EFFECTS OF A HYPOGLYCEMIC COMPONENT OF GINSENG RADIX ON INSULIN BIOSYNTHESIS IN NORMAL AND DIABETIC ANIMALS

ISAMI WAKI, HITONOBU KYO, MASATOSHI YASUDA AND MASAYASU KIMURA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

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DPG-3-2, a component of ginseng radix, which lowers the blood glucose level and stimulates insulin release in diabetic animals, was studied for its effects on insulin biosynthesis in different preparations of pancreas from animals with normoglycemia and with hyperglycemia (alloxan diabetic rats and genetically diabetic mice, KK-CAy). We measured incorporation of radioactive leucine into insulin and other protein fractions during a 2-h perfusion of rat pancreas, and into insulin during a 3-h incubation of mouse islets. Biosynthesis of insulin during a long-term culture of islets from KK-CAy mice was also measured. DPG-3-2 was found practically not to increase the incorporation into insulin in the pancreatic preparations from animals with normoglycemia, but in such preparations from animals with hyperglycemia DPG-3-2 (0.2–1.0 mg/ml) caused 1.5–1.8-fold incorporation into insulin. In addition, a long-term treatment of DPG-3-2 (0.5 mg/ml) was shown to stimulate insulin biosynthesis in islets from KK-CAy mice. Ginsenoside-Rb1 and -Rg1 decreased the insulin content of islet to the undetectable level. Thus, DPG-3-2 was shown to stimulate insulin biosynthesis in different preparations of pancreas from animals with hyperglycemia.

**Keywords** — ginseng radix, hypoglycemic component, insulin biosynthesis, perfused rat pancreas, pancreatic islets, islet culture, ginsenoside

Ginseng radix has long been used in Chinese traditional medicine. Various blended preparations including ginseng radix are available for a therapy of diabetes mellitus.1) A hypoglycemic component was partially purified from ginseng radix by various methods of fractionation based on an assay system to evaluate hypoglycemic effect in alloxan diabetic mice.1) The component was shown to increase the blood level of insulin in alloxan diabetic mice, to stimulate insulin release from perfused rat pancreas, and to enhance glucose-stimulated insulin release from the pancreas.20

The purpose of this study was to determine whether the hypoglycemic component would influence basal and glucose-stimulated insulin biosynthesis in pancreases from normal and diabetic animals. We measured incorporation of radioactive leucine into insulin and other protein fractions during perfusion of pancreases from normal and alloxan diabetic rats, and during incubation of pancreatic islets from mice with normoglycemia (KK) and with hyperglycemia (KK-CAy).3) Biosynthesis of insulin during culture of islets from KK-CAy mice was measured to evaluate a long-term effect of the hypoglycemic component.

MATERIALS AND METHODS

**Animals** — Male Wistar rats weighing 150–300 g, male KK mice 10–15 weeks old, weighing 20–30 g and having a blood glucose level of 100–150 mg/100 ml, and KK-CAy mice 12–15 weeks old, weighing 35–50 g and having a blood glucose level over 200 mg/100 ml were used. Some rats were injected with alloxan as previously reported.2) The rats and the mice were fasted for about 16 and 13 h, respec-
tively, before experiments.

**Radiolabeling of Proteins during Perfusion of Pancreas**—Isolated rat pancreas was perfused by the previously described method with some modifications. The perfusate was a modified Krebs-Ringer bicarbonate buffer solution (pH 7.4) (KRB) supplemented with 1% bovine serum albumin (BSA), 5% dextran and 6 mM Mg<sup>2+</sup>. Ca<sup>2+</sup> was not included to suppress insulin release from perfused pancreases. This modified KRB (30 ml), after addition of 1 μCi (2.87 nmol) of 14C-leucine, was circulated at 38°C at the rate of 2.3 ml/min for 2 h. After approx. 10-times circulation, the pancreas was quickly frozen and stored at −20°C. The homogenate was prepared in 10% trichloroacetic acid (TCA) solution. The TCA-precipitate separated by centrifugation at 15000 g for 20 min was extracted 3 times with 1 ml of acidified ethanol (ethanol : conc. HCl : H<sub>2</sub>O = 47.5:1:16.5 in volume). By the extraction procedure, 125I-insulin was recovered by approx. 60% from TCA-precipitates. A portion (0.4 ml) of each extract was measured for radioactivity, and 2 ml portion was pooled. Pooled extract with the same treatment was dialyzed against 1 N acetic acid, and 2 ml portion was applied to a Sephadex G-50 (fine) column (17 × 480 mm) equilibrated with 1 N acetic acid, and eluted with the same solvent. The eluate was collected in 3 ml fractions to be measured for radioactivity. The column was calibrated with BSA and bovine insulin, and the BSA-detectable, the intermediate and the insulin-detectable fractions were expressed as I, II and III, respectively.

