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Gliclazide protects 3T3L1 adipocytes against insulin resistance induced by hydrogen peroxide with restoration of GLUT4 translocation

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Abstract

Increased oxidative stress under hyperglycemia may contribute to progressive deterioration of peripheral insulin sensitivity. In this study, we investigated whether gliclazide, a second-generation sulfonylurea, can protect 3T3L1 adipocytes from insulin resistance induced by oxidative stress, and whether gliclazide can restore insulin-stimulated glucose transporter 4 (GLUT4) translocation under oxidative stress. We incubated 3T3L1 adipocytes in hydrogen peroxide to produce oxidative stress, then administered various concentrations of gliclazide, *N*-acetylcystein (NAC), or glibenclamide. Cells treated with these drugs were next exposed to insulin, subsequent glucose uptake was measured, and the insulin-stimulated GLUT4 translocation was monitored in living cells. We found that hydrogen peroxide treatment alone suppressed glucose uptake by insulin stimulation to 65.9% ± 7.8% of the corresponding controls ($P < .01$). However, addition of 0.1 to 10 μmol/L gliclazide to hydrogen peroxide–treated cells dose-dependently restored glucose uptake, with 5 μmol/L gliclazide significantly restoring glucose uptake to 93.3 ± 6.6% ($P < .01$) even under hydrogen peroxide. Treatment with the known anti-oxidant NAC also dose-dependently (0.1-10 mmol/L) restored insulin-induced glucose uptake in the presence of hydrogen peroxide. However, glibenclamide (0.1-10 μmol/L), another second-generation sulfonylurea, failed to improve glucose uptake. Similarly, treatment with 5 μmol/L gliclazide or 10 mmol/L NAC significantly overcome the reduction in insulin-stimulated GLUT4 translocation by hydrogen peroxide ($P < .01$), whereas 5 μmol/L glibenclamide did not. Therefore our data regarding gliclazide further characterize its mechanism of hypoglycemic effect: the observed improvements in insulin sensitivity and in GLUT4 translocation indicate that gliclazide counters the hydrogen peroxide–induced insulin resistance in 3T3L1 adipocytes and also would further augment the hypoglycemic effect of this drug as insulinotropic sulfonylurea.
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1. Introduction

Type 2 diabetes mellitus has been shown to be associated with increased oxidative stress, measurable as an accelerated production or decreased scavenging of oxygen free radicals [1,2]. Hyperglycemia promotes nonenzymatic protein glycation through the Maillard reaction, yielding Schiff bases, Amadori products, and advanced glycation end products. During this process, reactive oxygen species (ROS) are produced, and a possible role of ROS accumulation has been proposed in the development of diabetic complications [3,4].

Defects in insulin secretion from pancreatic beta cells together with insulin resistance of all major target tissues have been known to cause type 2 diabetes mellitus [5]. It has been recently recognized that the oxidative stress–induced tissue damage is responsible for progressive deterioration of insulin secretory capacity under hyperglycemia [6]. Treatment with anti-oxidant chemicals such as cystein or *N*-acetylcystein (NAC) has been shown to protect beta cells from damage by ROS [7,8]. In addition, oxidative stress has been shown to represent a causative factor for insulin resistance in peripheral target tissues. It has been recently reported that release of hydrogen peroxide, an inducer of oxidative stress, is significantly higher in the white adipose tissue of obese diabetic KKAy mice with peripheral insulin resistance than in control mice [9]. The insulin-induced

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cellular compartmentalization of phosphatidylinositol (PI)-3-kinase and IRS-1 in 3T3L1 adipocytes has been suggested to be oxidative stress–sensitive [10].

Gliclazide is a second-generation sulfonylurea commonly used in type 2 diabetes mellitus treatment. It exhibits a hypoglycemic effect through enhancing insulin secretion from beta cells by binding to high-affinity sulfonylurea receptors [11]. Besides this effect, gliclazide has been observed to possess anti-oxidant properties, for example, to decrease the oxidation of low-density lipoprotein [12]. In addition, our recent observation that gliclazide can protect beta cells from damage due to hydrogen peroxide supports an anti-oxidant role for this drug [13]. Since a free radical–scavenging activity of gliclazide has been demonstrated in vitro and in vivo [14-16], the anti-oxidant effect appears independent of gliclazide's insulinotropic action. In this study, we further investigated whether gliclazide can counter insulin resistance induced by hydrogen peroxide in 3T3L1 adipocytes. Specifically, we evaluated whether gliclazide affects insulin-stimulated glucose uptake and glucose transporter 4 (GLUT4) translocation under hydrogen peroxide treatment.

2. Materials and methods

2.1. Materials

Gliclazide was obtained from Dainippon Pharmaceutical (Osaka, Japan). Glibenclamide and NAC were purchased from Sigma (St Louis, Mo), and 2-deoxy-D-glucose (2-DOG) was from Wako (Osaka, Japan). 2-Deoxy-D-[U-^{14}C]glucose (300 mCi/mmol) was obtained from Dupont-NEN (Boston, Mass). Human GLUT4 cDNA was a generous gift from Dr GI Bell (University of Chicago). The GLUT4–enhanced green fluorescent protein (eGFP) construct was prepared by subcloning the full-length GLUT4 cDNA in frame into the *Hin*dIII and *Eco*RI sites of the peGFP vector (CLONTECH, Palo Alto, Calif) to make a C-terminal eGFP fusion.

