EFFECTS OF HYPOGLYCEMIC COMPONENTS IN GINSENG RADIX ON BLOOD INSULIN LEVEL IN ALLOXAN DIABETIC MICE AND ON INSULIN RELEASE FROM PERFUSED RAT PANCREAS

MASAYASU KIMURA, ISAMI WAKI, TADASHI CHUJO, TAKEO KIKUCHI, CHIZUKO HIYAMA, KAZUO YAMAZAKI AND OSAMU TANAKA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama, 930-01 Japan and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, ** Hiroshima, Japan

(Received July 16, 1980)

Some fractions extracted from ginseng radix (HAKUSAN) caused hypoglycemic effect on alloxan diabetic mice. The effect was abolished by the i.v. injection of antiserum against bovine insulin. The same doses of the ginseng fraction (10—50 mg/kg) produced an increase in the blood insulin level in alloxan diabetic mice. Normal mice loaded i.p. with glucose (2 g/kg or more) showed also such an increase. Insulin release from perfused rat pancreases was stimulated by the ginseng fraction (0.2 mg/ml), but the potency was not stronger than that of the sulfonylureas. It was demonstrated that glucose-induced insulin release was marked in the presence of the ginseng fraction. Impaired insulin responses to glucose in alloxan diabetic rats were increased by the fraction (0.5 mg/ml) to or above the control responses in normal rats. The enhanced effect was observed also in the presence of 100 μg/ml cycloheximide. These results indicate that some ginseng fractions stimulated insulin release, especially glucose-induced insulin release from pancreatic islets and thereby lowered the blood glucose level.

**Keywords**—ginseng radix; hypoglycemic effect; alloxan diabetic mice; blood insulin; insulin release; glucose; perfused pancreas

Byakko-ka-Ninjin-tô, one of Kanpô Hôzais (blended medicines) used with expectation of the treatment of diabetes in traditional medicine, is effective in lowering the blood glucose level in alloxan diabetic mice. The hypoglycemic effect is considered to be derived from combined actions of the constituent crude drugs and mainly dependent on ginseng radix which is capable of exhibiting a hypoglycemic action by itself. We attempted to isolate hypoglycemic components from ginseng radix and partial purification of the active principle has been carried out. The present experiment was conducted to determine whether or not the hypoglycemic effect was mediated by an increase in blood insulin and to determine whether or not the ginseng extract enhanced glucose effect on insulin release from pancreases. All the patients with diabetes mellitus have a high blood level of glucose and may have impaired insulin responses to glucose. Enhancement of glucose effect on insulin-release and -biosynthesis appears to play a significant role in the therapeutic effect of ginseng radix on diabetes mellitus.

MATERIALS AND METHODS

**Animals**—Male mice of dd-strain weighing 20—25 g and male rats of Wistar strain weighing 150—300 g were used. The animals were fasted for about 13—16 hr before each experiment.

**Alloxan Diabetic Animals**—Alloxan monohydrate was injected into a tail vein of mice at 84.5 mg/kg, and that of rats at 60 mg/kg. Seven days later, blood glucose levels were determined at fasting and the rodents with a glucose level of 150 mg% or more were used as alloxan diabetics.
Assay for Blood Levels of Glucose and Insulin — Blood samples (20 μl for glucose and 50 μl for insulin) were obtained by puncturing the orbital venous plexus with capillary glass tubes. The glucose level was determined by the method of Momose et al.6) and the level of insulin as immuno-reactive insulin (IRI) by the radioimmunoassay using talcum powder.7) The hypoglycemic activity in alloxan diabetic mice was evaluated as described previously.2) The anti-insulin serum chosen for the insulin assay was obtained from one of guinea pigs sensitized with bovine insulin.

Tests on Insulin Disappearance from Blood — Alloxan diabetic mice were given 20 ng insulin containing 0.3 μCi 125I-insulin via a tail vein. At predetermined intervals blood samples of 50 μl were withdrawn by the orbital puncture followed by the addition of trichloroacetic acid (TCA) at a final concentration of 10%. All the TCA-precipitable radioactivity were measured and assumed to represent intact insulin.

