Antihyperglycemic Effect of Puerarin in Streptozotocin-Induced Diabetic Rats

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The antihyperglycemic action of puerarin, purified from the roots of *Pueraria lobata*, was investigated in streptozotocin-induced diabetic rats (STZ-diabetic rats). Bolus intravenous injection of puerarin decreased the plasma glucose concentrations in a dose-dependent manner in STZ-diabetic rats. Similar treatment with puerarin also decreased the plasma glucose in normal rats, although the effect was not as great as that in STZ-diabetic rats. Puerarin at the effective dose (15.0 mg/kg) significantly attenuated the increase of plasma glucose induced by an intravenous glucose challenge test in normal rats. In the isolated soleus muscle of STZ-diabetic rats, puerarin enhanced the uptake of radioactive glucose in a concentration-dependent manner. Moreover, the mRNA and protein levels of the subtype 4 form of glucose transporter (GLUT4) in soleus muscle were increased after repeated intravenous administration of puerarin in STZ-diabetic rats for 3 days. These results suggest that puerarin can increase the glucose utilization to lower plasma glucose in diabetic rats lacking insulin.

Insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) rank highly among the top 10 causes of mortality throughout the world. Diabetes often leads to disability from the vascular complications of coronary artery disease and cerebrovascular disease, renal failure, blindness, and limb amputation in addition to neurological complications and premature death.[1,2] With the rapid advancements in medicine, novel treatments with fewer side effects became feasible for the long-term control of this disorder. Of the herbal medications used in diabetic disorders, Puerariae Radix is mentioned as one of the important crude materials in Oriental medicine.[3] Some isoflavones, such as puerarin (the chemical structure shown in Figure 1), daidzein, and daidzin, have been isolated as the main component of *Pueraria lobata* (Leguminosae).[4] Actually, puerarin has been screened to decrease the serum cholesterol and glucose levels[5] in addition to increasing coronary artery blood flow.[6] In the present study, we purified puerarin and investigated its effect on glucose metabolism.

Results and Discussion

The present study purified puerarin from the roots of *P. lobata*. The obtained substance has been identified as the same as that supplied by Professor Juntian Zhang (Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China). To rule out the pharmacokinetic effects, puerarin was studied by intravenous injection into animals. We found that bolus injection of puerarin could lower plasma glucose concentrations effectively in STZ-diabetic rats with insulin deficiency. As shown in Figure 2, the basal plasma glucose concentration in STZ-diabetic rats was 25.6 ± 2.2 mmol/L. A dose-dependent reduction of plasma glucose was observed in STZ-diabetic rats after the intravenous injection of puerarin at a dose of 5.0−20.0 mg/kg. The maximal plasma glucose lowering activity of

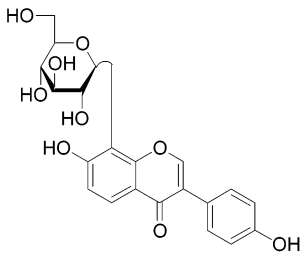

Figure 1. Chemical structure of puerarin.

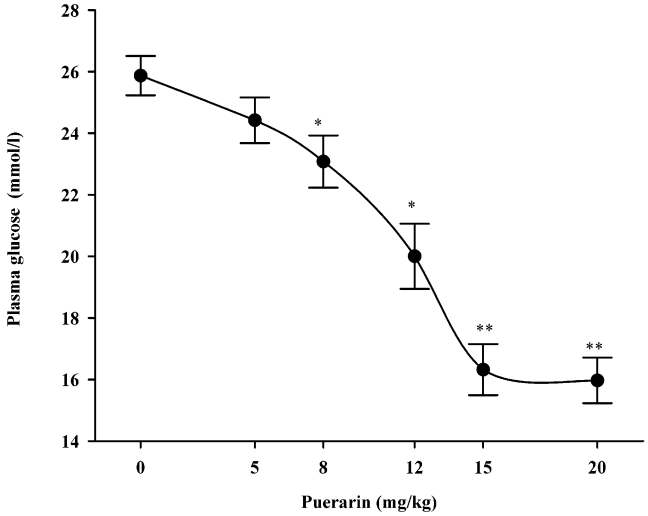

Figure 2. Effect of purearin on plasma glucose concentration in STZ-diabetic rats. Means ± SEM (bars) were obtained from each group of eight animals. *P < 0.05 and **P < 0.01 vs animals treated with vehicle only.

