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Effect of glimepiride (HOE 490) on insulin receptors of skeletal muscles from genetically diabetic KK-Ay mouse

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Abstract

A new sulfonylurea, glimepiride (HOE 490), has been developed for the glycemic control in non-insulin-dependent diabetes mellitus. We examined the effect of glimepiride on glucose and insulin levels in KK-Ay mice, an animal model of non-insulin-dependent diabetes mellitus, which is characterized by hyperglycemia and hyperinsulinemia. Administration of glimepiride (0.5 mg/kg/day) for 8 weeks to KK-Ay mice resulted in decrease in glucose (297 ± 36 to 250 ± 51 mg/dl) and insulin (76 ± 14 to 41 ± 14 μU/ml) levels. To clarify the mechanism of the agent, we examined the effect of this new drug on insulin receptors in the skeletal muscles. There was no difference in insulin binding to the receptors from both glimepiride-treated and -untreated KK-Ay mice muscles. The insulin-stimulated autophosphorylation of insulin receptors from KK-Ay mice was decreased compared to that from normal mice (5 ± 1 vs. 39 ± 13% over basal). Glimepiride did not ameliorate impaired insulin-stimulated insulin receptor autophosphorylation. To determine the effect of glimepiride on post-insulin receptor signaling pathway, we measured 2-[^{3}H]glycerol incorporation into diacylglycerol in the cultured rat fibroblast cell line overexpressing human insulin receptors. Glimepiride (100 μM) as well as insulin (10 nM) significantly stimulated diacylglycerol production. These results suggest that glimepiride has a potent extrapancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway, but not through insulin receptor kinase signaling pathway.

Keywords: Glimepiride; KK-Ay mouse; Sulfonylurea; Insulin receptor

1. Introduction

Sulfonylurea has widely been used in non-insulin-dependent diabetes mellitus patients who are characterized by decreased insulin secretion and/or insulin resistance. Thus, the best way to treat these patients is to increase insulin secretion and/or insulin sensitivity. The most prominent action of sulfonylureas is a stimulation of insulin secretion from pancreatic cells (Gylfe et al., 1984). Recently, new hypoglycemic agents to potentiate insulin action without stimulating insulin secretion have been reported to be useful for the treatment of hyperglycemia (Kobayashi et al., 1992; Nolan et al., 1994). Glimepiride; 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamide)ethyl]-phenyl]sulfonyl]-3-(4-methylcyclo-hexyl) urea (C24H34N4O5S), although it belongs to the sulfonylureas, is also believed to have ability to ameliorate insulin resistance of non-insulin-dependent diabetes mellitus since it does not greatly stimulate insulin secretion but still has a remarkable glucose lowering effect (Kaneko et al., 1993).

To clarify the mechanism of glucose lowering effect and amelioration of insulin resistance by glimepiride, we examined the effects of glimepiride on insulin binding and insulin-stimulated autophosphorylation of insulin receptors from skeletal muscles of KK-Ay mouse, an animal model of non-insulin-dependent diabetes mellitus (Iwatsuka et al., 1970). Since insulin-stimulated phospholipid synthesis is believed to lead to the activation of glucose transporter system (Romero et al., 1988; Müller et al., 1993a), we measured synthesis of diacylglycerol, one of the important

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second messengers in the phospholipid signaling pathway in rat-1 fibroblast cells overexpressing human insulin receptors, and in which insulin receptor mediated signaling pathway has been meticulously analyzed (McClain et al., 1987; Suzuki et al., 1993; Zhao et al., 1994).

2. Materials and methods

2.1. Experimental animals

Five-week-old male KK-Ay mice were purchased from CLEA Japan and were maintained on a usual laboratory chow. We used 36 mice for the experiment and their blood glucose levels were greater than 170 mg/dl at non-fasting state. At 9 weeks of age, they were divided into four groups with almost the same levels of blood glucose, glycated hemoglobin and plasma insulin. Thereafter, oral treatments with gliclazide, glimepiride or only vehicle, i.e. control, was started. Diet and water were given ad libitum during the treatment. Animals were sacrificed by decapitation at 8 weeks after the drugs were started. The blood was saved for measurement of glucose and insulin. The hindlimb muscles were removed and quickly frozen in liquid nitrogen.

