Effects of M16209, a New Antihyperglycemic Agent, on Insulin Sensitivity In Vivo: Euglycemic Clamp Studies in Rats

Masahiko Ohta, Nobuya Murakami, Katsuaki Kato, Masahiro Mizota, Ichitomo Miwa, Jun Okuda, Yasunao Yoshimasa, Hideshi Kuzuya, Kazuwa Nakao, and Hiroo Imura

The effects of M16209 (1-(3-bromobenzo[b]furan-2-ylsulfonyl)hydantoin) on the in vivo insulin sensitivity of rats were studied by euglycemic clamp methods after 1 week of administration (10 or 100 mg/kg/d). M16209 increased both the glucose infusion rate (GIR) and metabolic clearance rate (MCR) of 3-[^{3}H]-glucose, but did not suppress hepatic glucose output. M16209 also increased the [^{3}H]-2-deoxyglucose utilization rate, rate of incorporation of [^{14}C]-glucose into glycogen, and glycolytic flux in the soleus and red gastrocnemius muscles, but not in the extensor digitorum lungus and white gastrocnemius muscles. M16209 affected neither the [^{3}H]-2-deoxyglucose utilization rate nor the rate of incorporation of [^{14}C]-glucose into lipids in epididymal adipose tissue. In the soleus muscle, M16209 decreased glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) content, but did not affect fructose-1,6-bisphosphate (F-1,6-BP) content. Moreover, M16209 increased glycogen synthase-I activity and fructose-2,6-bisphosphate (F-2,6-BP) content in the soleus muscle. These results suggest that M16209 increases insulin-stimulated glucose uptake in peripheral tissues, particularly oxidative muscles, through potentiation of insulin action on glycogen synthesis and glycolysis. Glycogen synthase and phosphofructokinase (PFK) appear to be major targets of the action of M16209.

THE HYDANTOIN derivative, M16209 (1-(3-bromobenzo[b]furan-2-ylsulfonyl)hydantoin (Fig 1), was initially reported to be a potent aldose reductase inhibitor (IC_{50} for recombinant human aldose reductase, 51 nmol/L) and to prevent diabetic neuropathy and sugar cataract in rats.[1-3] M16209 also proved to manifest antihyperglycemic activity in streptozotocin-induced mildly diabetic rats and neonatally streptozotocin-induced non–insulin-dependent diabetic rats without causing hypoglycemia.[4] Since M16209 accelerates the disappearance of serum glucose in intravenous (IV) glucose tolerance testing of normal rats and stimulates insulin release from isolated and perfused rat pancreas only at high glucose concentrations, the potentiation of glucose-induced insulin release seemed to participate in the antihyperglycemic activity of the compound.[5] Further studies have suggested that M16209 improves hyperglycemia and hyperinsulinemia in genetically obese diabetic rats and mice by increasing insulin sensitivity in peripheral tissues.[6] In the present study, the in vivo potentiating effects of M16209 on insulin action were assessed by the hyperinsulinemic-euglycemic clamp technique in an attempt to clarify the mechanisms and target tissues of these effects.

MATERIALS AND METHODS

Experimental Animals and M16209 Administration

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 280 to 380 g were provided food and water ad libitum. The rats were reared under controlled lighting (lights on from 7 AM to 6 PM), temperature (23° ± 2°C), and moisture (55% ± 5%) for all experiments. Rats received suspensions of M16209 or vehicle (5% gum arabic or 0.5% carboxymethylcellulose sodium) orally for 7 days, and were deprived of food for 21 to 28 hours after the last administration until commencement of the experiment.