puerarin in STZ-diabetic rats was 33.5 ± 2.2% at 15.0 mg/kg. Thus, 15.0 mg/kg of puerarin was employed in subsequent experiments. However, the plasma glucose lowering activity at the same dose of puerarin in normal Wistar rats was slightly lower than that in STZ-diabetic rats (Figure 3). Actually, the plasma insulin level in STZ-diabetic rats was obtained as only about 1/120 of the normal rats as described in our previous report.[7] Also, it has been docu-

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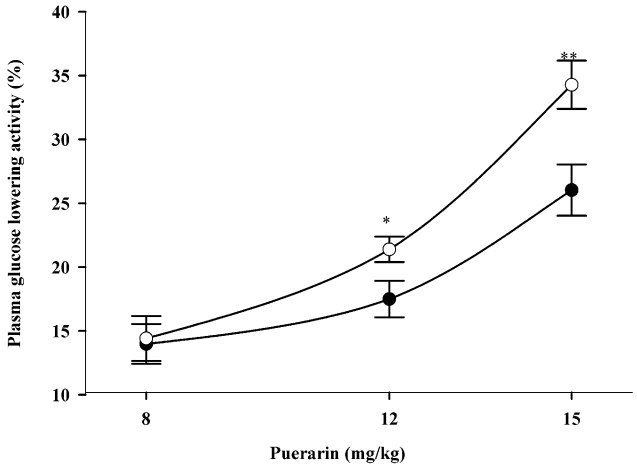

Figure 3. Plasma glucose lowering activity produced by intravenous injection of puerarin in normal Wistar rats (filled circles) and STZ-diabetic rats (open circles). Means ± SEM (bars) were obtained from each group of eight animals. *P < 0.05 and **P < 0.01 vs normal animals (filled circles) at same dose, respectively.

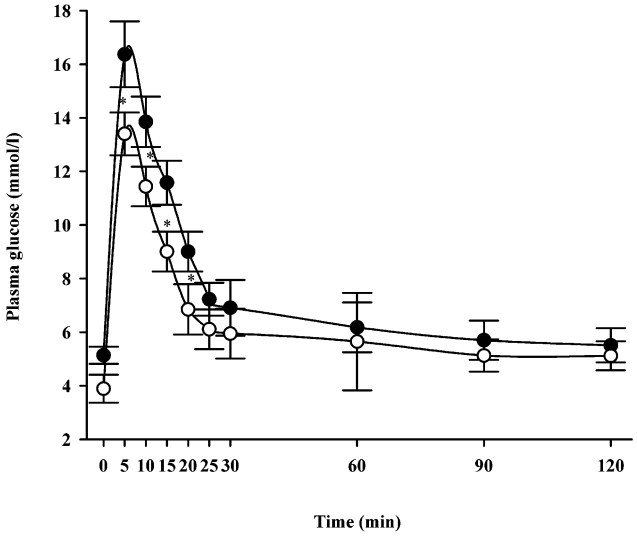

Figure 4. Effect of purearin on plasma glucose concentration in normal rats receiving an intravenous glucose challenge test (IVGCT). Purearin (15.0 mg/kg) (open circles) vs saline at the same volume (filled circles). *P < 0.05 vs data from control group (means ± SEM of 8 rats in each group).

mented that repeated oral administration of puerarin for 4 to 5 days significantly lowered the plasma glucose concentrations in alloxan-induced diabetic mice.[5] The metabolic changes in alloxan-induced diabetes have been mentioned as the insulin-deficient conditions.[8] Thus, an insulin-independent action of puerarin can be considered.