2.2. Chemicals

Glimepiride (HOE 490) was provided by Hoechst (Germany). Gliclazide was purchased from Dainippon Pharmaceutical (Japan). The doses used for the experiment were 0.5 mg/kg/day of glimepiride, 20 mg/kg/day of gliclazide, which are based on the equipotency in the previous studies (Kaneko et al., 1993). All drugs suspended in 0.5% carboxy-methylcellulose sodium (CMC-Na) were orally administrated to mice by a stomach tube in the morning. Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum were purchased from Gibco. Electrophoresis and protein assay reagents were purchased from Bio-Rad (Richmond, CA). [γ-^{32}P]ATP, [^{125}I]insulin and 2-[^{3}H]glycerol were obtained from Amersham (UK). Other chemicals purchased were of analytical grade. Anti-insulin receptor antibodies to the carboxyl-terminus of insulin receptors and a rat-1 cell line overexpressing human insulin receptors (HIRc-B) were a generous gift from Dr. J.M. Olefsky. Silica gel was purchased from Merck.

2.3. Analysis of blood samples

Blood samples were obtained from the tail vein at 15:00–16:00 h. Blood glucose was analyzed by the glucose oxidase method. Plasma insulin level was analyzed by double-antibody radioimmunoassay using a commercial kit (Pharmacia Biosystems, Japan). Glycated hemoglobin A1c was determined by affinity chromatography using a commercial kit (Seikagaku Kogyo, Japan).

2.4. Preparation of partially purified insulin receptor

As previously described (Kobayashi et al., 1992), the frozen muscles were finely ground and homogenized in a 40 ml ice-cold solubilizing buffer (pH 7.4) containing 50 mM Hepes, 2 mM dichloroacetic acid, 150 mM NaF, 20 mM sodium pyrophosphate, 20 mM EDTA, 300 kIU/ml aprotinin, 4.5 mM phenylmethylsulfonyl fluoride, 4 mM orthovanadate, and 2% Triton X-100. The frozen slurry was homogenized and solubilized on ice for 30 min. After centrifugation at $2200 \times g$ for 15 min, the soluble extract was centrifuged at $200\,000 \times g$ for 40 min. The supernatant was applied twice over a 1 ml wheat germ agglutinin column. The column was washed with buffer A (pH 7.4), containing 25 mM Hepes, 120 mM NaCl, 1 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM NaF, 10 mM sodium pyrophosphate, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 2 mM orthovanadate, 10% glycerol, and 0.05% Triton X-100. Insulin receptors were eluted with 0.3 M *N*-acetylglucosamine in the buffer A and preserved at -70°C until analysis.

2.5. Insulin binding to solubilized receptors

Partially purified receptors (20 μl) were incubated with [^{125}I]insulin at 4°C for 16 h in the presence of various concentrations of unlabeled insulin in 100 μl of 50 mM Hepes (pH 7.8) containing 0.1% Triton X-100 and 0.2% bovine serum albumin. After the addition of γ-globulin as a carrier protein, receptor-bound insulin was precipitated with polyethylene glycol as previously described (Watarai et al., 1988).

2.6. Autophosphorylation of insulin receptors

Partially purified receptors with equal insulin binding capacity were preincubated with insulin (100 nM) at 4°C for 24 h. The phosphorylation was initiated by adding a solution composed of 50 mM Hepes, 100 mM $MnCl_2$, 40 mM CTP, 1 mM ATP, and [γ-^{32}P]ATP (pH 7.8). After incubation at 4°C for 10 min, the reaction was terminated by adding stopping solution containing 0.2% Triton X-100, 10 mM EDTA, 100 mM NaF, 20 mM sodium pyrophosphate, 20 mM ATP, and 20 mM Hepes (pH 7.6) as previously described (Sasaoka et al., 1988). Insulin receptors were incubated at 4°C for 16 h with anti-insulin receptor serum (Sasaoka et al., 1988). Immune complexes were precipitated by adding 200 μl of 10% suspension of pansorbin. After incubation for 2 h at 4°C, the immune complexes bound to pansorbin were sedimented by centrifugation at $10\,000 \times g$ for 10 min. After washing, the pellet was dissolved in the sample buffer containing 2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.01% bromophenol blue and 10 mM sodium phosphate with 100 mM dithiohreitol, and boiled for 5 min. After the mixture was centrifuged at $10\,000 \times g$ for 5 min, the supernatant

was applied to 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (Watarai et al., 1988). The gel was stained with Coomassie blue, dried and autoradiographed with Kodak X-Omat film for 24 h. The bands at 95 000 kDa protein were scanned by a two-dimensional densito-pattern analyzer.