Experiment 1: Whole-Body Glucose Uptake and Hepatic Glucose Output

Rats were anesthetized by intraperitoneal administration of 52 mg/kg α-chloralose and 640 mg/kg urethane. The left carotid artery (for blood sampling), right jugular vein (for 3-[^{3}H]-glucose infusion), left femoral vein (for exogenous glucose infusion), and right femoral vein (for insulin infusion) were catheterized with fine silicon tubes (Silastic; Dow-Corning, Midland, MI) filled with 50 U/mL heparin. A tracheotomy was systematically performed to avoid respiratory problems during anesthesia. After completion of the surgery, the rats were transferred to a heating stand (37°C) and hyperinsulinemic-euglycemic clamp studies combined with 3-[^{3}H]-glucose infusion were performed in accordance with the method reported by Kergort and Portha[7] with some modifications. 3-[^{3}H]-glucose (New England Nuclear, Boston, MA) was administered as an initial IV priming dose (48 kBq) followed immediately by continuous IV infusion at a rate of 1.1 kBq/min using an infusion pump (syringe infusion pump 235; Atom, Tokyo, Japan) until the entire experiment was completed. At 45 to 50 minutes after commencement of the labeled glucose infusion, blood was sampled for measurement of both the specific activity of labeled glucose and the plasma insulin level during the basal steady state. Insulin (3 or 30 mU/kg/min) was then infused in the priming mode (initially 6 or 60 mU/kg/min, respectively, for 10 minutes) to obtain suspended hyperinsulinemia. In previous experiments, we have shown that plasma insulin levels reach steady state within 20 minutes with this priming mode (data not shown). Infusion of exogenous glucose (6% or 17% solution in saline) was begun 5 minutes after initiation of the insulin infusion. Using 9 μL whole blood sampled every 5 or 2.5 minutes, plasma glucose was determined within 70 seconds by ANTSENSE (Bayer Sankyo, Tokyo, Japan), a table-top blood glucose analyzer based on an immobilized enzyme membrane/H_2O_2 electrode method. The infusion rate of exogenous glucose was varied to maintain euglycemia for 75 minutes. Blood was sampled at 55 and 75 minutes after the start of insulin infusion during the hyperinsulinemic steady state to measure the mean

From the Department of Pharmacology, Fuji Central Research Laboratory, Mochida Pharmaceutical, Tokyo; the Departments of Clinical Biochemistry and Pathobiochemistry, Faculty of Pharmacy, Meijo University, Nagoya; and the Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto, Japan.

Submitted October 5, 1995; accepted April 3, 1996.

Address reprint requests to Katsuaki Kato, PhD, Fuji Central Research Laboratory, Mochida Pharmaceutical Co, Ltd, 1-1-1 Kamiya, Kita-ku, Tokyo, Japan.

0026-0495/96/4509-0009$03.00/0

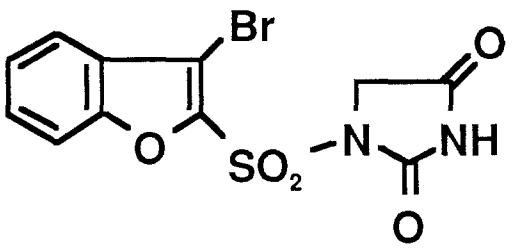

Fig 1. Chemical structure of M16209.

plasma insulin level, and at 75 minutes to measure the specific activity of labeled glucose. Plasma glucose level during the hyperinsulinemic steady state was the mean of levels measured during the last 25 minutes of the steady state (six points). The specific activity of labeled glucose, whole-body glucose uptake (metabolic clearance rate [MCR] of glucose), and hepatic glucose output (endogenous glucose production rate) were measured in accordance with the procedure of Steele[8] and Terrettaz and Jeanrenaud.[9] Plasma insulin level was measured by an enzyme-linked immunoassay (Insulin SEIKEN EIA; Sanko Junyaku, Tokyo, Japan) using human insulin (or rat insulin for basal samples) as standard.