On the basis of the fact that the intravenous glucose challenge test (IVGCT) was performed to characterize the ability of rats to clear glucose from the circulation,[9] the IVGCT was carried out in the present study. As shown in Figure 4, the basal plasma glucose concentration in Wistar rats was 5.2 ± 2.2 mmol/L. Thirty minutes after treatment, the plasma glucose concentration was decreased to 3.9 ± 0.5 mmol/L in rats receiving an intravenous injection of puerarin (15.0 mg/kg). However, the plasma glucose concentration was 5.1 ± 0.3 mmol/L in vehicle-treated rats, which was not different from the basal level of plasma glucose. Five minutes after glucose injection, the plasma glucose concentration was elevated to 16.4 ± 1.2 mmol/L in vehicle-treated rats and was 13.4 ± 0.8 mmol/L in puerarin-treated rats (Figure 4). The increase of plasma glucose produced by the glucose injection was significantly

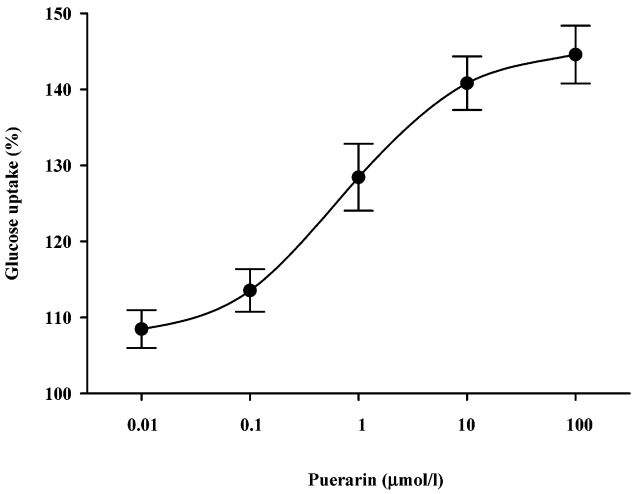

Figure 5. Effect of purearin on the uptake of radioactive glucose into soleus muscle isolated from STZ-diabetic rats. Results are expressed as percentage change from control.

lowered in the rats pretreated with puerarin 5 min after glucose injection when compared to the vehicle-treated rats. The plasma glucose in rats pretreated with puerarin remained significantly lower 15 min after glucose injection compared to the vehicle-treated group (Figure 4). No statistical difference (P > 0.05) was obtained for the plasma glucose concentration in rats receiving glucose injection 25 min later between the puerarin-treated group and vehicle-treated controls. The obtained finding shows that puerarin can enhance glucose utilization in vivo.

Skeletal muscle is a major site of glucose disposal.[10] Glucose transport, which depends on insulin-stimulated translocation of glucose carriers to the cell membrane, is the rate-limiting step in carbohydrate metabolism of skeletal muscle.[11] Under basal conditions, the rate of glucose uptake in skeletal muscle is low. Insulin-stimulated glucose utilization is the major site for regulation of plasma glucose concentrations.[12] IDDM is an abnormal metabolic state characterized by an insulin defect involving muscle and other tissues. Thus, glucose uptake stimulated by puerarin was studied to investigate the mechanism of changes in glucose utilization. In the present study, specific glucose uptake (2-DG uptake) into soleus muscle of STZ-diabetic rats was increased about 212% after incubation with 1 nmol/L bovine insulin when the basal level of glucose uptake into the soleus muscle isolated from STZ-diabetic rats without bovine insulin stimulation was taken as 100%. A similar stimulation with puerarin for 30 min increased the specific glucose uptake into isolated soleus muscle in a concentration-dependent manner from 0.01 μmol/L (about 108% of basal level) to 100.0 μmol/L (about 145% of basal level), although the activity was lower (P < 0.05) than that of bovine insulin (Figure 5). These results suggest that puerarin can increase the utilization of glucose in peripheral tissue via an insulin-independent mechanism. Further studies are needed to elucidate the molecular mechanisms of puerarin to regulate plasma glucose concentrations in the absence of insulin.