2.7. Assay for diacylglycerol

HIRc-B cells were maintained in culture as described previously (McClain et al., 1987). Confluent monolayers in 3.5-cm culture dishes were incubated for 72 h in serum-free DMEM. 2-[^{3}H]glycerol (20 μCi/ml) was added and incubated for 2 h. After removing the medium, cells were rinsed with phosphate buffered saline and incubated at 37°C with insulin or glimepiride in DMEM for the indicated time. After termination of the reaction by removing the medium, the lipids of the cells were extracted as described previously (Suzuki et al., 1991). Briefly, chilled extraction mixture (methanol : chloroform : 0.01 N HCl = 1 : 2 : 1) was added to the cells. The mixture was centrifuged at 2000 × *g* for 10 min and the lipid phase was dried by N_2 stream. The lipid extracts were resolved in chloroform and analyzed by silica gel thin-layer chromatography (TLC) using solvent system 1 (chloroform : acetone : methanol : glacial acetic acid : H_2O = 10 : 4 : 4 : 2 : 1). Neutral lipid band was scraped and the silica powders were extracted with chloroform : methanol (2 : 1). The liquid fractions were dried and resolved in chloroform, which were analyzed by TLC using solvent system 2 (petroleum ether : diethylether : acetic acid = 70 : 30 : 2). ENHANSE (DuPont) was sprayed on gel surface and the plate was exposed to a Kodak X-Omat film for the appropriate time (4–7 days). After developing the film, the corresponding band to the diacylglycerol on the gel was scraped and the radioactivity was determined by liquid scintilation counter.

2.8. Statistical analysis

The data are presented as mean ± S.E. *P* values were determined by unpaired *t*-test and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Profile of experimental animals

Table 1 shows the results of blood glucose and insulin concentration levels of normal and KK-Ay mice with or without sulfonylurea drug treatment. KK-Ay mice showed hyperglycemia with hyperinsulinemia. The blood glucose level of control group (KK-Ay CMC-Na) was 299 ± 38 mg/dl before the treatment and gradually increased to 458 ± 29 mg/dl at the end of treatment. Gliclazide treatment did not change this deterioration of glucose metabolism. In contrast, glimepiride treatment prevented the increase of blood glucose level and the decreased the insulin concentration of the KK-Ay mice. Thus, glimepiride ameliorated not only hyperglycemia, but also hyperinsulinemia in KK-Ay mice.

Table 1
Changes in blood glucose and plasma insulin levels before and 8 weeks after administration of CMC-Na (control), gliclazide and glimepiride in KK-Ay mice

Animal	Treatment	Blood glucose (mg/dl)		Plasma insulin (μU/ml)	
		0	8 weeks	0	8 weeks
Normal			108 ± 3		7 ± 1
KK-Ay	CMC-Na (control)	299 ± 38	457 ± 29	69 ± 9	87 ± 7
	Gliclazide	295 ± 35	423 ± 40	71 ± 12	103 ± 15
	Glimepiride	297 ± 36	250 ± 51 [a]	76 ± 14	41 ± 14 [b]

Each value represents mean ± S.E. ($n = 8$–9).
[a] $P < 0.01$, [b] $P < 0.05$ vs. control (KK-Ay).

3.2. Insulin binding to solubilized receptors

Insulin binding to partially purified insulin receptor from muscles is shown in Fig. 1. Insulin binding was decreased in KK-Ay mice with or without sulfonylurea drugs compared to that in normal mice, probably due to a down-regulation by hyperinsulinemia. Furthermore, there was no difference in insulin binding between glimepiride-treated and -untreated KK-Ay mice.

