FURTHER PURIFIED GINSENG EXTRACT FRACTION (D-O-ANA) FOR INSULIN RELEASE AND ITS MODE OF ACTION COMPARED WITH THE ISOLATED RESIDUAL COMPONENTS

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ABSTRACT

A further purified fraction (D-O-ANa) was obtained from DPG 3-2 fraction of Ginseng Radix by complete removal of saponins, nucleosides, nucleic acid bases, amino acids, and sugars. D-O-ANa - induced insulin release was investigated to compare with that of DPG 3-2 and other isolated components. Among the subfractions of DPG 3-2, D-O-ANa exhibited the most potent release of insulin with or without high concentrations of glucose, and it particularly enhanced the second phase of glucose-induced insulin release. DGP 3-2 potentiated significantly the glucose-induced insulin release from the isolated islets of diabetic mice at increasing concentrations of extracellular calcium ions (0.16 - 2.5 mM). A definite relationship was found between calcium (^{45}Ca) uptake and insulin release. Ginsenoside (G)-Rb_1 and G-Rg_1 did not enhance the glucose-induced insulin release. The effect of ginseng saponins was blocked by glucose (16.7 mM), being distinctly different from the glucose-potentiated effect of DPG 3-2. The insulin release effect of G-Rg_1 was unaffected by the presence or absence of extracellular Ca^{2+} and theophylline.

Adenosine also increased insulin release from isolated islets, but had no effect on perfused rat pancreas. Arginine stimulated insulin release less evidently than D-O-ANa, though arginine- and adenosine-induced glucagon releases were more remarkable. In conclusion, D-O-ANa appears to be a major fraction in insulin release activity of ginseng and its mode of action may be related to Ca^{2+} ion uptake. This physiological mechanism was distinct from that of the abnormal release induced by ginseng saponins.

INTRODUCTION

It has been previously reported by us that the hypoglycemic fraction of ginseng (DPG 3-2) increased insulin release from isolated pancreatic islets.[1,2,3] From DPG 3-2, recently a further purified fraction (D-O-ANa) was obtained by removing completely saponins, nucleosides, nucleic acid bases, amino acids, and sugars. D-O-ANa-induced insulin release was investigated to compare with that of DPG 3-2 and other residual components by the methods of pancreas perfusion, in addition to the isolated pancreatic islets.

The extraordinarily increased release of insulin induced by ginseng saponins was found when the high doses of the ginseng fractions contamined with saponins were employed.[4] This saponin-induced release, which was ultimately proved to be not physiological, and the insulin

release induced by DPG 3-2 were investigated in this study. The latter release was depended on the dynamics of Ca^{2+} ions.[5]

MATERIALS AND METHODS

Animals

Genetically diabetic KK-CA^y mice[6] and Wistar rats were used. Wistar rats (male, 250 - 350 g) were perchased from Shizuoka Laboratory Animal Centre (Hamamatsu). Genetically diabetic KK-CA^y mice (male, 40 - 60 g, more than 250 mg/dl of blood glucose) were obtained from the closed colony maintained in our laboratory. General features, pharmacological basis, and the breeding method of KK-CA^y mice were previously reported.[6]

Assay Procedure of Insulin Release

Insulin release effect was determined by the following two methods. 1) Pancreatic islets of KK-CA^y mice were isolated by the collagenase technique[7] and incubated in Krebs-Ringer bicarbonate buffer (KRBB, pH 7.4) containing 0.2% bovine serum albumin (BSA). Released insulin (IRI) was measured by the radioimmunoassay using bovine insulin as a standard.[6] 2) Isolated rat pancreas was perfused with KRBB according to the method of Grodsky with minor modifications.[8] Released glucagon (IRG) was simultaneously determined with IRI.[6]

Measurement of Ca^{2+} Uptake

^{45}Ca net uptake was measured by a washing procedure.[9] Batches of 5 - 10 islets were incubated in KRBB containing 0.2% BSA and 2.5 μCi $^{45}CaCl_2$. An aliquot of the medium was used for IRI assay. Islets were then washed a few times and dissolved in hyamine^R. The radioactivity was counted by a liquid scintillation counter.

Fractionation Method of Ginseng Extract

The crude water extract of Ginseng Radix (HAKUSAN, Korea) was fractionated by the method shown in Fig. 1.