A family of glucose transporters (GLUT) mediates glucose transport across the cell membrane, and the subtype 4 form (GLUT4) is predominant in skeletal muscle.[13,14] Reduction in insulin-mediated glucose uptake caused by decreasing expression of GLUT4 mRNA and protein in diabetes was observed.[15,16] It is possible that puerarin can enhance the glucose uptake via an effect on gene expression of GLUT4. Figure 6 shows the representative response of

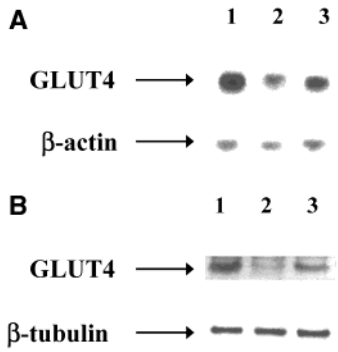

Figure 6. (A) Representative response of mRNA level for GLUT4 or β-actin in soleus muscle isolated from normal or STZ-diabetic rats receiving the repeated treatment with purearin (15.0 mg/kg) or the same volume of vehicle three times for 3 days. (B) Identification of GLUT4 through a single band at 45 kDa using immunoblotting analysis. Lane 1, vehicle-treated normal rats; lane 2, vehicle-treated STZ-diabetic rats; lane 3, purearin-treated STZ-diabetic rats.

Table 1. Quantification of the Responses of MRNA and Protein Levels for GLUT4 in Isolated Soleus Muscle from Rats Treated with Puerarin or Vehicle

	GLUT4 (arbitrary units)	
group	mRNA/β-actin	protein/β-tubulin
vehicle-treated normal rat	1.10 ± 0.05	1.09 ± 0.06
vehicle-treated STZ-diabetic rat	0.56 ± 0.05**	0.47 ± 0.04**
puerarin-treated STZ-diabetic rat	1.01 ± 0.04	0.88 ± 0.07*

[a] Means ± SEM were obtained from each group of four animals. *P < 0.05 and **P < 0.01 vs vehicle-treated normal rat.

mRNA level for GLUT4 in isolated soleus muscle from the vehicle or puerarin-treated STZ-diabetic rats determined by Northern blot analysis using β-actin as the internal standard. The mRNA level of GLUT4 in isolated soleus muscle obtained from the vehicle-treated STZ-diabetic rats was only about 51% of that from the vehicle-treated normal rats. It has been documented that long-term exposure is required for the activation of mRNA level in cells.[17] Repeated treatment of STZ-diabetic rats with puerarin (15.0 mg/kg) three times daily (tid) for 3 days resulted in a marked elevation of GLUT4 mRNA level in isolated soleus muscle to a level near that of vehicle-treated normal rats (Figure 6A). The quantification of the mRNA levels for change of GLUT4 is shown in Table 1. Plasma glucose concentrations of STZ-diabetic rats were also significantly (P < 0.01) decreased to 16.5 ± 3.1 mmol/L after repeated treatment with puerarin (15.0 mg/kg, tid) for 3 days, as compared to vehicle-treated STZ-diabetic rats (26.1 ± 3.3 mmol/L). However, the plasma glucose concentration in these puerarin-treated rats was still higher than that in the vehicle-treated normal rats (5.4 ± 0.6 mmol/L, P < 0.01).

A similar action of puerarin was also found in the protein level of GLUT4 in isolated soleus muscle determined by Western blot (Figure 6B). The protein level of GLUT4 in the soleus muscle of vehicle-treated STZ-diabetic rats was only about 43% of vehicle-treated normal rats (Figure 6B). After puerarin (15.0 mg/kg, tid) treatment for 3 days, the GLUT4 protein level was elevated to about 80% of the vehicle-treated normal rats. The quantification of the protein levels for change of GLUT4 is shown in Table 1. From the increase in both protein and mRNA levels of GLUT4 by puerarin, the increase of GLUT4 gene expression can be considered as one of the action mechanisms for puerarin.

