolol and pentolamine on gly-

Tab 1. Effects of GGP on PG, LG, and c-AMP levels, and AC and PP activities in liver tissue of mice. *n*=8. Mean±SD. ^a*P*>0.05, ^b*P*<0.05, ^c*P*<0.01 vs control.

GGP/ mg·kg ⁻¹	PG/g·L ⁻¹	LG/ mg·g ⁻¹ Wt	cAMP/ nmol·g ⁻¹ Wt	AC/%	PP/%
Control	1.12±0.15	66±8	1.58±0.12	100±80	100±20
100	0.96±0.14 ^b	52±10 ^c	1.65±0.01 ^a	200±90 ^b	116±17 ^a
200	0.80±0.13 ^c	50±11 ^c	1.77±0.14 ^b	260±100 ^b	132±10 ^c

Wt: wet tissue; GGP: ginseng glycopeptides; PG: plasma glucose; LG: liver glycogen; AC: adenylate cyclase; PP: phosphorylase.

cogen-lowering activity of GGP Forty-eight mice were equally and randomly divided into 6 groups. Groups 1 and 2 were injected ip with saline 5 mL/kg, Groups 3 and 4 were treated with propranolol (PPN) 40 mg/kg, and Groups 5 and 6 with pentolamin (PTL) 10 mg/kg. At 40 min after the dosing, Groups 1, 3, and 5 were injected iv saline 5 mL/kg, and the other 3 groups were given GGP 100 mg/kg. They were decapitated and liver tissues were taken for the measure liver glycogen content 20 min after giving GGP.

The pretreatments with PPN and PTL led to different effects on the action of GGP. PPN reversed the glycogen decrease, which was induced by GGP. However, PTL showed not only no adverse effect on GGP-induced decrease in glycogen, but also to some extent further reduced glycogen contents (Tab 2).

Tab 2. Effects of PPN and PTL on glycogen-lowering action of GGP in mice. *n*=8. Mean±SD. ^a*P*>0.05, ^b*P*<0.05, ^c*P*<0.01 vs saline.

Agents/mg·kg ⁻¹				Liver glycogen/mg·g ⁻¹ Wt
Saline	GGP	PPN	PTL	
+	-	-	-	23±5
+	100	-	-	16±5 ^c
-	-	40	-	21±5 ^a
-	100	40	-	22±5 ^a
-	-	-	10	16±10 ^b
-	100	-	10	13±10 ^b

Wt: wet tissue. SL: Saline. PPN: propranolol. PTL: pentolamine.

Effects of GGP on β -adrenoceptor of duck erythrocyte membrane [³H] dihydroalprenolol ([³H]DHA), a potent adrenergic antagonist of β -adrenoceptor, was used to identify binding sites of GGP on the erythrocytic membrane of Beijing duck. It was found that GGP could compete with [³H]DHA for the binding sites in the erythrocytic membrane and its IC₅₀ of GGP was 63 nmol/L, therefore GGP showed agonist-like activity on β -adrenoceptor (Fig 1).

Effects of GGP on plasma lactic acid content and LDH activity in blood and liver tissue of mice Twenty-four mice were equally divided into 3 groups and two groups were injected iv with GGP at the dosage of 50 and 100 mg/kg, respectively. The third group was injected with saline at 2 mL/kg as control. Following decapitation, blood and liver tissue were taken for

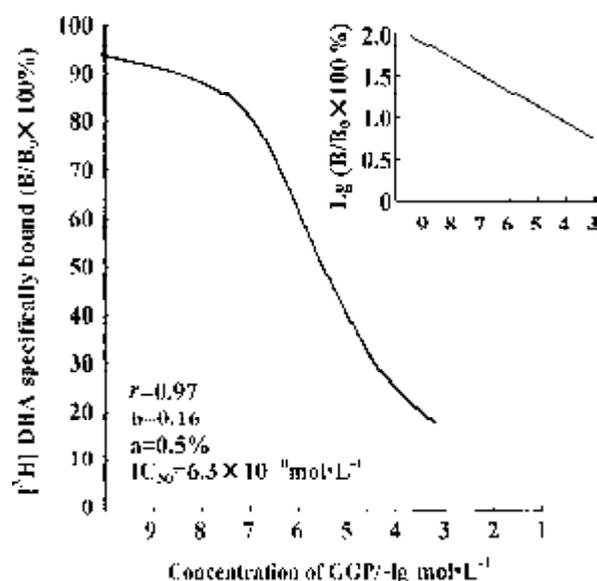


Fig 1. Competition curve of GGP in duck erythrocytic membranes. Membranes were incubated with [³H]DHA in the presence of different concentrations of GGP at 25 °C for 15 min.

the measurement of lactic acid content and LDH activity at 60 min after administration of GGP (Tab 3).