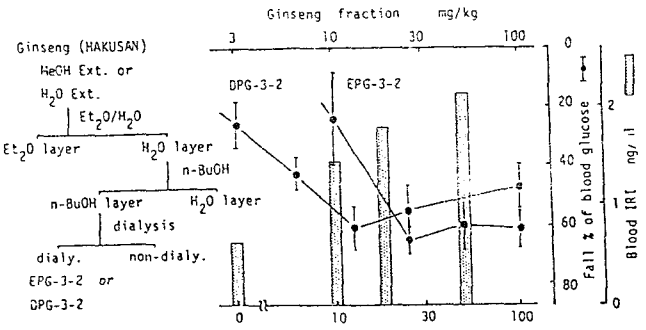

Fig. 1. Fractionation method of ginseng extract.

RESULTS AND DISCUSSION

Effect of a Further Purified Ginseng Extract Fraction (D-O-ANa) on Insulin Release

D-O-A fr., D-O-AN fr., and D-O-ANa fr. influenced insulin releasing activity more evidently than the other part of subdivided fractions of DPG 3-2 (Fig. 1). D-O-ANa exhibited the most potent release of insulin with or without high concentrations of glucose (Fig. 2). D-O-ANa dose-dependently enhanced the glucose-induced release of insulin, particularly the second phase of release (Fig. 3). This glucose-potentiated effect was much more remarkable than the effect of D-O-ANa alone.

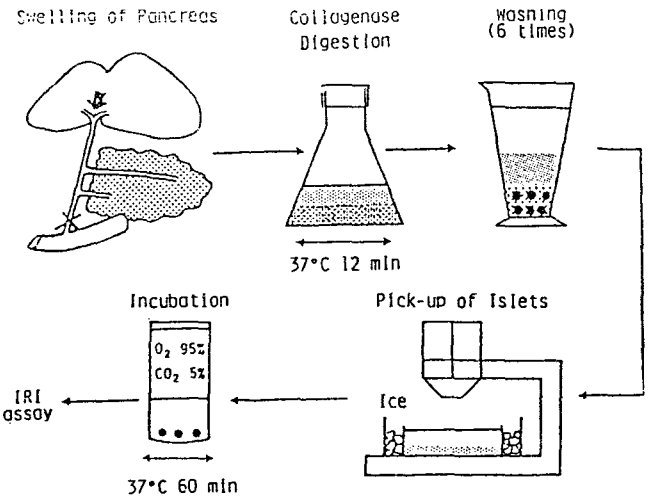

Fig. 2. Effect of D-O-ANa on the glucose-potentiated insulin release in perfused rat pancreas. Shaded area shows the effect of D-O-ANa alone.

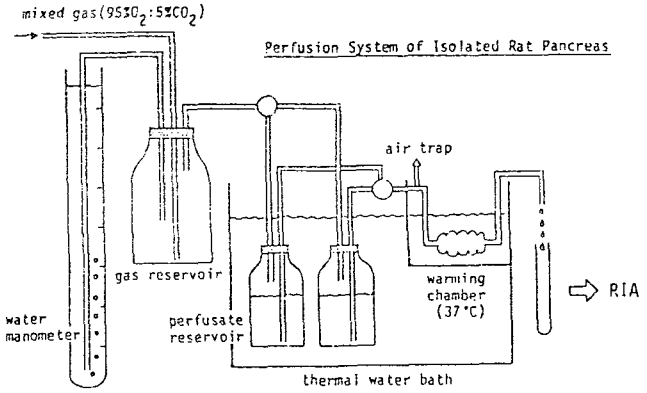

Fig. 3. Effect of D-O-ANa on the second phase of glucose-induced insulin release in perfused rat pancreas.

Adenosine also stimulated insulin release from isolated islets, but did not stimulate in the perfusion of rat pancreas. Arginine stimulated insulin release less evidently than D-O-ANa, though arginine- and adenosine-induced glucagon releases were more remarkable (Table 1).

Though only partially purified, D-O-ANa contains the principle component in the insulin releasing activity, and is characterized by the glucose-potentiated effect and the specific effect on the insular cells.

Abnormal Release of Insulin Induced by Ginseng Saponins

The ginseng extract fraction (DPG 3-2) from which the majority of saponins was removed, greatly stimulated insulin release from the isolated pancreatic islets of mice at higher doses (more than 4 mg/ml). Ginseng saponins [ginsenoside (G)-Rb_1, -Rg_1] which still contaminate DPG 3-2 also stimulated insulin release. However, G-Rb_1 and G-Rg_1 did not enhance the glucose-induced insulin release. The effect of G-Rb_1 or G-Rg_1 *per se* was blocked by glucose (16.7 mM), which was distinguished from the glucose-potentiated effect of DPG 3-2 (Fig. 4). The insulin releasing effect of G-Rg_1 was unaffected by the presence or absence of extracellular Ca^{2+} ions and theophylline (Table 2).