In conclusion, the data obtained suggest that intravenous injection of puerarin can lower plasma glucose in STZ-diabetic rats through an increase of glucose utilization. Thus, puerarin may develop to be an attractive adjuvant for the treatment of diabetic patients in the future.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-500 spectrometer in DMSO-d_6 solution, using the corresponding solvent as internal standard. Optical rotation was measured on a Jasco DIP-1020 digital polarimeter. Column chromatography was carried out with Sephadex LH-20 (25−100 μm, Pharmacia Fine Chemicals), MCI-gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries), and silica gel (70−230 mesh, Merk). TLC on silica gel plates (60 F-254, Merk) and 10% sulfuric acid solution was used as visualizing agent on heating.

Plant Material. The plant, *Pueraria lobata*, was collected in Ping-Tung, Taiwan, in March 2000. It has been identified by Dr. Hsien-Chang Chang (Division of Pharmacognosy, National Laboratories of Food and Drugs, Department of Health, Taiwan). A voucher specimen was deposited at the School of Pharmacy, Taipei Medical University.

Extraction and Isolation. The sliced stem root of *Pueraria lobata* (Willd.) Ohwi (3 kg) was extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated in vacuo and partitioned to yield n-BuOH (63 g) and aqueous extracts (84 g). The n-BuOH extract was fractionated on a Sephadex LH-20 column and eluted with MeOH to yield saponin (3 g) and flavonoid fractions (47 g). The flavonoid fraction was subjected to silica gel column chromotography, eluting with a mixture of $CHCl_3$−MeOH (9:1−7:3), to provide five fractions. Fraction 5 was further separated by Sephadex LH-20 column chromatography with H_2O−MeOH (1:0−0:1). Fractions eluted from MeOH were purified by an open column (MCI-gel CHP 20P) and eluted with H_2O−MeOH (1:0−3:2) to yield two fractions. Finally, fractions eluted from H_2O−MeOH (3:2) were rechromatographed over silica gel eluting with $CHCl_3$−MeOH−H_2O (7:3:0.5) to give 1.5 g of puerarin.

Puerarin: off-white solid, mp 187 °C (dec); $[\alpha]_D$ +18.1° (c 1.0, MeOH); ^{1}H NMR δ 3.20−3.28 (3H, m, glc H-3, 4, 5), 3.69 (1H, brd, J = 11.4 Hz, glc H-6), 4.00 (1H, m, glc H-2), 4.80 (1H, d, J = 9.4 Hz, glc H-1), 6.79 (2H, d, J = 8.5 Hz, H-3′, 5′), 6.97 (1H, d, J = 8.8 Hz, H-6), 7.37 (2H, d, J = 8.5 Hz, H-2′, 6′), 7.92 (1H, d, J = 8.8 Hz, H-5), 8.31 (1H, s, H-2); ^{13}C NMR δ 70.2 (d, C-4), 70.8 (d, C-2), 73.5 (d, C-1), 78.8 (d, C-3), 81.3 (d, C-5), 112.7 (s, C-8), 115.0 (d, C-3′,5′, 6), 116.7 (s, C-4a), 122.6 (s, C-1′), 123.1 (s, C-3), 126.3 (d, C-5), 130.1 (d, C-2′, 6′), 152.7 (s, C-2), 157.2 (s, C-4′, 8a), 161.2 (s, C-7), 175.0 (s, C-4).