**Radiolabeling of Insulin in Mouse Pancreatic Islets**—Pancreatic islets of Langerhans were isolated by the collagenase method from KK and KK-CA<sup>y</sup> mice. The pancreas was swollen by injecting Hank’s solution into the pancreatic duct, then isolated and minced with scissors. BSA and collagenase (1000 U/mg protein) were added to give final concentrations of 20 mg/ml and 4 mg/ml, respectively. The reaction mixture (up to 5 ml) was shaken at 200 strokes/min for 12−15 min at 37°C, then diluted to 50 ml with ice-cold Hank’s solution and allowed to settle for about 3 min. The upper portion (20 ml) of the dilution was removed and the same volume of fresh ice-cold medium was added. This washing procedure was repeated 8 times until the supernatant became clear. Then, under a stereoscopic microscope, islets of Langerhans were picked up by means of a micropipet and transferred to ice-cold KRB. Radiolabeling of insulin in pancreatic islets was performed by the method of Sando et al. with some modifications. Ten islets were incubated under an atmosphere of 95% O<sub>2</sub>−5% CO<sub>2</sub> at 37°C for 3 h in 200 μl of KRB supplemented with BSA (2 mg/ml), 125I-leucine (2 μCi/ml, 3.4 μg/ml) and 18 other amino acids (20 μg/ml each). After incubation, the islets were washed twice with 0.5 ml of KRB containing 50 μg/ml of non-radioactive leucine, and homogenized by sonication (500 W, 95 kHz, 15 min) in 0.5 ml of acidified ethanol at 0°C. After centrifugation, the supernatant (0.2 ml corresponding to that of 4 islets) added with or without bovine insulin (200 μg/0.2 ml) was neutralized and incubated at 37°C with 1.0 ml of the 1/10 dilution of antirat insulin serum obtained from a guinea pig. After a 60-min incubation, 1 ml of 30% solution of polyethylene glycol (PEG) was added to the reaction mixture and the PEG-precipitate, after being washed, was dissolved in water to be measured for radioactivity. Bound 125I-insulin (0.2 ng) to the diluted antisera was 58% of the total in the presence of 0−5 μg insulin, but 200 μg or more insulin inhibited it to 10%, which was a nonspecific binding activity. Therefore, biosynthesized radioactive insulin was estimated as immunoreactive insulin (IRI, insulin and related-proteins in immunoreactivity) by subtracting the bound radioactivity in the presence of 200 μg insulin from the bound radioactivity in the absence of it.

**Culture of Pancreatic Islets**—Pancreatic islets of KK-CA<sup>y</sup> mice were isolated using 8 mg/ml collagenase (190−210 U/mg protein) under sterile conditions. Then, 3 islets were incubated at 37°C under a water-saturated atmosphere of
Ginseng Effect on Insulin Biosynthesis

95% air-5% CO₂ in 1 ml of culture medium consisting of 90% Eagle's MEM and 10% bovine serum. Bovine serum was inactivated by heating at 56°C for 30 min. The incubation medium containing a test compound was changed every 5 d. The concentration of insulin of culture medium was determined by radioimunoassay with bovine insulin as standard. The insulin content of islet was determined by extracting insulin with acidified ethanol followed by the radioimunoassay.

Compounds—Purified collagenase (1000 U/mg protein) and partial purified collagenase (190–210 U/mg protein) were obtained from Amano Pharmaceuticals Co. and Worthington Biochemical Corp., respectively. Insulin, leucine, leucine, and leucine-4,5,3H (specific activity 1 Ci/mmol, RCC Amersham) were obtained via Japan Radioisotope Association. Eagle's MEM from Nissui Pharmaceuticals Co. and bovine crystalline insulin from Shimizu Seiyaku Co., Ltd. A hypoglycemic component of ginseng radix (DPG-3-2) and guinea pig antitoxin sera were obtained as previously reported. Ginsenoside-Rb₁ and -Rg₁ were isolated and purified according to the current methods.