Experiment 2: Insulin Action on Individual Tissues

Hyperinsulinemic-euglycemic clamp studies combined with [^{3}H]-2-deoxyglucose and [^{14}C]-glucose administration were performed in accordance with the method of Ferre et al[10] and James et al[11] with some modifications. Anesthesia and cannulation surgery were performed as described for experiment 1. Blood was sampled for measurement of basal plasma insulin and glucose levels at 20 minutes after cannulation surgery. The hyperinsulinemic-euglycemic clamp was then started and continued for 90 minutes as described earlier, except for exclusion of 3-[^{3}H]-glucose infusion. At 45 minutes after initiation of the insulin infusion, blood was sampled for determination of plasma insulin, and 1.4 MBq [^{3}H]-2-deoxyglucose and 1.4 MBq [^{14}C(U)]-glucose (both from New England Nuclear) were administered together via the right jugular vein. Blood samples for determination of plasma glucose and plasma tracer were obtained 2, 5, 10, 20, 30, and 45 minutes after bolus administration of the tracers. Blood samples were obtained to determine plasma insulin 20 and 45 minutes after tracer administration. Plasma insulin in the steady state during hyperinsulinemia was the mean of levels measured 0, 20, and 45 minutes after tracer administration, and plasma glucose in the steady state during hyperinsulinemia was the mean of levels measured during the last 45 minutes after tracer administration. Immediately after the last blood sampling, rats were killed, and the soleus, red and white gastrocnemius, and extensor digitorum longus muscles, as well as the epididymal white adipose tissue, were rapidly removed and frozen at −40°C. In muscle tissues, glucose uptake (accumulation of [^{3}H]-2-deoxyglucose-6-phosphate), glycogen synthesis (rate of incorporation of [^{14}C]-glucose into glycogen), and glycolytic flux (difference between glucose uptake and glycogen synthesis) were measured in accordance with the method of James et al.[11] In epididymal adipose tissues, glucose uptake (accumulation of [^{3}H]-2-deoxyglucose-6-phosphate corrected by dividing by 0.61) was determined by the method of Ferre et al.[10] Lipid synthesis (incorporation of [^{14}C]-glucose into Folch's extract)[12] was measured by a procedure similar to that used for determining incorporation of [^{14}C]-glucose into glycogen.

Experiment 3: Assays of Glycolytic Intermediates and Enzyme Activity

Rats were killed, and the soleus and white gastrocnemius muscles were rapidly removed and frozen at −80°C for assay of glycogen synthase-I (EC 2.4.1.11),[13] hexokinase (EC 2.7.1.1),[14] glucose-6-phosphate (G6P),[15] fructose-6-phosphate (F6P),[15] fructose-1,6-bisphosphate (F-1,6-BP),[15] and fructose-2,6-bisphosphate (F-2,6-BP).[16] Glycogen synthase-I activity was measured with 0.11 mmol/L G6P and 0.018 mmol/L UDP-glucose.

Statistical Analysis

The data are expressed as the mean ± SE. Differences between two groups were analyzed by Student's *t* test, and differences between three or more groups were analyzed by ANOVA and Bonferroni's multiple comparison method.

RESULTS

Experiment 1

In the hyperinsulinemic-euglycemic clamp study, M16209 (10 or 100 mg/kg/d for 7 days) significantly increased the glucose infusion rate (GIR) by 21% and 46%, respectively, at 3 mU/kg/min insulin infusion without affecting basal and steady-state plasma insulin levels, and also tended to increase the GIR at 30 mU/kg/min insulin infusion, although the increase was not significant (Fig 2 and Table 1). M16209 did not affect the body weight of rats (data not shown). M16209 increased whole-body glucose uptake (MCR of glucose) by 17% to 30% at both 3 and 30 mU/kg/min insulin infusion without any effect on hepatic glucose output (Fig 3).

Experiment 2

Also in this study, M16209 (10 mg/kg/d for 7 days) significantly increased the GIR by 14% in the hyperinsulinemic (3 mU/kg/min insulin infusion)-euglycemic clamp study (Table 2). M16209 significantly increased glucose uptake, glycogen synthesis, and glycolytic flux in soleus muscles by 37%, 46%, and 32%, respectively, compared with the insulin control. In red gastrocnemius muscles,

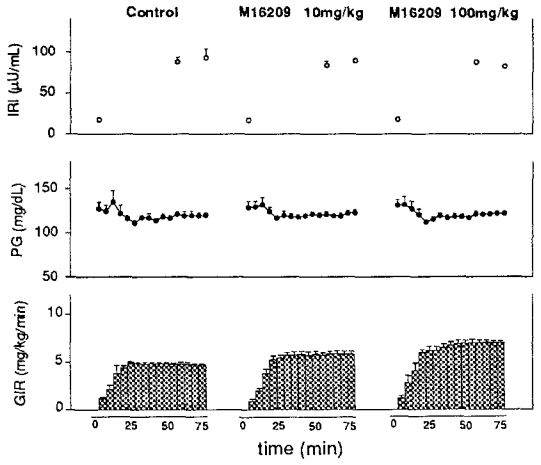

Fig 2. A typical pattern of plasma insulin (IRI), plasma glucose (PG), and glucose infusion rate (GIR) during glucose clamp study.

