Troglitazone Enhances Glycolysis and Improves Intracellular Glucose Metabolism in Rat Mesangial Cells

Tamotsu Asano, Masanori Wakisaka, Mototaka Yoshinari, Shin Nakamura, Yasufumi Doi, and Masatoshi Fujishima

To clarify the action of the new antidiabetic agent, troglitazone, on rat mesangial cells, we assessed its effect on the uptake and intracellular metabolism of glucose. Troglitazone increased the uptake of 2-deoxyglucose (2DOG) in a dose-dependent manner with an upregulation of glucose transporter 1 (GLUT1) mRNA, whereas it had no effect on the uptake of α-methyl glucoside (AMG). This troglitazone-induced glucose uptake was not suppressed by phlorizin. In 5 mmol/L glucose, 2 μg/mL (4.5 μmol/L) troglitazone increased glucose consumption 2.9-fold, similar to that in 20 mmol/L glucose. Troglitazone increased the production of pyruvate and lactate as a consequence of the increase in glycolysis, but did not increase the cellular ATP content. Troglitazone improved the high-glucose–induced accumulation of intracellular sorbitol and fructose and elevation of the cellular redox potential. These data suggest that troglitazone enhances glucose uptake through GLUT1 with an acceleration of glycolysis, and improves the abnormal intracellular glucose metabolism under high-glucose conditions in rat mesangial cells.

IT IS CONCEIVABLE that hyperglycemia causes metabolic abnormalities that mediate functional changes of mesangial cells.[1,2] Mesangial cells, which play an important role in diabetic nephropathy, possess 2 types of glucose transporters, facilitated glucose transporter 1 (GLUT1) and sodium-coupled glucose transporter (SGLT).[3] An increase in glucose uptake by mesangial cells under high-glucose conditions or by cells with an overexpression of GLUT1 under the normal glucose condition has been reported to increase cellular sorbitol content, redox potential, and extracellular matrix production.[4-9] We have recently reported that a suppression of glucose entry by inhibition of SGLT with phlorizin, a specific inhibitor for SGLT, ameriolates the high-glucose–induced overproduction of extracellular matrix and decrease of cell proliferation in bovine retinal pericytes.[10] These observations suggest the significance of the increase in glucose uptake and consecutive intracellular abnormal metabolism by mesangial cells in diabetic nephropathy.

Troglitazone, a thiazolidinedione derivative, ameliorates hyperglycemia and hyperinsulinemia in animals and humans.[11-14] This effect of troglitazone is mainly explained by an upregulation of the insulin receptor, an enhancement of insulin action, and a direct insulin-like action in insulin-sensitive cells.[15-18] However, troglitazone is reported to stimulate glucose uptake through an upregulation of GLUT1 in cells other than insulin-sensitive cells, such as mouse 3T3-L1 fibroblasts and human smooth muscle cells.[19,20] Moreover, troglitazone is reported to inhibit the increased activity of MAP kinase in smooth muscle cells[21] and protein kinase C in cardiomyocytes,[22] which suggests that troglitazone may have beneficial effects on diabetic complications. However, the mechanisms underlying its effects are not fully understood, and it is unknown whether troglitazone has any effects on glucose uptake and metabolism in rat mesangial cells.

From the Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Submitted March 21, 1999; accepted August 25, 1999.

Address reprint requests to Tamotsu Asano, MD, Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8586, Japan.

0026-0495/00/4903-0005$10.00/0

To study the effects of troglitazone on mesangial cells, we investigated glucose uptake and intracellular glucose metabolism by the cells in the presence of troglitazone.

MATERIALS AND METHODS

Isolation and Culture of Rat Mesangial Cells

Mesangial cells from the kidneys of 4-week-old Sprague-Dawley rats were isolated by a differential sieving procedure as previously reported.[18] The cells were cultured in 100-mm plates (Nunc, Roskilde, Denmark) in a 1:1 mixture of Dulbecco's modified Eagles's medium (DMEM) and Ham's F-12 (both from Sigma, St Louis, MO) containing 10% fetal bovine serum (GIBCO, Grand Island, NY), 10% Nu-serum (Collaborative Research, Bedford), penicillin (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL). After reaching greater than 90% confluency, mesangial cells from 5 to 9 passages were cultured for 2 days in DMEM/F-12 containing 5% fetal calf serum (FCS), 5% Nu-serum, and 5 mmol/L glucose with a daily change of medium, before the following experiments.