Pancreatic Perfusion — This was performed according to the methods of Grodsky et al.8) and Penhos et al.9) Rats were injected i.v. with heparin (2000 U/kg) and decapitated 5 min later under ether anesthesia. The superior mesenteric, gastric and hepatic arteries, common bile duct and the duodenum at the upper and lower portions distal to the pancreas were ligated. The celiac artery was cannulated for arterial inflow and the portal vein to collect the venous effluent. The perfusate used was Krebs-Ringer bicarbonate buffer solution containing 1% bovine serum albumin and 5% dextran (M.W. 40000). This buffer was equilibrated with 95% O2-5% CO2 gas and adjusted to pH 7.4. The flow rate was kept at 1.3 ml/min and the buffer and perfusion apparatus were maintained at 37°. The perfusate alone was perfused for 15 min until a test compound dissolved in the buffer was perfused. Total insulin release from the pancreas into the venous effluent was calculated from the concentration of insulin over initial level and the effluent volume.

Preparations of EPG-3-2 and DPG-3-2 — Ginseng fractions lowering blood glucose level in alloxan diabetic mice were obtained from ginseng radix (HAKUSAN) (Fig. 1). Both the hypoglycemic fractions contained unknown substances as major components and ginsenoside Rg6 as minor components.

Compounds used — 125I-insulin was obtained

![Graph](image)

FIG. 1. Isolation and partially Purification of Hypoglycemic Fractions from Ginseng Radix (HAKUSAN) and their Dose-response Curves in Alloxan Diabetic Mice

Each point represents the mean ± S.E. from 8—10 mice. Fall% was calculated according to the equation2) that 100% is obtained when a blood glucose level in mice injected with a test compound decreased to 85 mg%, a mean blood glucose level in normal dd-mice fasted.
from Japan Radioisotope Association, Tokyo. Tolbutamide and metformine were obtained from Toyama Chemical Industry, Co., Ltd. and glibenclamide from Yamanouchi Pharmaceutical Co., Ltd., carbutamide from Sumitomo Chemical Co., Ltd. and bovine crystalline insulin from Shimizu Seiyaku Co., Ltd.

RESULTS
Effect of Anti-insulin Sera on the Hypoglycemic Activity of Ginseng Fraction

EPG-3-2 and DPG-3-2 lowered in a dose-dependent manner the blood glucose level in alloxan diabetic mice as shown in Fig. 1 and the ED\textsubscript{50} with 95% confidence limits were 17.9 (11.4–28.0) and 8.9 (4.9–17.9) mg/kg, respectively. The hypoglycemic effect of 50 mg/kg of EPG-3-2 (Fig. 2) and 180 mg/kg of the compound was inhibited by the anti-insulin sera. The hypoglycemic effect of carbutamide (at a near lethal dose of 800 mg/kg, i.p.) was also inhibited by the same anti-insulin sera, whereas metformin was not affected at all (Fig. 2). The hypoglycemic effect of EPG-3-2 appeared to be dependent on the increase in blood insulin.

Effect of EPG-3-2 on Blood Insulin Level in Alloxan Diabetic and Normal Mice

Effect of EPG-3-2 on blood insulin level in alloxan diabetic and normal mice was compared with that of carbutamide as shown in Table I. In alloxan diabetic mice EPG-3-2 produced a dose-dependent increase in the blood insulin level but not so in normal mice, whereas carbutamide did so in normal animals.

| TABLE I. Effect of EPG-3-2 on Blood Insulin Levels in Alloxan Diabetic and Normal Mice |
|-------------------------------------|------------------|------------------|------------------|
| Compounds                  | mg/kg | Δ Blood IRI concentration, ng/ml | Alloxan diabetic | Normal          |
|---------------------------|-------|---------------------------------|------------------|----------------|---------------|
| EPG-3-2                   | 10    | 0.82±0.25                       | 0.97±0.61        |
|                           | 18    | 1.17±0.21                       | 0.81±0.29        |
|                           | 50    | 1.53±0.47                       | 0.72±0.23        |
| Carbutamide               | 25    | 0.18±0.17                       | 0.55±0.19        |
|                           | 50    |                                 | 0.97±0.37        |

Each value represents mean ± S.E. from 3–5 mice. Blood immunoreactive insulin (IRI) increased was shown as the concentration increased over the initial level during the 30–180 min period after the i.p. injection of compounds. The mean initial values of alloxan diabetic and normal mice were 0.63± 0.15 ng/ml and 0.64±0.21 ng/ml, respectively.
Normal mice loaded with glucose were used as a different model of hyperglycemia from alloxan diabetes. Table II shows that the increase in blood insulin level was markedly induced by EPG-3-2 in mice loaded with 2 g/kg or more of glucose.