RESULTS

Effects of DPG-3-2 on Incorporation of 14C-Leucine into Protein Fractions during Perfusion of Rat Pancreas

During a 2-h perfusion of pancreas of normal and alloxan diabetic rats, 14C-leucine was incorporated into protein fractions. The incorporation into the total protein of the acid-ethanol extract of pancreas from diabetic rats was more markedly done, compared with that seen in the case of pancreas from normal rats (Table I). Glucose and DPG-3-2 increased the latter incorporation. The total protein fraction of the extract of pancreas from normal rats was divided into I, II and III. Glucose was shown to stimulate the incorporation into III, whereas DPG-3-2 appeared to increase the incorporation into I, which was almost not sensitive to glucose.

<table>
<thead>
<tr>
<th>Pancreas</th>
<th>Compound</th>
<th>Incorporation (cpm/g wet pancreas, 2h)</th>
<th>Relative amount of III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acid-ethanol extracted protein fraction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Totala)</td>
<td>I b)</td>
</tr>
<tr>
<td>Normal rat</td>
<td>Control</td>
<td>2199 ± 314 (7)</td>
<td>1176</td>
</tr>
<tr>
<td></td>
<td>DPG-3-2 (0.2 mg/ml) (D)</td>
<td>4006 ± 322 (4)</td>
<td>2023</td>
</tr>
<tr>
<td></td>
<td>Glucose (2 mg/ml) (G)</td>
<td>4287 ± 535 (7)</td>
<td>1586</td>
</tr>
<tr>
<td></td>
<td>D+G</td>
<td>4928 ± 408 (3)</td>
<td>2301</td>
</tr>
<tr>
<td>Alloxan diabetic rat</td>
<td>Control</td>
<td>5773 ± 1020 (3)</td>
<td>3983</td>
</tr>
<tr>
<td></td>
<td>DPG-3-2 (0.2 mg/ml) (D)</td>
<td>5895 ± 1046 (3)</td>
<td>3424</td>
</tr>
<tr>
<td></td>
<td>Glucose (5 mg/ml) (G')</td>
<td>5373 ± 380 (3)</td>
<td>2939</td>
</tr>
<tr>
<td></td>
<td>D+G'</td>
<td>5427 ± 466 (2)</td>
<td>2610</td>
</tr>
</tbody>
</table>

a) Values represent the mean±S.E. followed by the number of animals in parentheses.
b) The value was obtained by dividing each mean total in the direct ratio of I:II:III determined by fractionating the radioactivity of pooled extract using Sephadex G-50 columns.
c) The percentage of control value.
d) p<0.05, compared with control value.
DPG-3-2, however, appeared to increase the incorporation into the insulin fraction of pancreas from diabetic rats, whereas the supramaximal concentration (5 mg/ml) of glucose never stimulated such incorporation (Fig. 1, Table I). When DPG-3-2 and glucose were combined, no additive effect on the incorporation into III was apparently observed.

**Effects of DPG-3-2 on Incorporation of $^3$H-Leucine into IRI during Incubation of Mouse Islets**

Tritium-labeled leucine was incorporated into IRI during a 3-h incubation of islets from mice with normoglycemia (KK) and with hyperglycemia (KK-CA). Glucose at the maximal concentration of 3 mg/ml markedly stimulated the incorporation into IRI in islets from both mice (Table II). Addition of DPG-3-2 (1 mg/ml) to 0.5 mg/ml of glucose showed no increase of the incorporation in islets from KK mice, but when islets from KK-CA mice were incubated, DPG-3-2 (0.5, 1 mg/ml) significantly stimulated the incorporation. A combination of DPG-3-2 (1 mg/ml) with glucose (3 mg/ml) in a modified KRB which could suppress insulin release from islets had no additive effect (Table II-b). In normal KRB, however, the stimulatory effect of glucose on the incorporation was apparently inhibited by DPG-3-2. This concentration-related effect of DPG-3-2 was observed more markedly in islets from KK mice, compared with that seen in islets from KK-CA mice.

**Effects of DPG-3-2 and Ginsenosides on Insulin Biosynthesis during Culture of Islets**

Pancreatic islets of KK-CA mice were cultured for 15 d in the presence of either 1 mg/ml or 3 mg/ml of glucose with or without a test compound. On the 5th, 10th and 15th day, the content of IRI per islet (IRI CONTENT) and the amount of IRI in culture medium per islet (MEDIUM IRI) were determined. In the presence of 3 mg/ml of glucose, MEDIUM IRI largely accumulated without a decrease in the IRI CONTENT (Fig. 2). However, 1 mg/ml of glucose allowed a decrease in IRI CONTENT and MEDIUM IRI. Addition of DPG-3-2 (0.5 mg/ml) to 1 mg/ml of glucose prevented the decrease in IRI CONTENT seen on the 10th day in the presence of glucose alone. In addition, MEDIUM IRI to the non-detectable level. than the decrement in IRI CONTENT. Especially, the amount of insulin synthesized during the second 5-day culture period was almost equal to the content of insulin of islet. The additive effect of DPG-3-2 and glucose (3 mg/ml) was apparently not observed in a long-term culture. Ginsenosides decreased IRI CONTENT and MEDIUM IRI to the non-detectable level.