Hexose Uptake and Consumption

For the determination of hexose uptake, mesangial cells were preincubated for 24 hours in DMEM/F-12 containing 1% FCS and 1% Nu-serum in 12-well plates (28-mm diameter; Nunc) with various concentrations of troglitazone, and were washed 3 times with 20 mmol/L Tris/HEPES (pH 7.4), 1 mmol/L $CaCl_2$, 5 mmol/L KCl, 2.5 mmol/L $MgSO_4$, and 145 mmol/L NaCl. Then, either 2-[^{3}H]-deoxy-D-glucose ([2-DOG] 0.1 mmol/L, 1 μCi/mL) or methyl-α-D-[^{14}C(U)]-glucoside ([AMG] 0.1 mmol/L, 1 μCi/mL; DuPont-NEN, Boston, MA) was added to 500 μL of the media and incubated for 30 minutes at 37°C as described previously.[3] The incubations were terminated by rapid aspiration of the media, followed by washing 3 times with ice-cold phosphate-buffered saline. The cells were solubilized at room temperature in 500 μL 0.5-mmol/L NaOH; 400 μL was neutralized with acetic acid for the measurement of radioactivity using Aloka Liquid Scintillation Counter LSC 1000 (Aloka, Tokyo, Japan). The remainder was used for determination of the protein concentration. Hexose uptake was normalized against the cell protein concentration.

For the determination of glucose consumption, mesangial cells were incubated for 48 hours in 6-well plates (35-mm diameter) in 2 mL medium containing 5% FCS, 5% Nu-serum, and 5 or 20 mmol/L glucose, plus the appropriate concentrations of troglitazone and 0.2 mmol/L phlorizin (Sigma), with a daily change of the media. Glucose concentrations in the media were determined by the glucose oxidase method using a Beckman Glucose Autoanalyzer 2 (Beckman, Fullerton, CA). The DNA content of each well was determined by the Hoechst

33258 dye method (Sigma) using a TKO 100 Spectrophotometer (Hoeffer Scientific Instruments, San Francisco, CA) and calf thymus DNA (Clontech Laboratories, Palo Alto, CA) as a standard.

Measurement of GLUT1 mRNA

Mesangial cells in 100-mm plates (Nunc) were incubated for 24 hours in 10 mL DMEM/F-12 (1:1), 1% FCS, 1% Nu-serum, and 5 mmol/L glucose in the presence or absence of troglitazone. Total RNA was harvested using a commercial preparation of guanidinium and phenol (Isogen; Nippon Gene, Tokyo, Japan). A rat GLUT1 cDNA fragment (490 bp) was amplified by the reverse transcription–polymerase chain reaction (PCR) using synthesized primer for GLUT1, forward 5'CCGTTTCACAGCCCGCACAG 3' and reverse 5' GACACCTCCCCCACATACAT 3'. In brief, 2 μg total RNA from rat mesangial cells was reverse-transcribed to cDNA with a reverse transcriptase (Promega, Madison, WI) at 37°C for 1 hour in a standard buffer. The PCR was performed in the standard buffer with 0.5 μmol/L of each primer, 1.5 mmol/L $MgCl_2$, and 0.5 U Taq polymerase (Toyobo, Osaka, Japan). The temperature program for the amplification was 35 cycles of 1 minute at 94°C, 30 seconds at 56°C, and 1 minute at 72°C. The rat GLUT1 cDNA fragment and rat GAPDH oligonucleotide probe (Calbiochem-Novabiochem International) labeled with ^{32}P-cytidine triphosphate and ^{32}P-adenosine triphosphate (^{32}P-ATP) by random prime labeling and end-labeling, respectively, were used as a Northern blotting probe. For Northern blot analysis, 20 μg RNA was subjected to electrophoresis on 1% agarose/6.3% formaldehyde gel and transferred to a nylon membrane (Amersham, Arlington Heights, IL), which was prehybridized and hybridized with either of the ^{32}P-labeled probes. After washing, autoradiographs were analyzed by the BAS 2000 system (Fuji Film, Tokyo, Japan) and quantified as the relative amount of glucose transporter versus GAPDH mRNA by the National Institutes of Health imaging program.

Measurement of Intracellular Lactate and Pyruvate

Mesangial cells in 100-mm plates were incubated for 24 hours in 10 mL DMEM/F-12 (1:1), 1% FCS, 1% Nu-serum, and 5 or 20 mmol/L glucose in the presence or absence of troglitazone. Incubations were terminated by rapidly adding 3N perchloric acid to the culture media with shaking. The tubes were then centrifuged, and the supernatants were removed to measure lactate and pyruvate content by the enzymatic method using a lactate and pyruvate assay kit (Sigma).