In the pancreas perfusion system, G-Rb_1 (more than 0.5 mg/ml) also stimulated insulin release, though G-Rg_1 (1 mg/ml) could not do so (Fig. 5). In this system the effect of G-Rb_1 was reduced in the high glucose condition, and was not influenced by the absence of extracellular Ca^{2+} ions.

Table 1. Effects of D-O-ANa and Other Ginseng Components on Insulin and Glucagon Releases in Perfused Rat Pancreas

Drugs	Doses (mg/ml)	Σ IRI (ng/20 min)		Σ IRG (ng/20 min)	Ratio (G/I)
D-O-ANa	1.0	38.97[a]	(92%)[b]	3.04[c]	0.08[d]
arginine	1.0	9.61	(15%)	41.31	4.30
adenosine	1.0	8.17	(13%)	4.32	0.53
ginsenoside					
– Rb_1	0.5	12.85	(25%)	5.25	0.41
– Rg_1	1.0	11.55	(14%)	3.69	0.32
glibenclamide	0.1μg/ml	8.65	(43%)	4.19	0.48

a) the calculated value obtained from a typical experiment by integrating the amounts of insulin released for 20 min.
b) relative potency compared with 16.7 mM glucose (100%).
c) calculated according to the case of insulin release.
d) the quotient of (Σ IRG) divided by (Σ IRI).

Table 2. Effect of Ginsenoside-Rg_1 on Insulin Release in Isolated Islets of KK–CA^y Mice

Glucose (mM)	$CaCl_2$ (mM)	Theophylline (mM)	Ginsenoside-Rg_1 (mg/ml)	IRI release (ng/islet/60 min)
2.8	0	0	0	2.50 ± 0.46*
2.8	0	0	2	7.34 ± 0.51
2.8	0	10	0	2.90 ± 0.51
2.8	0	10	2	8.81 ± 2.47
2.8	2.5	0	0	3.16 ± 0.36
2.8	2.5	0	2	9.23 ± 2.32
2.8	2.5	10	0	3.62 ± 1.25
2.8	2.5	10	2	7.06 ± 1.00
16.7	2.5	0	0	6.15 ± 1.17

*) Mean ± S.E. of three experiments.
Note: Effect of ginsenoside-Rg_1 on insulin release is not influenced by $CaCl_2$ and/or theophylline.

The effect of high doses of DPG 3-2 fraction resembles that of ginseng saponins contaminating the fraction and the mechanisms of action of the latter appeared to the distinct from those of usual insulin secretagogues in which Ca^{2+} ions and the cyclic AMP system are apparently involved.

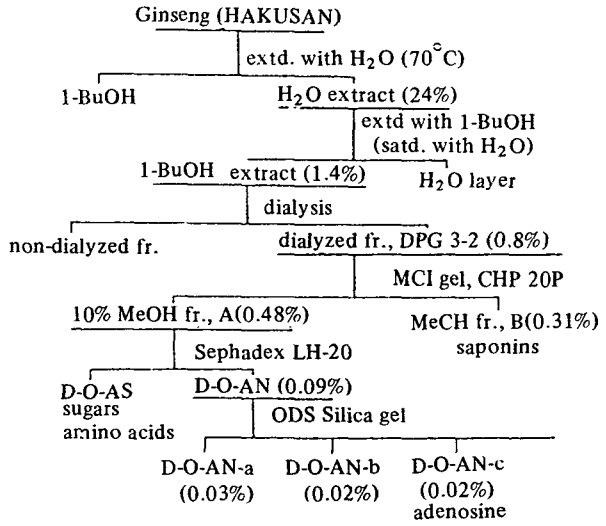

Fig. 4. Effect of ginsenoside-Rb_1 (A) and ginsenoside-Rg_1 (B) on insulin release from pancreatic islets of KK-CA^y mice incubated with 2.8 mM (○) or 16.7 mM (●) glucose.

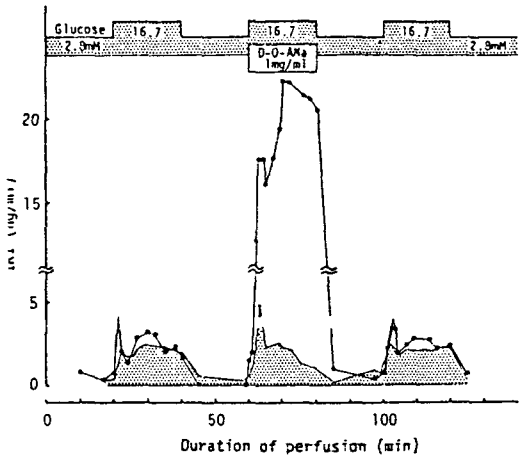

Fig. 5. Effect of ginsenoside-Rb_1 and ginsenoside-Rg_1 on insulin release in perfused rat pancreas.