Microscopy demonstrated that pancreatic islets had a sort of cell damage, when cultured in ginsenoside-containing medium. DPG-3-2 never changed microscopic features of living cultures.
DISCUSSION

In order to evaluate the effect of DPG-3-2 on insulin biosynthesis, we first measured the incorporation of radioactive leucine into protein fractions of rat pancreas. The incorporation into protein fractions of the acid-ethanol extract of pancreas from normal rats was increased by glucose or DPG-3-2 under conditions suppressing insulin release from the pancreas. The acid-ethanol extracted protein fractions were divided into I (BSA fraction), II (intermediate fraction) and III (insulin fraction) by applying to a Sephadex G-50 column as already reported.\textsuperscript{6,7} Glucose was shown to increase the incorporation into mainly III. Since glucose is a specific stimulator of insulin biosynthesis, the incorporation into III was compared for the purpose of evaluating the effect of DPG-3-2 on insulin biosynthesis. The stimulant effect of DPG-3-2 on the incorporation into III in normal rat pancreas was far less marked than that of glucose. DPG-3-2 was found to increase the incorporation into protein fractions which was almost insensitive to glucose. However, when pancreases of diabetic rats were perfused, DPG-3-2 appeared to increase the incorporation into III. In such experiments, in contrast, glucose showed no stimulation. Additive effect on incorporation into III between DPG-3-2 and glucose was not shown especially in pancreases of diabetic rats. Since we failed to isolate pancreatic islets from alloxan diabetic rats, further studies to evaluate the effect of DPG-3-2 on insulin biosynthesis were performed using islets from genetically diabetic mice.

During a 3-h incubation of islets from mice with normoglycemia (KK) and with hyperglycemia (KK-CA\textsuperscript{Y}), radioactive leucine was incorporated into IRI. Glucose caused 29- and 5.8-fold incorporation into IRI in islets from KK and KK-CA\textsuperscript{Y} mice, respectively. Glucose stimulation of the incorporation was shown to be more marked in islets from mice without hyperglycemia. Absolute values of the incorpora-

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
\textbf{Islet} & \textbf{DPG-3-2 (mg/ml)} & \textbf{Radioactive IRI (cpm/4 islets, 3h)} & \\
 & & \textbf{Glucose (mg/ml)} & \\
 & & 0.5 & 3 \\
\hline
KK & 0 & 35 ± 14 (5) & 1026 ± 101 (5) \\
 & a) 0.5 & 47 ± 18 (3) & 637 ± 32 (3)\textsuperscript{c} \\
 & 1.0 & 166 ± 26 (4) & 512 ± 40 (3)\textsuperscript{c} \\
 & a) 0 & 310 ± 29 (3)\textsuperscript{c} & \\
 & 1.0 & 495 ± 73 (5) & \\
KK-CA\textsuperscript{Y} & a) 0.5 & 750 ± 87 (3)\textsuperscript{c} & 2893 ± 175 (6) \\
 & 1.0 & 2768 ± 157 (3) & \\
 & b) 0 & 4596 ± 318 (4) & \\
 & 1.0 & 3740 ± 255 (3) & \\
\hline
\end{tabular}
\caption{Effects of DPG-3-2 on Incorporation of \textsuperscript{3}H-Leucine into IRI in Mouse Islets}
\end{table}

Values represent the mean ± S.E. followed by the number of experiments in parentheses. 
\textit{a}) Experiments in the medium as in Materials and Methods. 
\textit{b}) Experiments in a modified medium of which concentrations of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} were 0 mM and 6 mM, respectively, to suppress insulin release from pancreatic islets. 
\textit{c}) p<0.05, compared with each control value in the absence of DPG-3-2. The incorporation into IRI in the presence of 3 mg/ml of glucose was all significantly greater than the corresponding value in the presence of 0.5 mg/ml of glucose.
tion were higher in KK-CA\textsuperscript{Y} mouse islets, because KK-CA\textsuperscript{Y} mouse islets were greater in volume than KK mouse ones.\textsuperscript{31} In contrast, DPG-3-2 stimulated the incorporation into IRI