Animal Models. Male Wistar rats, age 8−10 weeks (200−250 g body weight), were obtained from the Animal Center of National Cheng Kung University Medical College. Diabetic rats were prepared by giving an intravenous injection of STZ (Sigma Chemical Co., St. Louis, MO) (60 mg/kg), into the fasting rats. Rats with plasma glucose concentrations of 20 mmol/L or greater in addition to polyuria and other diabetic features were considered as having IDDM. Also, the plasma insulin level in STZ-diabetic rats became 1.33 ± 0.8 pmol/L (n = 8) in a way markedly lower than that of the normal rats (161.1 ± 3.4 pmol/L; n = 8) showing IDDM. All studies were carried out 2 weeks after the injection of STZ. All animal procedures were performed according to the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

Effect of Puerarin on Plasma Glucose. The solution of puerarin was prepared by dissolving the purified puerarin into normal saline. The fasting STZ-diabetic rats received an intravenous injection of puerarin at the desired doses, and blood samples (0.1 mL) were collected under sodium pentobarbital anesthesia (30.0 mg/kg, ip) from the tail vein for measurement of plasma glucose. In the preliminary experi-

ments, puerarin at 20.0 mg/kg was found to produce the maximal plasma glucose lowering effect in STZ-diabetic rats 30 min after intravenous injection. Thus, the effect of puerarin on plasma glucose was determined using blood samples collected after 30 min. Control rats received a similar injection of vehicle at the same volume.

Intravenous Glucose Challenge Test. An intravenous glucose challenge test (IVGCT) was performed according to the method described previously.[9] Briefly, the basal plasma glucose concentration was obtained from samples from the tail vein of Wistar rats under anesthesia with sodium pentobarbital (30.0 mg/kg, ip) before the IVGCT. A solution of puerarin at 15.0 mg/kg or the same volume of saline was injected into the tail vein of rats. At 30 min later, blood samples (0.1 mL) from the tail vein were drawn and indicated as 0 min. Then, a glucose dose of 60.0 mg/kg was injected through the femoral vein of rats. Rats receiving a similar injection of saline at the same volume were taken as control. Blood samples (0.1 mL) from the tail vein were drawn at 5, 10, 15, 20, 25, 30, 60, 90, and 120 min following the glucose injection for the measurement of the plasma glucose concentrations. Rats were maintained under anesthesia by pentobarbital throughout the procedure.

Determination of Plasma Glucose. Blood samples (0.1 mL) were collected by a chilled syringe containing 10 IU heparin from the tail vein of rats under anesthesia with sodium pentobarbital (30.0 mg/kg, ip). Blood samples were then centrifuged at 13 000 rpm for 3 min, and an aliquot (15 μL) of plasma was added to 1.5 mL of Glucose Kit Reagent (Biosystems S.A., Barcelona, Spain) and incubated at 37 °C in a water bath (Yamato-BT-25, Tokyo, Japan) for 10 min. The concentration of plasma glucose was then estimated via an analyzer (Quik-Lab, Ames, Miles Inc., Elkhart, IN 46515) with samples run in duplicate.

Measurement of Glucose Uptake into Soleus Muscle. Glucose uptake was determined using the uptake of radioactive glucose analogue, 2-[1-^{14}C]-deoxy-D-glucose (2-DG) (New England Nuclear, Boston, MA), as described previously.[18] Animals were sacrificed by cervical dislocation and the soleus muscle was quickly excised by a pair of scissors, dissected free of any adjoining connective tissue, blotted, and divided into long longitudinal strips (35−25 mg per strip). Muscles were placed in 3 mL of Krebs-Ringer bicarbonate buffer (KRBB) (37 °C, pH 7.4) containing 1 mmol/L glucose, 1% fatty acid-free bovine serum albumin (BSA) under aeration with 5% CO_2 in O_2. After preincubation for 30 min, the muscle tissue was incubated with 1.0 nmol/L bovine insulin (Novo Industrias, Bagsvaerd, Denmark) or puerarin at the desired concentrations for 30 min and then with 50 μL KRBB containing 2-DG (1 μCi/mL) for 5 min at 37 °C in the shaking water bath under aeration. Reactions were terminated by quickly blotting the muscles and dissolving them in 0.5 mL of 0.5 N NaOH for 45 min before neutralization with 0.5 mL of 0.5 N HCl. After centrifugation, 800 μL of each supernatant was mixed with 1 mL of aqueous counting scintillant, and the radioactivity was determined using a β-counter (Beckman LS6000). Nonspecific uptake of 2-DG, assessed after incubation with 20 μmol/L cytochalasin B (Sigma Chemical Co., St. Louis, MO) to block transportation,[18] was subtracted from the total muscle-associated radioactivity. Specific 2-DG uptake was expressed as the percentage of basal uptake that was obtained from soleus muscle incubating with KRBB only.