Tab 3. Effects of GGP on plasma lactic acid content and LDH activity in blood and liver tissue of mice. *n*=8. Mean±SD. ^a*P*>0.05, ^b*P*<0.05, ^c*P*<0.01 vs control.

Group	Plasma LDH/ U·L ⁻¹	Liver LDH/ kU·g ⁻¹ protein	Plasma lactic acid/g·L ⁻¹
Control	13 680±3240	6999±963	28±9
GGP 50 mg·kg ⁻¹	7290±1270 ^c	6329±283 ^a	14±4 ^c
GGP 100 mg·kg ⁻¹	7180±1410 ^c	5940±490 ^b	12±6 ^c

Effects of GGP on CTS and MDH activities in mouse liver tissue Twenty-four mice were equally allocated into 3 groups. Group 1 was injected iv with saline at 10 mL/kg, Groups 2 and 3 were given GGP at the dosage of 50 and 100 mg/kg, respectively. The mice were decapitated and liver tissues were taken for the measurement of MDH and CTS activities 2 h after injection of GGP. The activities of CTS and MDH were enhanced by the application of GGP (Tab 4).

Effects of GGP on SDH and CCO activities in mouse liver tissue Sixty-four mice were equally divided into 2 groups: group 1 was given iv with GGP at

Tab 4. Effects of GGP on CTS and MDH activities in mouse liver tissue. *n*=8. Mean±SD. ^b*P*<0.05, ^c*P*<0.01 vs control.

Group	CTS/%	MDH/%
Control	100±10	100±14
GGP 50 mg·kg ⁻¹	110±9 ^b	123±27 ^b
GGP 100 mg·kg ⁻¹	122±11 ^c	126±26 ^b

GGP: ginseng glycopeptides; CTS: citrate synthetase; MDH: malate dehydrogenase.

100 mg/kg, and group 2 was given saline at 5 mL/kg as control. At 10, 20, 40, and 60 min after injection, 8 mice from control or treated groups were decapitated and liver tissue was quickly removed to measure activities of SDH and CCO. The results showed that at 10 min after administration, SDH activity began to rise and reached plateau stage at 40 min after injection of GGP. CCO activity of GGP-treated group was obviously higher than that of control group at 20 and 40 min after administration (Tab 5).

Tab 5. Effects of GGP on SDH and CCO activities in mouse liver tissue. *n*=8. Mean±SD. ^a*P*>0.05, ^b*P*<0.05, ^c*P*<0.01 vs control.

	GGP/ mg·kg ⁻¹	Time after injection of GGP/min			
		10	20	40	60
SDH/%	0	100±23	100±20	100±26	100±13
	100	160±35 ^b	107±18	144±18 ^c	140±18 ^c
CCO/%	0	100±29	100±9	100±10	100±29
	100	106±28 ^a	149±19 ^b	127±21 ^b	114±47 ^a

GGP: ginseng glycopeptides; SDH: succinate dehydrogenase; CCO: cytochrome oxidase.

DISCUSSION

Based on the results from the present study, we found that the hypoglycemic activity of GGP might not be due to the enhancement of insulin secretion, because the increase in insulin level occurred only at 20 min after administration of GGP, and the hypoglycemic action of GGP could last up to 16 h^[1]. Therefore, the effect of GGP on hyperglycemia can not be realized only through its influence on insulin level. In addition, GGP and insulin, although hypoglycemia could be in-

duced by both drugs, exhibited different effects on glycogen metabolism. GGP decreased liver glycogen content, but insulin increased it.

In the present study, administration of GGP decreased both the level of plasma lactic acid and the activities of plasma and liver LDH. Therefore, the hypoglycemic activities of GGP may not be through anaerobic glycolysis. On the other hand, GGP could enhance the activities of CTS, MDH, SDH, and CCO, and these four enzymes were the rate-limiting enzymes in aerobic glycolysis (tricarboxylic acid cycle). The increase in the activities of these rate-limiting enzymes will certainly speed up the utilization of glucose, which in turn will lower down the level of plasma glucose. Therefore, hypoglycemic activity of GGP may be realized mainly due to increasing aerobic glycolysis.