Measurement of Intracellular Sorbitol and Glucose

Mesangial cells in 100-mm plates were incubated for 24 hours in 10 mL DMEM/F-12 (1:1), 1% FCS, 1% Nu-serum, and 5 or 20 mmol/L glucose in the presence or absence of troglitazone. The cellular levels of sorbitol and glucose were determined as previously reported.[23] Briefly, after lyophilization of sonicated cells, samples were dissolved in 1 mL 5% $ZnSO_4$ and mixed with 1 mL 0.3N $Ba(OH)_2$. After centrifugation, the supernatants were lyophilized and silylated with 0.6 mL of a 3:2:1 mixture of pyridine:hexamethyldisilazone:trimethyl chlorosilane for 1 hour at 80°C. After centrifugation of the samples with 1 mL $CHCl_3$ and 2 mL distilled H_2O, the lower layers were dried and dissolved in 0.1 mL CS_2. The samples were assayed using a GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) on a DB-1 column (60 × 0.32 mm ID) with a 1-μm film thickness (J&W Scientific, CA) maintained at 190°C, using helium as a carrier gas at a flow rate of 3.5 mL/min. Detection was made by a flame ionizer at 320°C, and the area under the peak was calculated with a GC-Workstation (CLASS-GC10; Shimadzu). These hexose values are expressed as nanomoles per 10 mg protein.

Measurement of Cellular ATP

Cellular ATP levels were measured using a luciferase-luciferin reaction kit (Molecular Probes, Eugene, OR) after extraction of the cells with 5% trichloroacetic acid as described previously.[24] The chemiluminescence was measured using an LB953 (EG&G Beltold, Germany).

Statistical Analysis

The data are expressed as the mean ± SEM. ANOVA and Student's *t* test (2-tailed) were used for statistical analysis. A *P* level less than .05 was considered statistically significant.

RESULTS

Effect of Troglitazone on Hexose Uptake and Consumption

The effect of troglitazone on the cellular radioactivity of the unmetabolizable sugars 2DOG and AMG in rat mesangial cells after 24 hours of incubation with troglitazone is shown in Fig 1. The cellular radioactivity of ^{3}H-2DOG was increased in a dose-dependent manner. In the presence of 1, 2, and 10 μg/mL troglitazone, the cellular radioactivity of ^{3}H-2DOG was enhanced by 1.5-, 2.1-, and 4.8-fold, respectively (Fig 1A). In contrast, the cellular radioactivity of ^{14}C-AMG was not altered by troglitazone (Fig 1B). Glucose consumption during the first 24 hours of incubation with troglitazone was also increased in a dose-dependent manner, with 1, 2, and 10 μg/mL troglitazone producing an increase of 2.7-, 2.9-, and 4.3-fold, respectively (Fig 2). The same effect of troglitazone on glucose consumption was also observed after 48 hours of incubation (data not