Effect on Gene Expression. Both STZ-diabetic rats and normal rats received intravenous injection of puerarin at an effective dose (15.0 mg/kg) every 8 h, three times daily (tid), into the tail vein. In the preliminary experiments, puerarin (15.0 mg/kg) was found to significantly modify the mRNA and protein levels for GLUT4 after 3 days of treatment. Thus, the effect of puerarin on the mRNA and protein levels for GLUT4 was determined using samples collected after 3 days of treatment. After the final treatment, the fasting animals were sacrificed. Soleus muscle was immediately removed, frozen in liquid nitrogen, and stored at −70 °C for Northern and Western blot analysis. Blood samples were also collected from the tail vein of these rats before sacrificing.

Northern Blot Analysis. Total RNA was extracted from soleus muscle of experimental animals using the Ultraspec-II RNA extraction system (Bioteck, Houston, TX). The concentration of RNA was measured using the absorbance at 260 nm. For Northern blot analysis, RNA (20 μg) was denatured in a solution containing 2.2 mmol/L formaldehyde and 50% formamide (v/v) by heating at 55 °C for 15 min. Aliquots of total RNA were then size-fractionated in a 1.2% agarose/formaldehyde gel. Ethidium bromide staining was used to identify the position of the 18S and 28S rRNA subunits and to confirm that equivalent amounts of undegraded RNA had been loaded. The fractionated RNA was transferred to a Hybond-N membrane (Amersham Corp., Bucks, UK) and cross-linked by UV irradiation (Stratagene, CA).

Probes were labeled with [α-^{32}P]dCTP (New England Nuclear, Boston, MA) using the Medaprime labeling system kit (Amersham Corp., Bucks, UK). Hybridizations were carried out in medium containing denatured salmon sperm DNA (100 μg/mL) at 65 °C for 2 h. The membrane was washed twice for 20 min in 2 × sodium saline citrate (SSC)/0.1% SDS at room temperature and once for 20 min in 0.1 × SSC/0.1% SDS at 40 °C. Autoradiograms were prepared on Kodak X-ray (Rochester, NY) film using a single enhancing screen at −70 °C (laid down for 16 h). Intensities of the mRNA bands on the blot were quantified by scanning densitometry (Hoefer, San Francisco, CA). The response of β-actin was used as an internal standard.

Western Blot Analysis. Tissue homogenates were prepared by mechanical homogenization of soleus muscle and liver using a glass/Teflon homogenizer. Protein content was determined by the BioRad protein dye binding assay (Richmond, CA). A total of 50 μg of tissue samples was separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) (10% acrylamide gel) using the Bio-Rad Mini-Protein II system (55 and 130 V during the stacking and separation gels, respectively). Protein was transferred to the expanded poly(vinylidene difluoride) membrane (PIERCE, Rockford, IL) using a Bio-Rad Trans-Blot system (2 h at 20 V in 25 mmol/L Tris, 192 mmol/L glycein, and 20% MeOH). Following transfer, the membrane was washed with phosphate-buffered saline (PBS) and blocked for 1 h at room temperature with 5% (w/v) skim milk powder in PBS. Blots were then incubated overnight (16 h) at 4 °C with an immmunoglobulin G (IgG) monocolonal mouse anti-rat antibody (1:1000) (Genzyme Diagnostics, Cambridge, MA) in 5% (w/v) skim milk powder dissolved in PBS/Tween 20 (0.5% by vol) to bind GLUT4 in soleus muscle. The intensity of the blots incubated with mouse monoclonal antibody (1:500) (Zymed Laboratories, Inc.) to bind the β-tubulin were used to ensure that the amount of protein loaded into each lane of the gel was constant. After the removal of primary antibody, the blots were extensively washed with PBS/Tween 20. Blots were then incubated for 2 h at room temperature with the appropriate peroxidase-conjugated secondary antibody dilution in 5% (w/v) skim milk powder dissolved in PBS/Tween 20. Following removal of the secondary antibody, blots were washed as above and developed by autoradiography using the ELC-Western blot system (Amersham Corp., Bucks, UK). Densities of the obtained immunoblots at 45 kDa for GLUT4 and 50 kDa for β-tubulin were quantified using a laser densitometer.