Table 1. Changes in Parameters During Glucose Clamp Study in Experiment 1

State	No.	PG (mg/dL)	CV (%)	IRI (μU/mL)	GIR (mg/kg/min)
Basal					
Control	9	129 ± 4		23 ± 3	
M16209 10 mg/kg	9	123 ± 5		21 ± 2	
M16209 100 mg/kg	9	124 ± 5		19 ± 2	
Insulin 3 mU/kg/min					
Control	5	120 ± 4	2.6 ± 0.6	91 ± 8	4.82 ± 0.12
M16209 10 mg/kg	5	121 ± 3	1.5 ± 0.3	87 ± 4	5.81 ± 0.31*
M16209 100 mg/kg	5	121 ± 2	2.1 ± 0.3	85 ± 2	7.02 ± 0.25*
Insulin 30 mU/kg/min					
Control	4	124 ± 5	1.7 ± 0.5	2,520 ± 122	12.26 ± 0.81
M16209 10 mg/kg	4	115 ± 7	2.0 ± 0.6	2,430 ± 105	14.12 ± 0.58
M16209 100 mg/kg	4	120 ± 4	1.9 ± 0.4	2,210 ± 74	15.29 ± 1.25

NOTE. Results are expressed as the mean ± SE. M16209 was administered for 7 days, and the clamp study was performed 1 day after the last administration.

Abbreviations: PG, plasma glugocse; IRI, plasma insulin; GIR, glucose infusion rate; CV, coefficient of variation for steady-state plasma glucose levels during the last 25 minutes.

*$P < .01$ *v* control.

M16209 significantly increased glucose uptake and glycogen synthesis by 77% and 78%, respectively, compared with the insulin control, and also tended to increase glycolytic flux, but the increase was not significant. These effects were not seen in extensor digitorum lungus and white gastrocnemius muscles (Fig 4). Glucose uptake and lipid synthesis from glucose in epididymal adipose tissue were not affected by M16209 treatment (Fig 5).

Experiment 3

M16209 (10 mg/kg/d for 7 days) did not affect hexokinase activity in soleus muscle. M16209 increased glycogen synthase-I activity 52% in soleus muscle, but not in white gastrocnemius muscle. The drug decreased G6P and F6P content 25% and 15%, respectively, in the soleus, but not in white gastrocnemius muscle, and did not affect F-1,6-BP content in either muscle. M16209 increased F-2,6-BP content 35% in soleus muscle, but not in white gastrocnemius muscle (Fig 6). Hexokinase activity in soleus muscle of control rats was 26.7 ± 1.8 mU/mg protein. Glycogen synthase-I activity, G6P, F6P, F-1,6-BP, and F-2,6-BP levels in soleus muscle of control rats were 214 ± 21 pmol/mg protein/min, 0.60 ± 0.05, 1.07 ± 0.06, and 0.19 ± 0.01 μmol/g protein, and 0.85 ± 0.05 nmol/g protein, respectively, and in the white gastrocnemius muscle of control rats, 239 ± 16 pmol/mg protein/min, 1.14 ± 0.20, 1.03 ± 0.16, and 1.40 ± 0.11 μmol/g protein, and 2.21 ± 0.26 nmol/g protein, respectively. M16209 did not affect protein content in muscles (data not shown).