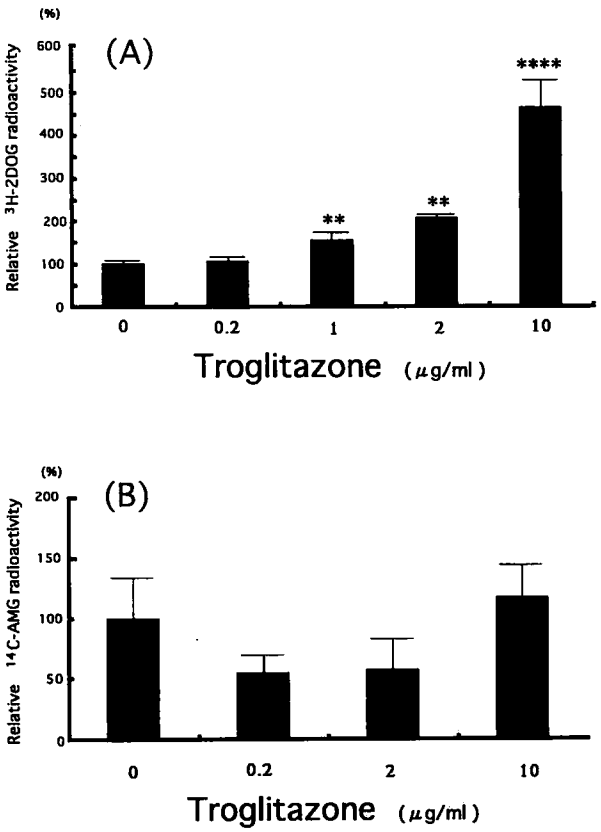

Fig 1. Effect of troglitazone on cellular radioactivity of ^{3}H-2DOG and ^{14}C-AMG in rat mesangial cells expressed relative to control cells (2DOG, 6.4 × 10^4 dpm/mg cell protein; AMG, 4.2 × 10^3 dpm/mg cell protein). Data are the mean ± SEM; n = 4 in each condition. **$P < .01$ *v* 5 mmol/L glucose, ****$P < .001$ *v* 5 mmol/L glucose.

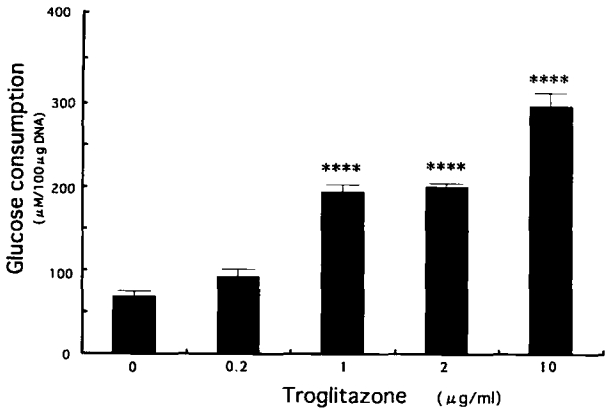

Fig 2. Effect of troglitazone on glucose consumption by rat mesangial cells. Glucose consumption was assessed by measuring the glucose concentration in the media before and after 24 hours' incubation. Data are the mean ± SEM; n = 4 in each condition. ****$P < .001$ *v* control.

shown). Glucose consumption in both 5 and 20 mmol/L glucose was increased by 2 μg/mL troglitazone. The increase in glucose consumption in 5 mmol/L glucose by troglitazone was similar to that in 20 mmol/L glucose in the absence of troglitazone, and was not reduced by the addition of phlorizin (Fig 3). GLUT1 mRNA was significantly increased by 1.4-fold after incubation with 2 μg/mL troglitazone for 24 hours (Fig 4).

Intracellular Pyruvate, Lactate, and Cellular Redox Potential in Mesangial Cells

The effect of 2 μg/mL troglitazone on the cellular content of the glycolytic products pyruvate and lactate is shown in Fig 5. The cellular lactate content in 20 mmol/L glucose was increased, which was not significant versus that in 5 mmol/L. Troglitazone at a concentration of 2 μg/mL increased the cellular pyruvate and lactate content by 2.3- and 1.4-fold in 5 mmol/L glucose and by 2.3- and 1.5-fold in 20 mmol/L glucose, respectively. The cellular redox potential (lactate to pyruvate ratio) was significantly increased in 20 mmol/L glucose compared with 5 mmol/L glucose; however, troglitazone signifi-

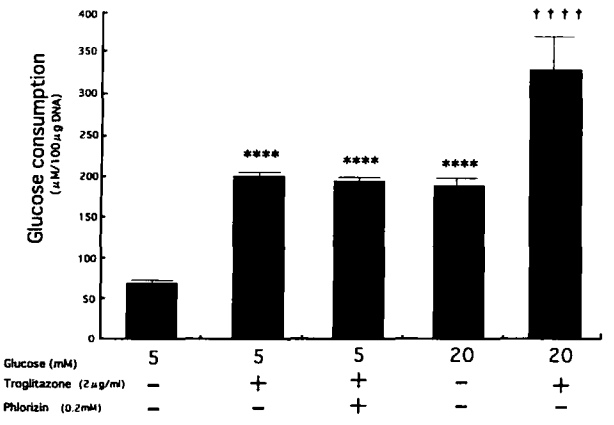

Fig 3. Effect of phlorizin on glucose consumption by rat mesangial cells. Data are the mean ± SEM; n = 4 in each condition. ****$P < .001$ *v* 5 mmol/L glucose, ††††$P < .001$ *v* 20 mmol/L glucose.