Statistical Analysis. The plasma glucose lowering activity was determined in fasted rats that received intravenous injection of puerarin under anesthesia. Data are expressed as the mean ± SEM for the number (n) of animals in the group as indicated in tables and figures. Repeated measures analysis of variance (ANOVA) was used to analyze the changes in plasma glucose and other parameters. Dunnett range post-hoc comparisons were used to determine the source of significant differences where appropriate. $P < 0.05$ was considered statistically significant.

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References and Notes

(1) Goldstein, D. A.; Massry, S. G. *Nephron* **1978**, *20*, 286−296.
(2) Weidmann, P.; Boehlen, L. M.; de Courten, M. *Am. Heart J.* **1993**, *125*, 1498−1513.
(3) Kaufman, P. B.; Duke, J. A.; Brielmann, H.; Boik, J.; Hoyt, J. E. *J. Altern. Complement. Med.* **1997**, *3*, 7−12.
(4) Kim, D. H.; Yu, K. U.; Bae, E. A.; Han, M. J. *Biol. Pharm. Bull.* **1998**, *21*, 628−630.
(5) Shen, Z. F.; Xie, M. Z. *Acta Pharm. Sin.* **1985**, *20*, 863−866.
(6) Fan, L. L.; O'Keefe, D. D.; Powell, W. J., Jr. *Acta Pharm. Sin.* **1984**, *19*, 801−807.
(7) Cheng, J. T.; Liu, I. M.; Chi, T. C.; Tzeng, T. F.; Lu, F. H.; Chang, C. J. *Diabetes* **2001**, *50*, 2815−2821.
(8) Szkudelski, T. *Physiol. Res.* **2001**, *50*, 537−546.
(9) Liu, I. M.; Chi, T. C.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. *Planta Med.* **1999**, *65*, 712−714.
(10) Baron, A. D.; Brechtel, G.; Wallace, P.; Edelman, S. V. *Am. J. Physiol.* **1988**, *255*, E769−E774.
(11) Ziel, F. H.; Venkatesan, N.; Davidson, M. B. *Diabetes* **1988**, *37*, 885−890.
(12) Hollenbeck, C.; Reaven, G. M. *J. Clin. Endocrinol. Metab.* **1987**, *64*, 1169−1173.
(13) Goodyear, L. J.; Hirshman, M. F.; Smith, R. J.; Horton, E. S. *Am. J. Physiol.* **1991**, *261*, E556−E561.
(14) Pessin, J. E.; Bell, G. I. *Annu. Rev. Physiol.* **1992**, *54*, 911−930.
(15) Berger, J.; Biswas, C.; Vicario, P. P.; Strout, H. V.; Saperstein, R.; Pilch, P. F. *Nature* **1989**, *340*, 70−72.
(16) Sivitz, W. I.; Desautel, S. L.; Kayano, T.; Bell, G. I.; Pessin, J. E. *Nature* **1989**, 3*40*, 72−74.
(17) Henriksen, E. J.; Bourey, R. E.; Rodnick, K. J.; KoranyI, L.; Permutt, M. A.; Holloszy, J. O. *Am. J. Physiol.* **1990**, *259*, E593−E598.
(18) Liu, I. M.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. *Br. J. Pharmacol.* **2000**, *129*, 631−636.

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