3.3. Effects on insulin receptor autophosphorylation

Insulin resistance in the cells was partly characterized by the decreased insulin receptor tyrosine kinase activity,

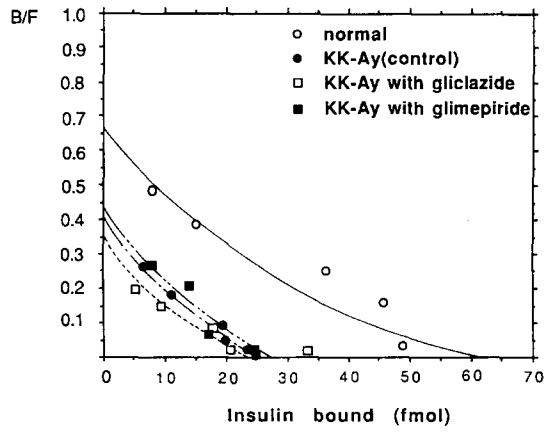
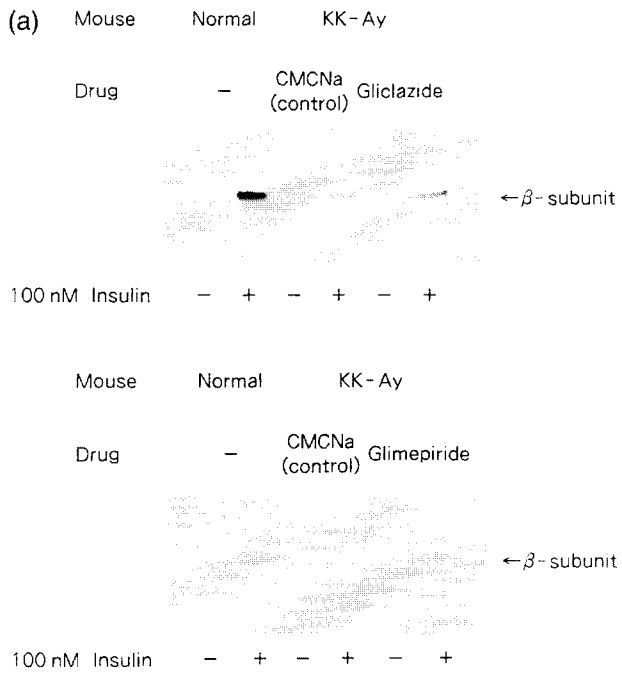


Fig. 1. Scatchard plot of insulin binding to partially purified insulin receptor from muscle. Results in normal mice (○———○), KK-Ay mice treated without sulfonylurea drugs (●–·–●), KK-Ay mice treated with glicrazide (□– – –□ and KK-Ay mice treated with HOE 490 (glimepiride) (■–·–·–■) are shown. The data are presented as the means of three separate experiments.



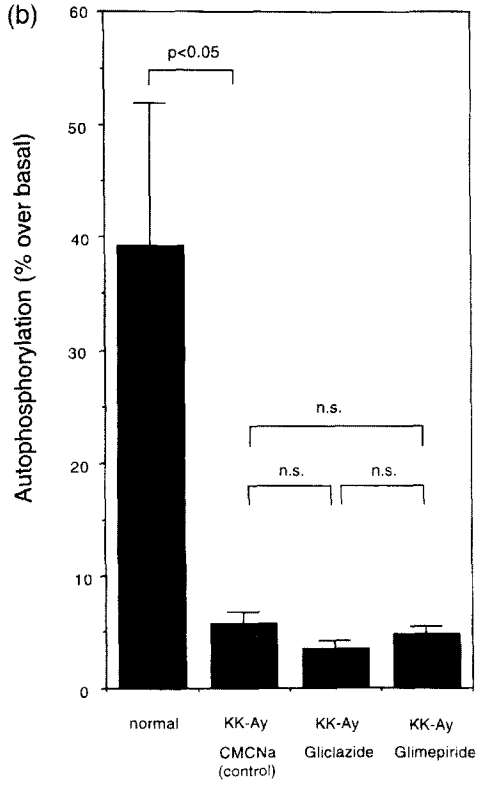


Fig. 2. Autophosphorylation of partially purified insulin receptors. Insulin receptors were prepared from muscle of normal and KK-Ay mice treated with or without sulfonylurea drugs, incubated in the presence or absence of 100 nM insulin as described in Materials and methods. A representative autoradiogram is shown. (b) The comparison of autophosphorylation among normal and KK-Ay mice with or without sulfonylurea drugs. The 95000 kDa bands on the autoradiogram were analyzed by densito-pattern analyzer and the values of insulin (100 nM) stimulated insulin receptor autophosphorylation is presented as percent over basal. The data are presented as mean ± S.E. of three separate experiments. n.s., statistically not significant.