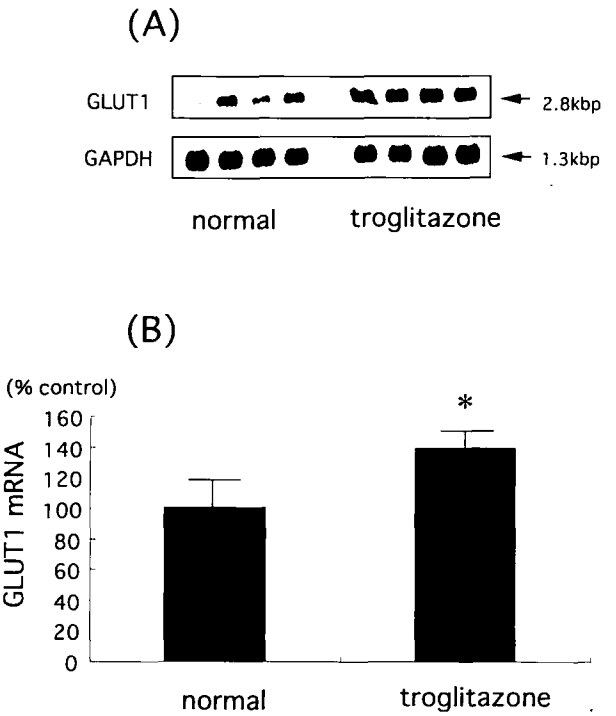

Fig 4. Effect of troglitazone on GLUT1 mRNA in rat mesangial cells. (A) Northern blots for GLUT1 and GAPDH from rat mesangial cells. (B) Relative density of GLUT1 *v* GAPDH mRNA. Data are the mean ± SEM; n = 4 in each condition. *$P < .05$ *v* control.

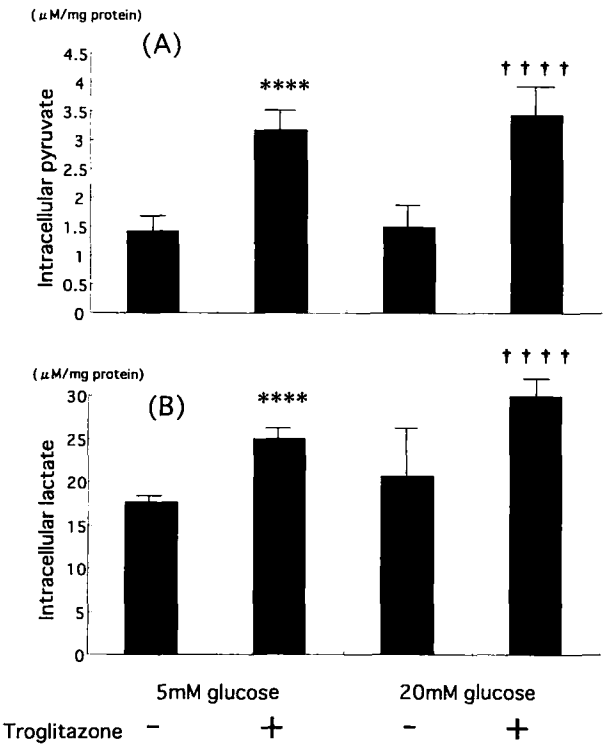

Fig 5. Effect of troglitazone on the intracellular content of (A) pyruvate and (B) lactate in rat mesangial cells. Data are the mean ± SEM; n = 8 in each condition. ****$P < .001$ *v* 5 mmol/L glucose, ††††$P < .001$ *v* 20 mmol/L glucose.

cantly reduced the ratio in 5 and 20 mmol/L glucose compared with the absence of troglitazone, respectively (Fig 6).

Intracellular Content of Glucose, Sorbitol, Fructose, and ATP in Mesangial Cells

The cellular content of glucose, sorbitol, and fructose was significantly increased in 20 mmol/L glucose compared with 5 mmol/L glucose (Fig 7A to C). Despite the increase in glucose influx by troglitazone, there was no difference in intracellular glucose in the presence or absence of troglitazone in both 5 and 20 mmol/L glucose. Troglitazone had a tendency to decrease the cellular levels of sorbitol and fructose in 5 mmol/L glucose, and significantly reduced the levels in 20 mmol/L glucose (Fig 7B and C). In contrast, there was no significant difference in the cellular content of ATP either in the presence or absence of troglitazone (Fig 8). There were significantly positive correlations among the intracellular levels of glucose, sorbitol, and fructose after incubation with each media containing 5 mmol/L glucose or 20 mmol/L glucose in the presence or absence of 2 μg/mL troglitazone (data not shown).