**FIG. 2. Effects of DPG-3-2 (0.5 mg/ml) and Ginsenosides (0.5 mg/ml) on Insulin Biosynthesis in Cultured Islets from KK-CA\textsuperscript{Y} Mice**

The content of IRI per islet (IRI CONTENT) (○) was determined on the 5th, 10th and 15th d in culture in addition to the initial value. Each point represents the mean of 2—6 islets. The S.E. was less than 1. The amount of IRI in culture medium per islet (MEDIUM IRI) was determined every 5 d. MEDIUM IRI was added to IRI CONTENT, and values of the total (●) are shown. Each point represents the mean of 2—4 dishes. Vertical bars represent the S.E. When the S.E. was less than 1, the bars are not shown. Symbols of (××) and (×××) are shown when MEDIUM IRI was more than and less than the decrement in IRI CONTENT, respectively. Ginsenoside-Rb\textsubscript{1} and -Rg\textsubscript{3} were tested, and the mean values are shown, because both effects were similar.
in islets not from KK mice with normoglycemia but from KK-CA\(^Y\) mice with hyperglycemia. When DPG-3-2 was added to glucose, the combined effect on the incorporation into IRI in islets from KK-CA\(^Y\) mice was similar to the effect of glucose alone under conditions suppressing insulin release from the islets. These findings were coincident with those seen in the case of perfused rat pancreas. When no treatment was done for suppressing insulin release, DPG-3-2 inhibited glucose-stimulated incorporation in islets from especially KK mice. When it became clear that potentiating effect of DPG-3-2 on glucose-stimulated insulin release\(^2\) would result in a marked decrease in the content of radiolabeled insulin in islets, it was proposed that influences of DPG-3-2 on insulin biosynthesis were weaker or slower in action than the activity on insulin release.

Pancreatic islets of KK-CA\(^Y\) mice were maintained in tissue culture for 15 d. The amount of newly-synthesized insulin was estimated every 5 days by subtracting IRI CONTENT obtained in the past 5 days from the total IRI (MEDIUM IRI plus IRI CONTENT). If some factors caused insulin degradation, we would have an underestimation of insulin biosynthesis. Glucose (3 mg/ml) increased the amount of insulin in the culture medium to 150% of IRI CONTENT without a decrease in the IRI CONTENT, whereas 1 mg/ml of glucose showed a decrease in the total IRI and IRI CONTENT. Addition of DPG-3-2 (0.5 mg/ml) to 1 mg/ml of glucose was shown to stimulate insulin biosynthesis. Above all, the amount of insulin synthesized in the second 5-day culture was nearly equal to the IRI CONTENT. When DPG-3-2 was combined with 3 mg/ml of glucose, a marked decrease in IRI CONTENT was observed in the first 5-day culture period. However, the insulin synthesis in the subsequent periods was not less remarkable than that seen in the case of 3 mg/ml of glucose. It remains to be solved whether some contamination included in DPG-3-2 would cause the decrease in IRI CONTENT during the first 5-day culture. In any case, long-term effect of DPG-3-2 would never result in an inhibition of glucose-stimulated insulin biosynthesis. In the presence of ginsenoside, on the other hand, IRI CONTENT decreased to the bottom value and MEDIUM IRI became undetectable even in the presence of 3 mg/ml of glucose. In addition, ginsenosides atrophied islet cultures whereas no atrophy was observed in the presence of DPG-3-2 (0.5 mg/ml).

Some ginsenosides have been reported to stimulate incorporations of radioactive leucine into mouse\(^1\) and rat\(^1\) serum proteins and rat bone marrow proteins,\(^2\) and of \(^14\)C-acetate into lipids\(^2\) in rat bone marrow and into cholesterol\(^2\) in rat liver. In those studies, radioactive compounds were injected i.p. into normal and ginsenoside-treated animals, and then the target organs were isolated to be measured for radioactivity. Ginsenoside-Rb\(_1\) which stimulated the \textit{in vivo} incorporation was ineffective in stimulating an \textit{in vitro} incorporation in liver slice.\(^2\) It is of great interest that DPG-3-2, a ginseng component, stimulated \textit{in vitro} incorporation into insulin (Table I, II) and other proteins (Table I) of pancreas and the insulin biosynthesis in islets maintained in tissue culture (Fig. 2).

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