Role of Ca^{2+} Ions in Glucose-Dependent Insulin Release Induced by the Hypoglycemic Component of Ginseng (DPG 3-2)

The role of Ca^{2+} ions in the insulin release effect of DPG 3-2 was studied on the isolated pancreatic islets of genetically diabetic KK-CA^y mice. The glucose-dependent insulin release by DPG 3-2 was selectively observed in the islets of diabetic mice. The effect was significantly poten-

tiated due to the increase in extracellular Ca^{2+} concentration (0.16 - 2.5 mM), as shown in Fig. 6. DPG 3-2 stimulated ^{45}Ca net uptake, which paralleled the insulin releasing activity (Fig. 7). A definite relationship was found between Ca^{2+} ion uptake and insulin release (Fig. 8). Furthermore, the effect of DPG 3-2 on Ca^{2+} ion uptake augumented more significantly at a high (16.7 mM) glucose concentration than at a low (2.8 mM) concentration (Table 3).

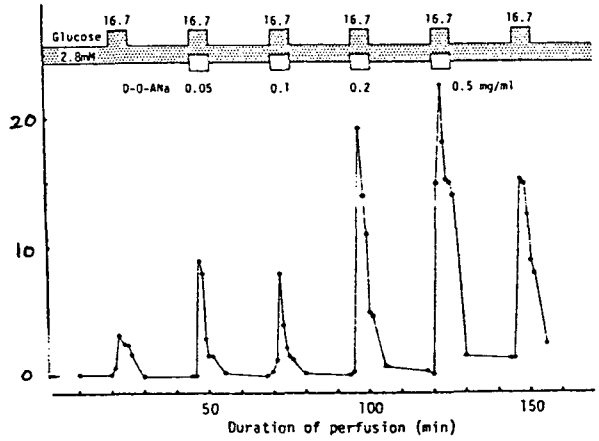

Fig. 6. Effect of $CaCl_2$ on DPG 3-2 - induced insulin release from pancreatic islets of KK-CA^y mice. Islets were incubated for 60 min at 37°C with 16.7 mM glucose and various concentrations of DPG 3-2 and $CaCl_2$.

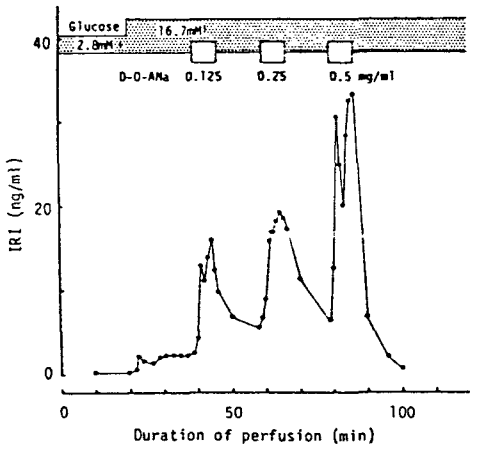

Fig. 7. Effect of DPG 3-2 on insulin release and Ca^{2+} uptake in pancreatic islets of KK-CA^y mice. Islets were incubated with 2.8 mM glucose, 2.5 mM $CaCl_2$ (2.5μCi ^{45}Ca) and various concentrations of DPG 3-2.

In comparison between the islets of diabetic male KK-CA^y mice and non-diabetic female KK-CA^y mice, which show a sex difference in the diabetes manifestation, the amount of insulin released by high doses of glucose in the diabetics was less than that in non-dibetics, although there was no difference between either group in the effect on Ca^{2+} ion uptake. This finding suggests that the impairment in insulin release in the diabetic KK-CA^y mice may not involve the

Table 3. Effects of DPG 3-2 on Calcium Uptake and Insulin Release in Isolated Pancreatic Islets of KK-CA^y Mice.

Glucose (mM)	$CaCl_2$ (mM)	DPG 3-2 (mg/ml)	Ca uptake[a] (pg/islet/ 60 min)	Δ	Insulin release[a] (ng/islet/ 60 min)	Δ
2.8	2.5	0	264 ± 40	] 238	1.62 ± 0.17[b]	] 0.34
2.8	2.5	2	502 ± 44		1.96 ± 0.12	
16.7	2.5	0	431 ± 42	] 323	2.25 ± 0.32	] 0.64
16.7	2.5	2	754 ± 62		2.89 ± 0.25	
16.7	6.25	0	2286 ± 425	] 1530	3.73 ± 0.41	] 0.82
16.7	6.25	2	3816 ± 848		4.55 ± 0.66	

a) Ca uptake and IRI release were assayed in the same islets under the condition of cycloheximide (0.1 mg/ml).
b) Mean ± S.E. of six to ten experiments.