DISCUSSION

Troglitazone induced an increase of glucose uptake by mesangial cells in a dose-dependent manner, as reported in other insulin-insensitive cells.[19,20] We used 2 μg/mL troglitazone to test its effect on glucose metabolism in mesangial cells, since this concentration increased glucose consumption to the same degree in 5 mmol/L glucose as in 20 mmol/L glucose. Troglitazone increased the uptake of 2DOG, which enters cells via GLUT1, and did not increase the uptake of AMG, which enters cells via SGLT.[3] Moreover, troglitazone-induced glucose consumption was not inhibited by phlorizin. These findings indicate that troglitazone has no effect on SGLT, but does have an effect to increase glucose uptake via GLUT1 with an upregulation of mRNA for GLUT1 in rat mesangial cells, as reported in other cells.[19]

Although troglitazone increased the rate of glucose entry in 5 mmol/L glucose to the same degree as in 20 mmol/L glucose, the cellular glucose content was significantly decreased. We also observed a significant increase of the cellular content of both pyruvate and lactate. In contrast, the cellular content of pyruvate and lactate in 20 mmol/L glucose alone was not increased despite the presence of an increase in cellular glucose content. These phenomena may be explained by the troglitazone-induced increase of glycolysis, since troglitazone is reported to have a relative stimulatory effect on glycolytic enzyme as a consequence of a direct suppression of the rate-limiting gluconeogenic enzyme in hepatocytes.[25] However, the effect of troglitazone on glycolysis seems limited in the process of nonoxidative glycolysis, since troglitazone did not induce ATP production.

The excessive glucose entry observed both in mesangial cells under high-glucose conditions and in cells overexpressing GLUT1 under the normal glucose condition is reported to induce sorbitol accumulation, which is one of the changes of intracellular glucose metabolism.[1,2,4,7] The increase of intracellular sorbitol induces an increase of the NADH/NAD$^+$ ratio (cytosolic redox potential) by the conversion of sorbitol to

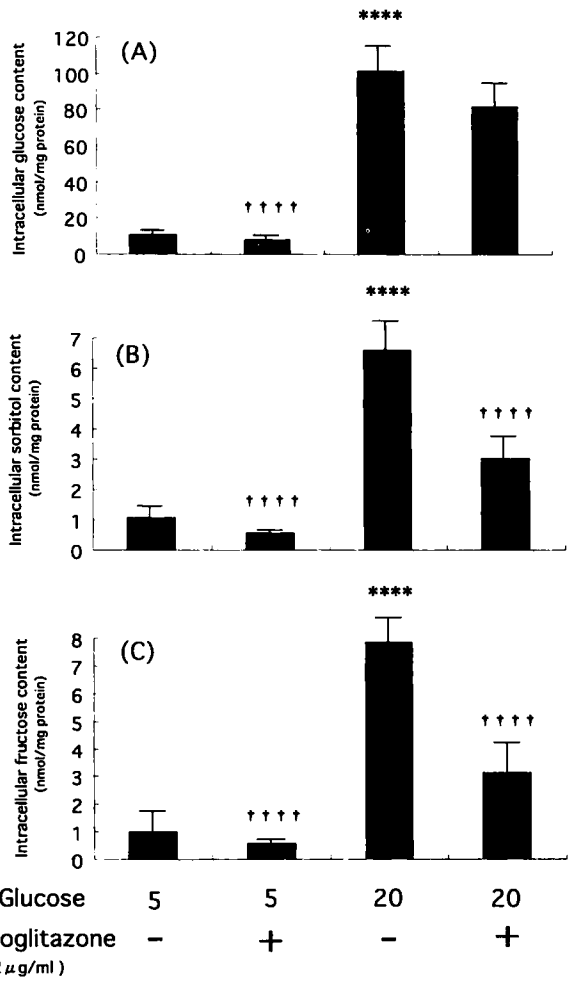

Fig 7. Effect of troglitazone on the intracellular content of (A) glucose, (B) sorbitol, and (C) fructose in rat mesangial cells. Hexose concentrations are expressed as nmol/mg protein. Data are the mean ± SEM; n = 4 in each condition. ****$P < .01$ *v* 5 mmol/L glucose, ††††$P < .001$ *v* 20 mmol/L glucose. Fig 8. Effect of troglitazone on cellular ATP content in rat mesangial cells. Data are the mean ± SEM; n = 4 in each condition.