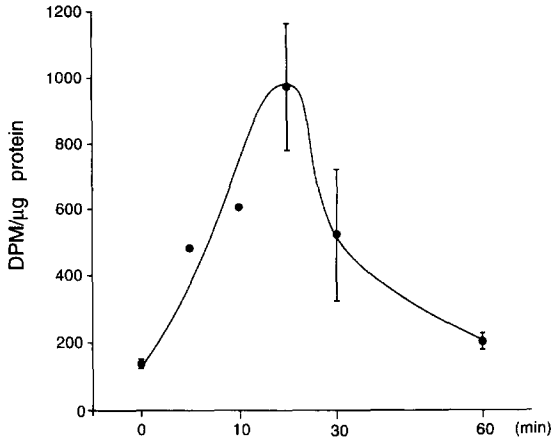


Fig. 3. Effect of glimepiride on the diacylglycerol synthesis. Cells were treated with glimepiride (100 μM) for the indicated time and the labeled glycerol into diacylglycerol was measured as described in Materials and methods. The data are expressed as dpm/μg protein and presented as mean ± S.E. of two to three experiments.

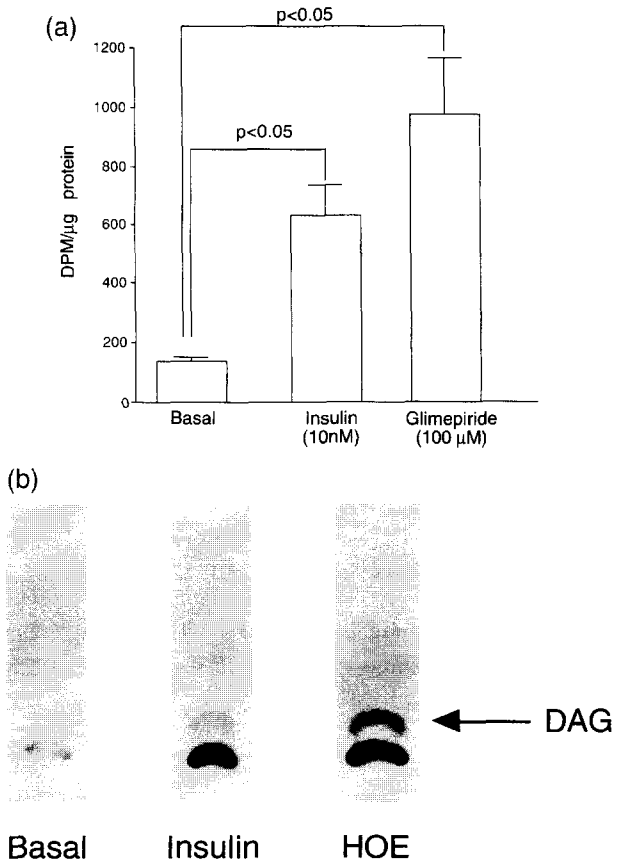


Fig. 4. Insulin or glimepiride stimulated glycerol incorporation into diacylglycerol in HIRc-B cells. (a) A representative autoradiogram of TLC plate is shown. Basal, buffer; insulin, insulin (10 nM); HOE, glimepiride (100 μM). (b) Insulin (10 nM) or glimepiride (100 μM) stimulated diacylglycerol synthesis. The labeled diacylglycerol on the TLC plate was counted as described in Materials and methods and is shown as dpm/μg protein. The results are show as mean ± S.E. from three independent experiments. There is no significant difference between the labeled diacylglycerol values in insulin treatment and glimepiride treatment.