Due to the properties of GGP, such as large molecular weight (M_r over 6000) and hydrophilia, we think it is nearly impossible that GGP can play its role through binding to nuclear receptors. Instead, it is conceivable that it exerts its function via binding to cell membrane receptors; the complex then triggers the transduction pathway of the second messenger (cAMP). Subsequently this signal will be transduced to mitochondria to cause the change in enzyme activities of tricarboxylic acid cycle. Finally this will speed up the process of aerobic glycolysis. In order to test this hypothesis, we examined the influence of α -receptor inhibitor, pentolamine, and β -receptor inhibitor, propranolol, on the effectiveness of GGP in decreasing liver glycogen. The results showed that β -adrenoceptor inhibitor, propranolol, could completely inhibit the action of GGP on decreasing liver glycogen. In addition, we found that at the same time of the decreases in blood glucose and liver glycogen after administration of GGP, the activities of AC, PP, and cAMP in mouse liver were increased. These results indicate that GGP causing decrease in plasma glucose and liver glycogen may be via binding to cell membrane β -adrenoceptor and transduce this signal via cAMP to mitochondria. Eventually, this will speed up aerobic glycolysis and liver glycogen catalysis. In order to confirm that the first step of GGP action is *via* binding to cell membrane β -adrenoceptor, we carried out an *in vitro* study using the red blood cells of Peking ducks. The results showed that GGP could compete cell membrane β -adrenoceptor with [³H]DHA (β -adrenoceptor antagonist) *in vitro*. Our data are well consistent with the recently published results. In their study, Zhu and Li^[14] provided evidence that oli-

gosaccharides could bind to β -adrenoceptors. GGP used in our study is a heterosaccharide, a critical part of which is structurally closely related with the oligosaccharides. Therefore, their results support our conclusion that GGP is the possible agonists of β -adrenoceptors. Further, de Souza *et al*^[15] and Weyer *et al*^[16] reported that some agonists of β -adrenoceptor are potent drugs for the treatment of diabetes. In consistent, we also found that GGP, as a kind of agonists of β -adrenoceptor, had the effectiveness in treating diabetes. Therefore, the effectiveness of GGP in treating diabetes is likely through β -adrenoceptor pathway.

To summarize these results from the entire study, we depicted the following mechanism underlying GGP action on hypoglycemia (Fig 2).

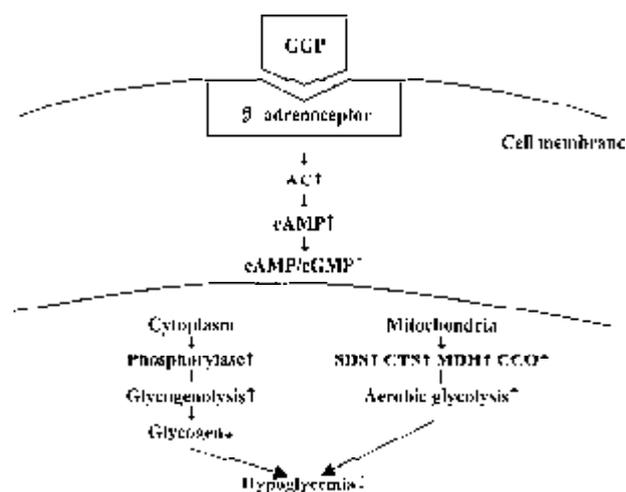


Fig 2. Possible transduction pathway of GGP on hypoglycemic activities.

In 1998, injection preparation of GGP had been issued as a new drug for treatment of diabetes by the State Drug Administration of China. This new drug was proved to have hypoglycemic effects on insulin-independent diabetes. Compared to insulin, GGP does not cause hypoglycemic shock or tolerance. Unlike biguanides or sulfonyureas, GGP does not cause acidosis. Since GGP has immunomodulatory function, GGP can reduce the manifestation of diabetes-associated infection diseases caused by impaired immune function. This drug has so far no obvious known side effects, provided that the method of drug administration is appropriate. To inject in this drug daily to diabetes patients will cause discomfortable. We are now trying to develop the slow-releasing preparation of GGP

for prolonged effects.

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