The responses of glucose-loaded mice were higher than those of alloxan diabetic mice. Effect of EPG-3-2 on the Disappearance of Insulin from Blood

The mechanism by which EPG-3-2 increased the blood insulin was considered as an increase in insulin release into blood and/or a decrease in insulin disappearance from blood. Fig. 3 shows that the effect of EPG-3-2 on the disappearance of insulin in alloxan diabetic mice was not significant at a dose of 50 mg/kg, producing an increase in the blood insulin and a decrease in the blood glucose.

FIG. 3. Effect of EPG-3-2 on Disappearance of Insulin from Blood in Alloxan Diabetic Mice

EPG-3-2 was applied i.p. 4 hr before the injection of insulin into a tail vein. Each point represents the mean ± S.E. from 4 mice of counts of TCA-precipitable radioactivity. AIS represents the disappearance curve of insulin in the presence of anti-insulin sera used as a positive control.

FIG. 4. Time Courses of IRI Release induced by Glucose with DPG-3-2 and Glucose Alone from Perfused Rat Pancreases

Columns represent the mean ± S.E. from 3 – 5 experiments for the insulin concentration over the initial level (3.6 ± 0.5 ng/ml) of venous effluent pooled at 5 min intervals.
Effect of DPG-3-2 on Insulin Release

The time courses of insulin release from perfused rat pancreases were examined. Fig. 4 shows that an increase in insulin release induced by 2 mg/ml glucose was evident in the first 5-min period of perfusion, while those induced by the glucose combined with 0.2 mg/ml DPG-3-2 were apparent in the periods from the second to the fifth 5-min. DPG-3-2 (0.2 mg/ml) alone increased insulin release by 6.5±1.9 ng/5 min (mean ±S.E., n =5) throughout the perfusion period. The effect of glucose (5 mg/ml) with or without DPG-3-2 was not inhibited by cycloheximide (Table III). Therefore, it is unlikely that the increase by DPG-3-2 and/or glucose of the insulin release from the perfused rat pancreas is dependent on insulin biosynthesis.

Glucose, tolbutamide and glibenclamide stimulated the insulin release, but metformin did not so as shown in Table IV. DPG-3-2 alone stimulated insulin release, but its potency was not so high as that of the sulfonylureas. In alloxan diabetic rats, DPG-3-2 scarcely caused insulin release.

**Potentiating Effect of DPG-3-2 on Glucose-induced Insulin Release**

Dose-response relationships of glucose for insulin release from perfused pancreas of normal and alloxan diabetic rats were shown in Fig. 5. The amount of insulin released from normal rat

| TABLE III. Effect of Cycloheximide on Stimulated Insulin Release from Perfused Rat Pancreases |

<table>
<thead>
<tr>
<th>Glucose</th>
<th>DPG-3-2</th>
<th>Period</th>
<th>IRI release, ng/5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/ml</td>
<td>mg/ml</td>
<td>min</td>
<td>Control</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0–5</td>
<td>41.0±5.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>5–25</td>
<td>20.1±2.6</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0–5</td>
<td>37.1±5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–25</td>
<td>37.1±3.9</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. from 5 experiments for the IRI/5 min released over the initial level during the perfusion period. The mean initial value was 2.7±0.3 ng/ml.

a) Cycloheximide was perfused at 0.1 mg/ml for 15 min before and throughout the perfusion period of stimulants.

| TABLE IV. Effect of Some Compounds on Insulin Release from Perfused Rat Pancreases |

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mg/ml</th>
<th>IRI released, ng/20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Control</td>
<td>0.05</td>
<td>5.0±3.5</td>
</tr>
<tr>
<td>DPG-3-2</td>
<td>0.2</td>
<td>26.5±7.0^a)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>40.0±7.5^a)</td>
</tr>
<tr>
<td>Glucose</td>
<td>1</td>
<td>29.3±18.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>52.5±11.5^a)</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>0.4</td>
<td>61.4±25.4^a)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>149.7±30.4^a)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.05</td>
<td>134.1±23.1^a)</td>
</tr>
<tr>
<td>Metformin</td>
<td>1</td>
<td>−4.0±10.5</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. from 3–5 experiments for the IRI released over the initial level during the first 20 min period of perfusion. The mean initial values in normal and alloxan diabetic rats were 3.0±0.3 ng/ml and 1.8±0.6 ng/ml, respectively.

a) Significantly different from control (p < 0.05).
Blood Insulin Released by Ginseng

FIG. 5. Effect of DPG-3-2 on Dose-response Relationships of Glucose for Insulin Release from Perfused Pancreases of Normal (broken line) and of Alloxan Diabetic (solid line) Rats

Each point represents the mean ± S.E. from 3 experiments of the IRI released over the initial level (3.0 ± 0.3 ng/ml in normal, 1.6 ± 0.3 ng/ml in diabetic) during the first 20-min period of perfusion.

pancreas was more than 2-fold that from alloxan diabetic animals. The height of response to 10 mg/ml glucose did not reach a plateau in normal rats but it did in alloxan diabetics.