and the improvement of the metabolic derangement ameliorated the insulin receptor tyrosine kinase activity (Watarai et al., 1988; Iwanishi and Kobayashi, 1993). We studied the effect of glimepiride on the insulin receptor autophosphorylation in KK-Ay mice muscles (Fig. 2a). Insulin (100 nm) stimulated phosphorus incorporation into 95 000 kDa insulin receptor ß-subunit. In untreated KK-Ay mice, insulin-stimulated phosphorylation was significantly decreased compared to that of normal mice ($P < 0.05$) (Fig. 2b). There was no difference in the signal intensity in those muscle samples in Fig. 2a in the immunoblot analysis with anti-insulin receptor antibody (data not shown). The treatment with either glimepiride or gliclazide did not change the autophosphorylation of the insulin receptor form muscles of KK-Ay mice. Therefore, neither glimepiride nor gliclazide ameliorated insulin binding and insulin receptor autophosphorylation in muscles of KK-Ay mice.

3.4. Effects of glimepiride on diacylglycerol production

As previously reported (Zhao et al., 1994), insulin stimulated diacylglycerol production in HIRc-B cells. Glimepiride also stimulated glycerol incorporation into diacylglycerol and its effect reached the maximum at 15 min after treatment (Fig. 3). Diacylglycerol produced by glimepiride treatment showed the same migrating distance in TLC as by insulin (Fig. 4a). The effect of glimepiride (100 μM) on glycerol incorporation into diacylglycerol was comparable to that of 10 nM insulin (Fig. 4b).

4. Discussion

KK-Ay mice were characterized by progressive increase in blood glucose and insulin level from 5 weeks of age. Insulin sensitivity of adipose tissue was decreased with age in these mice (Iwatsuka et al., 1970). We used 8- to 9-week-old KK-Ay mice with elevated glucose and insulin levels. Thus, these mice had insulin resistance along with glucose intolerance. Glimepiride, a new oral sulfonylurea, reduced plasma insulin concentrations and blood glucose levels in these mice (Table 1). No stimulatory effect on the insulin levels by gliclazide might indicate the insulin secretion was already maximally stimulated in KK-Ay mice or there might be a defect in the sulfonylurea-induced insulin secretion in this mouse. Even in this condition, glimepiride improved the hyperglycemia. It is well known that sulfonylurea drugs act on pancreatic β cells, in which membrane ATP-sensitive K^+ channels are inhibited, to promote the release of insulin and thereby reduce the blood glucose level (Panten et al., 1992). Prolonged drug treatment, however, revealed that plasma insulin levels often returned to pretreatment levels even though the sulfonylurea-induced hypoglycemic effects persisted (Beck-Nielsen et al., 1979). Therefore, it is believed that sulfonylureas may improve the diabetic patients' insulin activity by reducing post-insulin receptor defects, i.e. they may exert its extrapancreatic effect. It is likely that glimepiride may have more potent extrapancreatic hypoglycemic effects than gliclazide and other sulfonylureas to improve insulin resistance in peripheral tissue in KK-Ay mice.

We have previously demonstrated that pioglitazone, a thiazolidine derivative, ameliorated hyperglycemia and hyperinsulinemia in genetically obese Wistar fatty rats and high-fat-fed rats and suggested that pioglitazone might act directly on insulin receptors by increasing their insulin-stimulated tyrosine kinase activities (Kobayashi et al., 1992; Iwanishi and Kobayashi, 1993). Glimepiride, however, did not increase insulin-stimulated insulin receptor autophosphorylation and insulin binding ability was not affected in insulin receptors from glimepiride-treated KK-Ay mice muscles (Figs. 1 and 2). It has been already reported that sulfonylureas, such as chrolpropamide, glyburide and gliclazide, stimulate insulin action without affecting insulin binding and insulin-stimulated tyrosine kinase activities of insulin receptors (Jacobs et al., 1987; Caro et al., 1989; Bak et al., 1989). These observations suggested that glimepiride, as well as other sulfonylureas, had a common mode of extrapancreatic action to increase insulin sensitivity without affecting insulin receptor binding and tyrosine kinase activity.