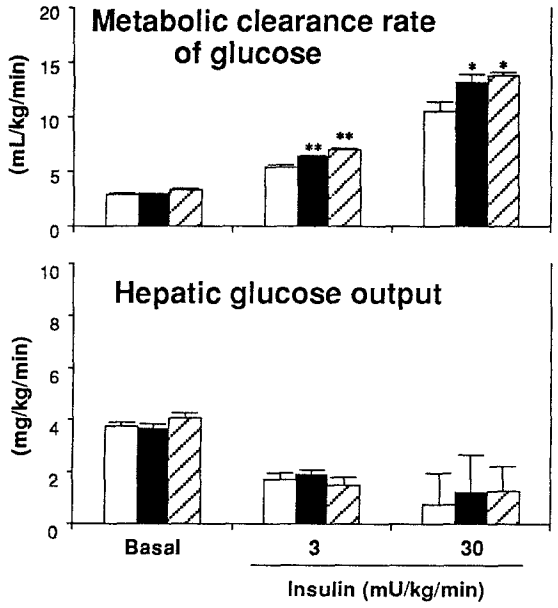

Fig 3. MCR of glucose and hepatic glucose output during glucose clamp study after 7 days of treatment with M16209 (n = 4 to 9). Hyperinsulinemic glucose clamp study with 3-[^{3}H]-glucose infusion was performed for 45 to 50 minutes (basal study) and 75 minutes (hyperinsulinemic study). (□) Control; (■) M16209 10 mg/kg; (▨) M16209 100 mg/kg. *$P < .05$, **$P < .01$: *v* control.

DISCUSSION

In experiment 1, we investigated the effect of M16209 on insulin sensitivity with respect to whole-body glucose uptake and hepatic glucose output by the hyperinsulinemic-euglycemic clamp method. Since M16209 increased the GIR without increasing steady-state plasma insulin levels, it appears that the increase in GIR was caused by potentiation of insulin action, but not by stimulation of insulin secretion from the pancreas. M16209 acts as an insulin secretagogue only at high blood glucose levels[4-5]; hence, the compound is probably ineffective in stimulating insulin secretion in the euglycemic clamp. By combining 3-[^{3}H]-glucose infusion with euglycemic clamp study, it was found that the increase in GIR induced by M16209 was due to an increase in whole-body glucose uptake (MCR) rather than to suppression of hepatic glucose output. This result is consistent with our previous observations that M16209 increases insulin-stimulated glucose uptake in isolated rat diaphragm and that it does not affect gluconeogenesis and glycogenolysis in isolated rat hepatocytes (Nagao and Ohta, unpublished data, May 1992). In this study using Wistar

Table 2. Changes in Parameters During Glucose Clamp Study in Experiment 2

Group	No.	Basal State		Steady State During Glucose Clamp			
		PG (mg/dL)	IRI (μU/mL)	PG (mg/dL)	CV (%)	IRI (μU/mL)	GIR (mg/kg/min)
Basal-control (no insulin infusion)	3	135 ± 7	24 ± 6	152 ± 4	5.1 ± 2.0	29 ± 3	
Insulin-control (insulin 3 mU/kg/min)	5	142 ± 5	24 ± 2	148 ± 4	2.7 ± 0.7	100 ± 1	5.43 ± 0.24
Insulin-M16209 10 mg/kg (insulin 3 mU/kg/min)	4	150 ± 3	25 ± 4	153 ± 5	2.5 ± 0.4	109 ± 5	6.21 ± 0.16*

NOTE. Results are expressed as the mean ± SE.

Abbreviations: PG, plasma glucose; IRI, plasma insulin; GIR, glucose infusion rate; CV, coefficient of variation for steady-state plasma glucose levels during the last 45 minutes.

*$P < .05$ *v* insulin-control.

rats, the effect of M16209 on MCR was comparatively small (17% to 30%), whereas in the study using Zucker fatty rats,[6] M16209 increased MCR by 50% and restored it to the level of lean rats. Thus, M16209, similar to thiazolidinedione compounds[17,18] known as insulin sensitizers, has more potent effects on insulin-resistant animals than on normal animals, and this may be a characteristic feature of insulin sensitizers.

In experiment 2, we attempted to examine which tissues are responsible for the increase in whole-body glucose uptake induced by M16209. The drug increased glucose uptake, glycogen synthesis, and glycolytic flux in soleus and red gastrocnemius muscles, both of which are mainly composed of oxidative muscle fibers,[19] but not in extensor digitorum lungus and white gastrocnemius muscles, both of which are mainly composed of glycolytic fibers.[19]