When DPG-3-2 was perfused together with glucose, an additive effect of DPG-3-2 on insulin release was observed in both normal and alloxan diabetic rats (Fig. 5). In particular the increase in glucose-induced insulin release from alloxan diabetic rat pancreas was marked when DPG-3-2 was added to 0.5 mg/ml. The height of response was quite high that the dose-response curve in alloxan diabetic rats was equivalent to the curve in normal rats treated with glucose alone. DPG-3-2 enhanced the effect of glucose on insulin release.

DISCUSSION

EPG-3-2 and DPG-3-2 fractions purified partially from the ginseng radix were found to lower the blood level of glucose in fasted alloxan diabetic mice. The decrement induced by the ginseng fraction tested never reached 100%, indicating that the ginseng component never lowered the blood glucose level below the mean level in normal fasted mice. In fact EPG-3-2 did not influence blood glucose level in fasted normal dd-strain mice.2) The hypoglycemic effect was abolished with i.v. injection of anti-insulin sera. The ginseng component appears to work in a different manner from that of the biguanides, since metformin was capable of exerting its effect in the presence as well as in the absence of anti-insulin antibody. Metformin did not lower the level in fasted diabetic mice until a near lethal dose was chosen. Similar results10,11) are reported in respect to effect of phenformin on the level in diabetic animals. Therefore, the hypoglycemic effect of ginseng component in fasted diabetic mice has a pharmacological property different from that of the biguanides.

EPG-3-2 fraction was found to increase blood IRI in alloxan diabetic mice. This dose-related effect was produced at the same doses that evoked the hypoglycemic effect. Normal mice failed to show such a dose-related increase, indicating that the mechanism of the ginseng component responsible for lowering blood glucose was not the same as that of the sulfonylureas.12-14) Normal mice, however, showed a marked increase in blood IRI when loaded i.p. with 2 g/kg or more of glucose. The increment of blood IRI in glucose-loaded normal mice was similar to that in diabetic mice. The IRI increase is due to an increase in IRI release into blood and/or a decrease in IRI disappearance from blood.15) The latter was not shown in the presence of EPG-3-2.

The hypoglycemic ginseng fraction was found to cause insulin release from perfused rat pancreas. The potency of the ginseng component which was not completely purified, was not stronger than that of the sulfonylureas reported.12-14) Furthermore the ginseng scarcely caused insulin release from alloxan diabetic pancreases. Marked insulin release was not demonstrated until the ginseng component mixed with glucose were infused into the celiac artery. This enhancing effect was not significantly reduced with 0.1 mg/ml cycloheximide which inhibits insulin biosynthesis.16) Secretion of newly synthesized, labeled insulin into the incubation medium
occurs about 1 hr after pulse labeling.\textsuperscript{17,18} Increment of insulin release from perfused rat pancreases during the first 20-min period of perfusion will not be dependent on insulin biosynthesis.\textsuperscript{10} Alloxan diabetic rat pancreases also showed such enhanced effect. It has been reported that insulin responses to glucose are impaired in diabetic islets.\textsuperscript{3–6} We observed that insulin responses in alloxan diabetic rats were reduced to less than a half as compared with those in normal rats. The diminished response was improved by DPG-3-2 fraction. A 0.5 mg/ml dose recovered it to or above the control insulin response in normal rats. The potentiation mechanisms of glucose effect will be investigated in relation to glucoreceptor mechanism\textsuperscript{20} in respect to insulin release and biosynthesis.

A new oral hypoglycemic agent, McN-3495 that differs structurally from both the sulfonylurea and phenformin has been introduced.\textsuperscript{10,11} The compound can lower the blood glucose level in fasted animals with or without diabetes. No hypoglycemic effect on fasted mice without diabetes is a characteristic of the ginseng component in addition to marked hypoglycemic effect on mice with diabetes. The ginseng component with hypoglycemic activity appears to belong to a new type of hypoglycemic agent, at least differs from the sulfonylureas, the biguanides and McN-3495, and may be useful in improving impaired glucoreceptor mechanism in diabetes.

Acknowledgement We acknowledge Dr. M. Goto of Takeda Chemical Industries, Ltd. for supplying ginseng roots (HAKUSAN). This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan.

REFERENCES
17) S.L.Howell and K.W.Taylor: Secretion of newly syn-