It has been reported that insulin stimulates hydrolytic enzymes, resulting in the generation of low molecular weight substances as insulin-mediators, such as inositol-phosphate-glycan, diacylglycerol, dimyristoylglycerol inositol glycan and alkylacylglycerol (Saltiel et al., 1986; Romero et al., 1988; Suzuki et al., 1993). Furthermore, diacylglycerol could stimulate protein kinase C activity and glucose transport activity (Cherqui et al., 1990). Our finding that glimepiride stimulated glycerol incorporation into diacylglycerol suggested that glimepiride might directly activate glucose transporter activity. Müller and coworkers also demonstrated that glimepiride activated a glycosyl phosphatidylinositol-specific phospholipase C in adipocytes (Müller et al., 1993b). As pioglitazone treatment in KK-Ay mice improved hyperglycemia and hyperinsulinemia together with an increase in diacylglycerol levels in muscles, a defect in phospholipid signaling may play a role in the insulin resistance in this mouse (Saha et al., 1994). Therefore, it is possible that glimepiride directly improve the defect to ameliorate hyperglycemia in these mice. Taken together, these results suggest that glimepiride exerts as a stimulator of phospholipid production and may stimulate glucose transporter 1 and glucose transporter 4 translocation. A recent report describing a defect in glycosyl phosphatidylinositol-dependent insulin signaling system in Zucker (fa/fa) rats may support the idea that phospholipid signaling is an important pathway associated with insulin action (Sanchez-Gutierrez et al., 1994).

In HIRc-B cells, the stimulatory effects of insulin on

membrane diacylglycerol production developed rapidly and the peak effect appeared within 5 min (Zhao et al., 1994). The effect of glimepiride was also rapid and reached the maximum around 15 min. The finding that the translocation of glucose transporters to the plasma membrane reached the maximum level at 20 min supports the idea that diacylglycerol synthesis may be the prerequisite for the translocation of the glucose transporters (Müller et al., 1993a,b). Although it is unclear whether the similar phenomenon occurs in the muscle, it is one possible mechanism for the extrapancreatic action of glimepiride.

We demonstrated that glimepiride, a new sulfonylurea, specifically ameliorate glucose intolerance without affecting insulin secretion. Furthermore, we demonstrated that glimepiride as well as insulin stimulated diacylglycerol production in the cultured cell line. Taken together, these results indicate that glimepiride may have more potent extrapancreatic effects than other sulfonylureas to effectively ameliorate insulin resistance caused by hyperglycemia. Further studies to prove the molecular process of glimepiride-stimulated generation of diacylglycerol in the biological action of this mediator may be necessary to clarify the molecular mechanisms of the glucose lowering effects of glimepiride.

References

Bak, J.F., O. Schmitz, N.S. Sorensen and A.O. Pedersen, 1989, Postreceptor effects of sulfonylurea on skeletal muscle glycogen synthase activity in type II diabetic patients, Diabetes 38, 1343.

Beck-Nielsen, H., O. Pedersen and H.O. Lindskov, 1979, Increased insulin sensitivity and cellular insulin binding in obese diabetics following treatment with glybenclamide, Acta Endocrinol. 90, 451.

Caro, J.F., O. Ittoop and M.K. Sinha, 1989, Glyburide but not ciglitazone enhances insulin action in the liver independent of insulin receptor kinase activation, Metabolism 37, 606.

Cherqui, G., C. Reynet, M. Caron, B. Melin, D. Wicek, E. Clauses, J. Capeau and J. Picard, 1990, Insulin receptor tyrosine redidues 1162 and 1163 control insulin stimulation of myristoyl-diacyl glycerol generation and subsequent activation of glucose transport, J. Biol. Chem. 265, 21254.

Gylfe, E., B. Hellman, J. Sehlin and I.B. Taljedal, 1984, Interaction of sulfonylurea with pancreatic beta-cell, Experientia 40, 1126.

Iwanishi, M. and M. Kobayashi, 1993, Effect of pioglitazone on insulin receptors of skeletal muscles from high-fat-fed rats, Metabolism 42, 1017.

Iwatsuka, H., A. Shino and Z. Suzuki, 1970, Gereral survey of diabetic features of yellow KK mice, Endocrinol. Jpn. 17, 23.

Jacobs, D.B., G.R. Hayes and D.H. Lockwood, 1987, Effect of chlorpropamide on glucose transport in rat adipocytes in the absence of changes in insulin binding and receptor-associated tyrosine kinase activity, Metabolism 36, 548.