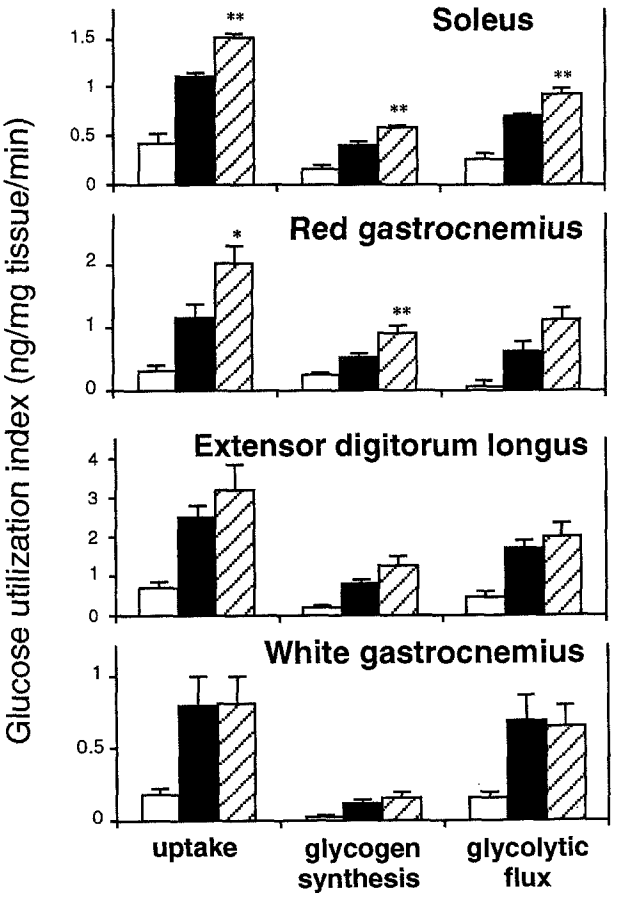

Fig 4. Glucose metabolism in muscles during glucose clamp study after 7 days of treatment with M16209 (n = 3 to 5). Hyperinsulinemic glucose clamp study combined with [^{3}H]-2-deoxyglucose and [^{14}C]-glucose administration was performed for 90 minutes. (□) Basal-control (no insulin infusion); (■) insulin-control (insulin 3 mU/kg/min); (▨) insulin-M16209 10 mg/kg (insulin 3 mU/kg/min). *$P < .05$, **$P < .01$: *v* insulin-control.

In experiment 3, we further investigated the mechanism of action of M16209 by focusing on two types of muscles, ie, the soleus muscle, sensitive to M16209, and the white gastrocnemius muscle, insensitive to M16209. The compound increased glycogen synthase-I activity in soleus muscle, but not in white gastrocnemius muscle. Glycogen synthase-I is one of the rate-limiting enzymes in the glycogen synthesis pathway, and the activation of this enzyme may result in an increased rate of incorporation of [^{14}C]-glucose into glycogen. In the soleus muscle, M16209 decreased both G6P and F6P, which are intermediates preceding the phosphofructokinase ([PFK] EC 2.7.1.11) step in the glycolytic pathway, whereas it did not affect the content of F-1,6-BP, which is the product of the PFK reaction. Moreover, M16209 increased the content of F-2,6-BP, which is believed to be the most potent activator of PFK.[20,21] However, M16209 did not affect the content of these phosphates in white gastrocnemius muscle.

These findings suggest that PFK in the soleus muscle may be activated by M16209 treatment. PFK is one of the rate-limiting enzymes in the glycolytic pathway, and activation of this enzyme may increase the glycolytic flux of [^{14}C]-glucose. Since it has been reported that insulin increases glycogen synthase-I activity[22] and F-2,6-BP con-

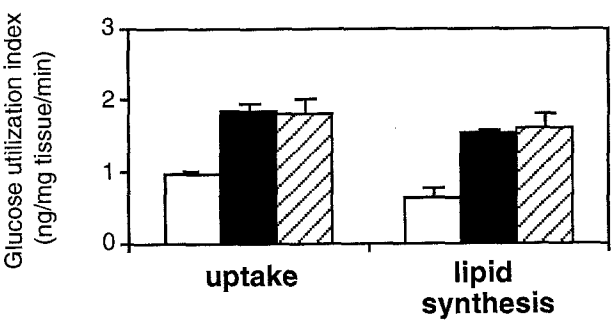

Fig 5. Glucose metabolism in epididymal adipose tissue during glucose clamp study after 7 days of treatment with M16209 (n = 3 to 5). (□) Basal control; (■) insulin-control; (▨) insulin-M16209 10 mg/kg.