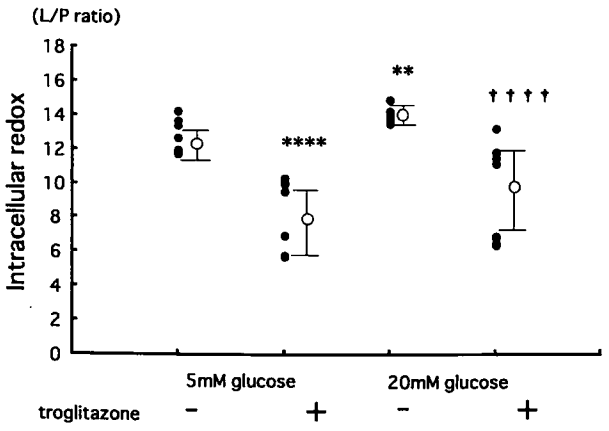

Fig 6. L/P ratio. Data are the mean ± SEM; n = 8 in each condition. **$P < .01$ *v* 5 mmol/L glucose, ****$P < .001$ *v* 5 mmol/L glucose, ††††$P < .001$ *v* 20 mmol/L glucose.

fructose in the polyol pathway, which is one of the mechanisms of diabetic complications.[26] The ratio of lactate to pyruvate (L/P ratio) represents the cytosolic redox potential in cells. An increase in the L/P ratio under high-glucose conditions was also reported.[6,27]

In this study, we also found a significant increase of the cellular content of sorbitol and fructose in 20 mmol/L glucose. Interestingly, troglitazone significantly reduced the cellular content of sorbitol and fructose in 20 mmol/L glucose by 55% and 60%, respectively. Troglitazone also reduced the L/P ratio in both 5 and 20 mmol/L glucose. The increase of glycolysis by troglitazone may be the reason for the reduced sorbitol and fructose content, since positive correlations among cellular glucose, sorbitol, and fructose content were observed. The troglitazone-induced improvement of abnormal intracellular glucose metabolism, such as sorbitol and fructose, in rat mesangial cells under a high-glucose condition seems to be one of the reasons for the reduced L/P ratio. This may support a recent report of the effect of troglitazone on the amelioration of albuminuria in streptozotocin-induced diabetic rats without a decrease in glucose.[28]

Troglitazone has been reported to induce mitochondrial uncoupling protein,[29,30] which plays an important role in the thermogenesis or lipid metabolism of cells, instead of producing ATP.[31] Although it is unknown whether troglitazone induces uncoupling protein in mesangial cells, the increase in glycolysis and decrease in sorbitol and fructose without an increase in ATP by troglitazone may be derived from the upregulation of uncoupling protein.

In conclusion, troglitazone increases glucose uptake by mesangial cells through GLUT, but not through SGLT. Troglitazone enhances nonoxidative glycolysis with decreasing cellular sorbitol and fructose content and improves the cytosolic redox potential in rat mesangial cells in a high-glucose condition. Troglitazone may be useful in the treatment of diabetic nephropathy by improving the intracellular glucose metabolism.