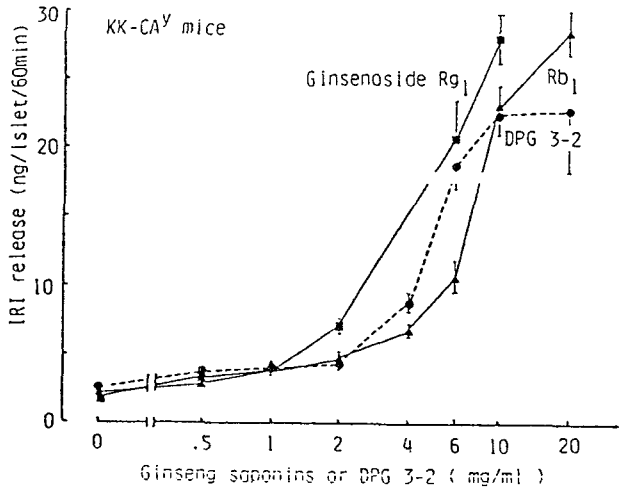

Fig. 8. Correlation between insulin release and Ca^{2+} uptake in pancreatic islets of KK-CA^y mice incubated with DPG 3-2. Each closed circle (●) refers to the experimental data, and open symbols represent the average of the effect of DPG 3-2; 0(○), 0.25 (△), 0.5 (▷), 1 (◁), 2 (□), 4 (◇) mg/ml. The ascending line was calculated by regression analysis. Data in this figure are the same as those used in Fig. 7.

process of Ca^{2+} ion uptake, but is related to the subsequent steps in Ca^{2+} ion dynamics. It can be speculated that DPG 3-2 stimulated the Ca^{2+} ion uptake, and the increase in the intracellular Ca^{2+} ions compensated for the defect.

CONCLUSION

D-O-ANa appears to be the principle component in insulin releasing activity in ginseng and its mode of action may involve Ca^{2+} ion uptake. This possible mechanism was evidently distinct from that of the abnormal release induced by ginseng saponins.

Acknowledgement

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Chong: You measured immunoreactive insulin. But did you have got an opportunity to actually measure the sugar levels as well, was it possible with your glucose infusions?

Kimura: No, in this method we measured insulin only.

Chong: In the perfusion model, did you measure insulin only?

Kimura: Yes, because glucose concentration was the same throughout the experiment.

Chong: Have you performed another experiment where you actually monitored the blood sugar level when you gave DOENA? Have you done an experiment where you actually monitored the blood sugar level in the animals you've studied? Have you done that?

Kimura: As I showed in the first slide, DPG-3.2 fraction lowered the blood glucose level but unfurtunately it was not done with this DOENA.

Sokabe: From your result on Ca^{++} uptake in the vascular muscle, I imagine it may act to increase blood pressure by constricting the vascular muscle. Have you ever tried to find out the effect of your compound on blood pressure?

Kimura: We have not yet determined the effect on blood pressure.

인삼성분 D-O-ANa이 인슐린 분비에 미치는 영향 및 작용기전에 관한연구

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인삼의 저혈당성 분획(DPG-3-2)이 적출한 랑겔한스섬에서 인슐린 분비를 증가시킨다고 일전에 본 저자들이 발표하였다. 근래에 이와 같은 DPG-3-2 분획으로부터 사포닌, 뉴크레오사이드, 뉴크레오타이드, 아미노산, 당 등을 완전히 제거시켜 더욱 정제한 D-O-ANa 성분을 추출하였다.

D-O-ANa 유발성 인슐린 분비효과를 DPG 3-2 분획과 그외 잔여성분과 비교 검토하였다.

D-O-ANa는 글루코우즈 농도의 고저에 무관하게 인슐린 분비를 가장 강하게 촉진하였다. 특히 D-O-ANa는 제 2 차증 글루코우즈 유발성 인슐린 분비를 촉진하였다.

DPG 3-2 분획은 당뇨병 쥐로부터 떼어낸 랑겔한스섬에서 세포외 체액의 칼슘이온이 증가됨에 따라 (0.16~6.25mM) 글루코우즈 유발성 인슐린 분비를 더욱 현저히 증가시켰다. 칼슘이온 흡수와 인슐린 분비의 상호관계가 뚜렷이 밝혀졌다. 이 관계는 single sucrose gap 방법에 의해 쥐의 적출 간 문맥에서 칼슘주파수가 증가되는 실험을 통해 증명되었다.

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