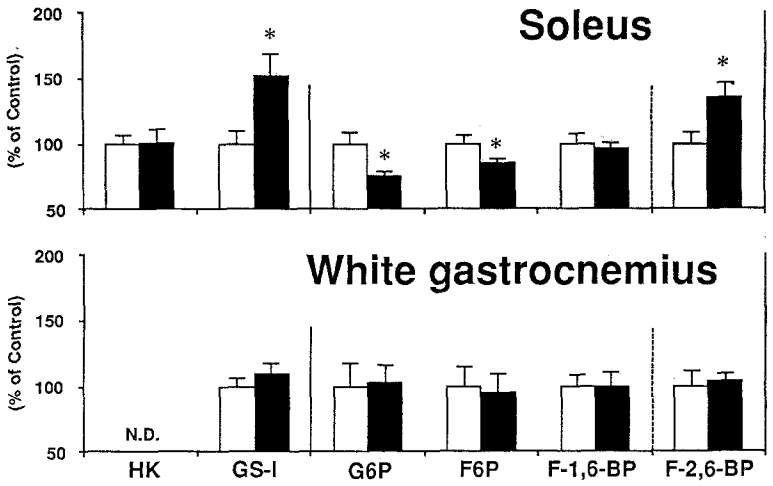

Fig 6. Hexokinase (HK) activity, glycogen synthase-I (GS-I) activity, glycolytic intermediates (G6P, F6P, and F-1,6-BP), and F-2,6-BP content in soleus and white gastrocnemius muscles (n = 6 to 12). M16209 was administered for 7 days to Wistar rats, which were killed after being starved for 21 to 28 hours after the last administration. (□) Control; (■) M16209 10 mg/kg. *$P < .05$ *v* control.

tent[23] in muscles and that oxidative muscles are more sensitive to insulin than glycolytic fibers,[24,25] it is likely that M16209 more effectively accelerates insulin action in oxidative fibers relative to glycolytic fibers. On the other hand, it has been reported that oxidative fibers have higher GLUT4 levels than glycolytic fibers[24,25] and that M16209 enhances insulin-induced translocation of GLUT4 from the intracellular membrane to the plasma membrane in 3T3 adipocytes.[26] It is therefore possible that M16209 causes an increase in GLUT4 content in the plasma membrane of muscles, as well as activation of enzymes such as glycogen synthase-I and PFK. M16209 did not affect glucose uptake and lipid synthesis from glucose in epididymal adipose tissue. CS-045, a thiazolidinedione compound known as an insulin sensitizer, has been reported to increase both glucose uptake in adipocytes[27] and body weight[28] of Zucker rats. M16209, by virtue of its action selectivity on skeletal muscle, appears to be more desirable as an antidiabetic drug than CS-045.

In conclusion, M16209 increases insulin-stimulated glucose uptake in peripheral tissues, particularly oxidative muscles, through potentiation of insulin effects on glycogen synthesis and glycolysis.

ACKNOWLEDGMENT

The authors wish to thank Michiko Machida and Michiko Kuratani for excellent assistance.

REFERENCES

1. Kato K, Nakayama K, Ohta M, et al: Effects of novel aldose reductase inhibitors, M16209 and M16287, on streptozotocin-induced diabetic neuropathy in rats. Eur J Pharmacol 193:185-191, 1991

2. Kato K, Nakayama K, Ohta M, et al: Effects of novel hydantoin derivatives with aldose reductase inhibiting activity on galactose-induced cataracts in rats. Jpn J Pharmacol 54:355-364, 1990

3. Kato K, Nakayama K, Mizota M, et al: Properties of novel aldose reductase inhibitors, M16209 and M16287, in comparison with known inhibitors, ONO-2235 and sorbinil. Chem Pharm Bull (Tokyo) 39:1540-1545, 1991

4. Nakayama K, Murakami N, Ohta M, et al: Antihyperglycemic effects of M16209, a novel aldose reductase inhibitor, in normal and diabetic rats. Eur J Pharmacol 276:77-83, 1995

5. Nakayama K, Murakami N, Ohta M, et al: Effect of M16209 on insulin secretion in isolated, perfused pancreases of normal and diabetic rats. Eur J Pharmacol 276:85-91, 1995

6. Murakami N, Ohta M, Hashimoto K, et al: Amelioration of insulin resistance in genetically obese rodents by M16209, a new antidiabetic agent. Eur J Pharmacol (in press)