Kaneko, T., N. Sakamoto, S. Nakagawa, S. Nakagawa, Y. Goto, Y. Hirata, Y. Akanuma, N. Hotta, S. Tarui, S. Baba, K. Kaku and G. Mimura, 1993, Optimal dose finding study of HOE 490 (glimepiride) in non-insulin dependent diabetes mellitus, J. Clin. Ther. Med. 9, 1107.

Kobayashi, M., M. Iwanishi, K. Egawa and Y. Shigeta, 1992, Pioglitazone increases insulin sensitivity by activating insulin receptor kinase, Diabetes 41, 476.

McClain, D.A., H. Maegawa, J. Lee, T.J. Dull, A. Ulrich and J.M. Olefsky, 1987, A mutant insulin receptor with defective tyrosine kinase displays no activity and does not undergo endocytosis, J. Biol. Chem. 262, 14663.

Müller, G., E.A. Dearey and J. Punter, 1993a, The sulfonylurea drugs, glimepiride, stimulates release of glycosylphosphatidylinositol-anchored plasma-membrane proteins from 3T3 adipocytes, Biochem. J. 289, 509.

Müller, G and S. Wied, 1993b, The sulfonylurea drug, glimepiride, stimulates glucose transport, glucose transporter translocation, and dephosphorylation in insulin-resistant rat adipocytes in vitro, Diabetes 42, 1852.

Nolan, J.J., B. Ludrik, P. Beerdsen, M. Joyce and J.M. Olefsky, 1994, Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone, New Engl. J. Med. 331, 1188.

Panten, U., M. Schwanstecher and C. Schwanstecher, 1992, Pancreatic and extrapancreatic sulfonylurea receptors, Horm. Metab. Res. 24, 549.

Romero, G., L. Luttrell, A. Rogol, K. Zeller, E. Herolett and J. Larner, 1988, Phosphatidylinositol-glycan anchors of membrane protein: potential precursors of insulin mediators, Science 240, 509.

Sanchez-Gutierrez, J.C., J.A. Sanchez-Arias, J.C. Valle, A. Guadano, B. Samper, J.M. Mato and J.E. Felise, 1994, Insulin resistance in genetically obese (fa/fa) rats: changes in the glycosyl-phosphatidylinositol signaling system in isolated hepatocytes, Endocrinology 134, 1485.

Saha, A.K., T.G. Kurowski, J.R. Colca and N.B. Ruderman, 1994, Lipid abnormalities in tissues of the KKAy mouse: effects of pioglitazone on malonyl-CoA and diacylglycerol, Am. J. Physiol. 267, E95

Saltiel, A.R., J.A. Fox, P. Sherline and P. Cuatrecasas, 1986, Insulin stimulated hydrolysis of a novel glycolipid generates modulation of cAMP phosphodiesterase, Science 233, 967.

Sasaoka, T., Y. Shigeta, Y. Takata, M. Sugibayashi, A. Hisatomi and M. Obayashi, 1988, Binding specificity and intramolecular signal transmission of uncleaved insulin proreceptor in transfected lymphocyte from patient with extreme insulin resistance, Diabetologia 32, 371.

Suzuki, S., K. Sugawra, Y. Satoh and T. Toyota, 1991, Insulin stimulated the generation of two putative insulin mediators, inositol-glycan and diacylglycerol in BC3H-1 myocytes, J. Biol. Chem. 266, 8115.

Suzuki, S., Y. Aneda, S. Hirai, Y. Satoh and T. Toyota, 1993, Insulin stimulates hydrolysis of plasmanylinositol-glycan and phophatidyl inositol-glycan in rat adipocytes. Insulin-induced generation of inositol glycan, alkylacylglycerol, and diacylglycerol, Diabetes 42, 988.

Watarai, T., M. Kobayashi, Y. Takata, T. Sasaoka, M. Iwasaki and Y. Shigeta, 1988, Alteration of insulin-receptor kinase activity by high-fat feeding, Diabetes 37, 1397.

Zhao, L., M.L. Standaert, D.R. Cooper, A. Avignon and R.V. Farese, 1994, Effects of insulin on protein kinase-C (PKC) in HIRC-B cells: specific activation of PKCϑ and its resistance to phorbol ester induced down-regulation, Endocrinology 135, 2504.