REFERENCES

1. Wardle EN: How does hyperglycemia predispose to diabetic nephropathy? Q J Med 89:943-951, 1996
2. Ziyadeh FN: Mediators of hyperglycemia and the pathogenesis of matrix accumulation in diabetic renal disease. Miner Electrolyte Metab 21:292-302, 1995
3. Wakisaka M, He Q, Spiro MJ, et al: Glucose entry into rat mesangial cells is mediated by both Na^+-coupled and facilitative transporters. Diabetologia 38:291-297, 1995
4. Kikkawa R, Umemura K, Haneda M, et al: Identification and characterization of aldose reductase in cultured rat mesangial cells. Diabetes 41:1165-1171, 1992
5. Pugliese G, Pricci F, Pugliese F, et al: Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells. Diabetes 43:478-490, 1994
6. Tilton RG, Baier LD, Harlow JE, et al: Diabetes-induced glomerular dysfunction: Links to a more reduced cytosolic ratio of NADH/NAD^+. Kidney Int 41:778-788, 1992
7. Heilig CW, Concepcion LA, Riser BL, et al: Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. J Clin Invest 96:1802-1814, 1995
8. Wakisaka M, Spiro MJ, Spiro RG: Synthesis of type IV collagen by cultured glomerular cells and comparison of its regulation by glucose and other factors with that of type IV collagen. Diabetes 43:95-103, 1994
9. Danne T, Spiro MJ, Spiro RG: Effect of high glucose on type IV collagen production by cultured glomerular epithelial, endothelial, and mesangial cells. Diabetes 42:170-177, 1993
10. Wakisaka M, Yoshinari M, Tamotsu A, et al: Normalization of glucose entry under the high glucose condition by phlorizin attenuates the high glucose–induced morphological and functional changes of cultured bovine retinal pericytes. Biochim Biophys Acta 1453:83–91, 1999
11. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045: Studies in KK and of/of mice and Zucker fatty rats. Diabetes 37:1549-1558, 1998
12. Lee M-K, Miles PDG, Khoursheed M, et al: Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. Diabetes 43:1435-1439, 1994
13. Suter SL, Nolan JJ, Wallace P, et al: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. Diabetes Care 15:193-203, 1992
14. Nolan JJ, Ludvik B, Beerdsen P, et al: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 331:1188-1193, 1994
15. Stevenson RW, Gibbs EM, Parker JC, et al: Thiazolidinedione antidiabetics, in Frontiers in Diabetes Research, vol 8. Lessons From Animal Diabetes. Smith-Gordon, 1995, pp 181-195
16. Swanson ML, Bleasdale JE: Antidiabetic agent pioglitazone increases insulin receptors on 3T3-L1 adipocytes. Drug Dev Res 35:69-82, 1995
17. Ciaraldi TP, Gilmore A, Olefsky JM, et al: In vitro studies on the action of CS-045, a new antidiabetic agent. Metabolism 39:1056-1062, 1990
18. Murano K, Inoue Y, Emoto M, et al: CS-045, a new oral antidiabetic agent, stimulates fructose-2,6-bisphosphate production in rat hepatocytes. Eur J Pharmacol 254:257-262, 1994
19. Tafuri SR: Troglitazone enhances differentiation, basal glucose uptake, and GLUT1 protein levels in 3T3-L1 adipocytes. Endocrinology 137:4706-4712, 1997
20. Kihara S, Ouchi N, Funahashi T, et al: Troglitazone enhances glucose uptake and inhibits mitogen-activated protein kinase in human aortic smooth muscle cells. Atherosclerosis 136:163-168, 1998
21. Graf K, Xi XP, Hsueh WA, et al: Troglitazone inhibits angiotensin II–induced DNA synthesis and migration in vascular smooth muscle cells. FEBS Lett 400:119-121, 1997
22. Bahr M, Spelleken M, Bock M, et al: Acute and chronic effects of troglitazone (CS-045) on isolated rat ventricular cardiomyocytes. Diabetologia 39:766-774, 1996
23. Kikkawa R, Umemura K, Haneda M, et al: Evidence for existence of polyol pathway in cultured rat mesangial cells. Diabetes 36:240-243, 1987
24. Webster KA, Discer DJ, Bishopric NH: Regulation of *fos* and *jun* immediate-early genes by redox or metabolic stress in cardiac myocytes. Circ Res 74:679-686, 1994
25. Fujiwara T, Okuno A, Yoshioka S, et al: Suppression of hepatic gluconeogenesis in long-term troglitazone–treated diabetic KK and C57BL/KsJ-db/db mice. Metabolism 44:486-490, 1995
26. Williamson JR, Chang K, Frangos M, et al: Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42:801-813, 1993
27. Yasunari K, Kohno M, Kano H, et al: Aldose reductase inhibitor prevents hyperproliferation and hypertrophy of cultured rat vascular smooth muscle cells induced by high glucose. Arterioscler Thromb Vasc Biol 15:2207-2212, 1995
28. Fujii M, Takemura R, Yamaguchi M, et al: Troglitazone (CS-

045) ameliorates albuminuria in streptozotocin-induced diabetic rats. Metabolism 46:981-983, 1997

29. Camirand A, Marie V, Rabelo R, et al: Thiazolidinediones stimulate uncoupling protein-2 expression in cell lines representing white and brown adipose tissues and skeletal muscle. Endocrinology 139:428-431, 1998

30. Shimabukuro M, Zhou YT, Lee Y, et al: Induction of uncoupling protein-2 mRNA by troglitazone in the pancreatic islets of Zucker diabetic fatty rats. Biochem Biophys Res Commun 237:359-361, 1997

31. Boss O, Muzzin P, Giacobino JP: The uncoupling proteins, a review. Eur J Endocrinol 139:1-9, 1998