7. Kergort M, Portha B: In vivo hepatic and peripheral insulin sensitivity in rats with non–insulin-dependent diabetes induced by streptozocin: Assessment with the insulin-glucose clamp technique. Diabetes 34:1120-1126, 1985

8. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

9. Terrettaz J, Jeanrenaud B: In vivo hepatic and peripheral insulin resistance in genetically obese (fa/fa) rats. Endocrinology 112:1346-1351, 1983

10. Ferre P, Leturque A, Burnol A, et al: A method to quantify glucose utilization in vivo in skeletal muscle and white adipose tissue of the anesthetized rat. Biochem J 228:103-110, 1985

11. James DE, Burleigh KM, Storlien LH, et al: Heterogeneity of insulin action in muscle: Influence of blood flow. Am J Physiol 251:E422-E430, 1986

12. James DE, Jenkins AB, Kraegen EW: Heterogeneity of insulin action in individual muscles in vivo: Euglycemic clamp studies in rats. Am J Physiol 248:E567-E574, 1985

13. Mandarino LJ, Wright KS, Verity LS, et al: Effects of insulin infusion on skeletal muscle pyruvate dehydrogenase, phosphofructokinase, and glycogen synthase. J Clin Invest 80:655-663, 1987

14. Frank SK, Fromm HJ: Effect of streptozocin-induced diabetes and insulin treatment on the synthesis of hexokinase II in skeletal muscle of the rat. Arch Biochem Biophys 249:61-69, 1986

15. Segel GB, Feig SA, Baehner RL, et al: Fluorometric analysis

of glycolytic intermediates and pyridine nucleotides in peripheral blood cells. J Lab Clin Med 78:969-976, 1971

16. Schaftingen EV: D-Fructose 2,6-bisphosphate, in Bergmeyer HU (ed): Methods of Enzymatic Analysis, vol 6. New York, NY, Academic, 1974, pp 335-341

17. Fujita T, Sugiyama Y, Taketomi S, et al: Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone), a new antidiabetic agent. Diabetes 32:804-810, 1983

18. Sugiyama Y, Shimamura Y, Ikeda H: Effects of Pioglitazone on hepatic and peripheral insulin resistance in Wistar fatty rats. Arzneimittel-forschung Drug Res 40:436-440, 1990

19. Armstrong RB, Phelps RO: Muscle fiber type composition of rat hindlimb. Am J Anat 171:259-272, 1984

20. Uyeda K, Furuya E, Luby LJ: The effect of natural and synthetic D-fructose 2,6-bisphosphate on the regulatory kinetic properties of liver and muscle phosphofructokinases. J Biol Chem 256:8394-8399, 1981

21. Matsuda M, Kaku K, Kaneko T: Regulation of muscle fructose-2,6-bisphosphate levels by sulfonylureas. Endocrinol Jpn 33:913-917, 1986

22. Dimitriadis G, Parry-Billings M, Samantha S, et al: Effects of insulin-like growth factor I on the rates of glucose transport and utilization in rat skeletal muscle in vitro. Biochem J 285:269-274, 1992

23. Rossetti R, Hu M: Skeletal muscle glycogenolysis is more sensitive to insulin than glucose transport/phosphorylation. J Clin Invest 92:2963-2974, 1993

24. Goodyear LJ, Hirshman MF, Smith RJ, et al: Glucose transporter number, activity, and isoform content in plasma membranes of red and white skeletal muscle. Am J Physiol 261:E556-E561, 1991

25. Kainulainen H, Breiner M, Schurmann A, et al: In vivo glucose uptake and glucose transporter proteins GLUT1 and GLUT4 in heart and various types of skeletal muscle from streptozotocin-diabetic rats. Biochim Biophys Acta 1225:275-282, 1994

26. Murakami N, Inoue G, Okamoto M, et al: Antihyperglycemic mechanism of M16209, an antidiabetic agent, 3T3-L1 adipocytes. Life Sci (submitted)

27. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37:1549-1558, 1988

28. Yoshioka S, Nishino H, Shiraki T, et al: Antihypertensive effects of CS-045 treatment in obese Zucker rats. Metabolism 42:75-80, 1993