2.2. Preparation of cells

3T3L1 cells were obtained from the cell bank of Japanese Collection of Research Bioresources (Tokyo, Japan). Cells were seeded and fed every 2 to 3 days in Dulbecco's Modified Eagle's Medium (DMEM) high glucose supplemented with 50 U/mL penicillin, 50 μg/mL streptomycin, 100 mmol/L MEM sodium pyruvate, and 10% fetal calf serum, and were grown under 5% CO_2 at 37°C. At confluence, differentiation was started by addition of medium containing 500 μmol/L isobutyl-methylxanthine (Sigma), 250 nmol/L dexamethasone (Sigma), and 1.7 μmol/L insulin. After 48 hours, this mixture was replaced with fresh medium. Between days 7 and 10 after induction of differentiation, glucose uptake after insulin stimulation was determined by measuring 2-deoxy-D-[U-^{14}C]glucose uptake, and the dynamics of the GLUT4-eGFP fusion protein were monitored as a measure of GLUT4 translocation in living cells.

2.3. 2-DOG uptake assay

Differentiated 3T3L1 adipocytes (10^6 cells per dish) were cultured with various concentrations of hydrogen peroxide in DMEM for 3 hours. To study the ability of gliclazide to protect 3T3L1 cells from oxidative stress, 0.1 to 10 μmol/L gliclazide was also added to the hydrogen peroxide–DMEM mix for the 3-hour incubation. This same protocol was used in other glucose uptake experiments here described, but with 0.1 to 10 mmol/L NAC or 0.1 to 10 μmol/L glibenclamide added where designated instead of gliclazide. After the 3-hour incubation with hydrogen peroxide and the test agent, the medium was removed and the cells were washed 3 times using serum-free Hank's buffer. The cells were maintained at 37°C for 2 hours in the serum-free Hank's buffer, 0.2 μCi 2-deoxy-D-[U-^{14}C]glucose was added to the medium containing 1 mmol/L nonradioactive 2-DOG, and cells were incubated for 20 minutes with 100 nmol/L insulin to stimulate glucose uptake. Uptake was stopped by aspiration of the buffer. Cells were rapidly washed several times with 1 mL of ice-cold phosphate buffered saline and were solubilized by the addition of 0.2 mol/L NaOH. As a control, nonspecific uptake was determined in parallel in the presence of 10 μmol/L cytochalasin B. The radioactivity associated with the cells was measured using a scintillation counter (LSC-3100, Aloca, Tokyo, Japan), as previously described [17].

2.4. Cell microinjection of GLUT4-eGFP cDNA

The 3T3L1 adipocytes were injected with cDNA of GLUT4-eGFP using an Eppendorf microinjector system (Femtojet, Eppendorf, Hamburg, Germany) fitted to a Zeiss Axiovert microscope. In each coverslip, the plasmid of GLUT4-eGFP cDNA was adjusted to 50 to 200 μg/mL in Tris-EDTA buffer and injected into the nucleus of ~50 cells. After injection, the cells were washed twice with DMEM containing 10% fetal calf serum and incubated for 16 to 24 hours under 5% CO_2 at 37°C. The efficiency of gene transfer into cells was approximately 8% to 27%.

2.5. Image capture and analysis of GLUT4 translocation

The 3T3L1 adipocytes microinjected with GLUT4-eGFP were analyzed by a laser-scanning confocal microscope LSM 510 (Carl Zeiss, Jena, Germany) to monitor the dynamics of GLUT4 translocation. Injected cells were placed on a heated stage adjusted to provide a temperature of 37°C in the bathing KRB buffer (pH 7.4 adjusted by 10 mmol/L HEPES) containing 0.3% BSA. Cells were incubated with 100 μmol/L hydrogen peroxide or vehicle alone, as described above, and 5 μmol/L gliclazide was added during the incubation period. For comparison, 10 mmol/L NAC or 5 μmol/L glibenclamide was used to assay protection against oxidative stress. Images were collected from living cells using 488-nm excitation wave-

length before, and 4, 10, and 20 minutes after stimulation with 100 nmol/L insulin. The eGFP signals were analyzed with NIH image software (version 1.61, National Institute of Mental Health, NIMH Public Inquiries, Bethesda, Md). To quantitate the extent of GLUT4-eGFP translocation to the plasma membrane, we calculated the ratio of fluorescence intensity in the peripheral region to that in the remaining cellular fluorescence. The peripheral/cellular ratio in basal state (before insulin stimulation) was expressed as 100%, and the value during insulin stimulation was calculated relative to this basal level.

2.6. Statistical analysis

Statistical analysis was performed by paired and unpaired *t* test or by analysis of variance using the StatView computer software (Abacus, Berkeley, Calif). Results are expressed as mean ± SEM, and $P < .01$ was considered significant.

3. Results

3.1. Induction of insulin resistance in differentiated 3T3L1 adipocytes by hydrogen peroxide

Micromolar concentrations of hydrogen peroxide have been shown sufficient to inhibit insulin signaling in differentiated 3T3L1 adipocytes [10,18]. To establish conditions for this basal effect, we pretreated 3T3L1 adipocytes with various concentrations of hydrogen peroxide for 3 hours and then evaluated insulin-stimulated glucose uptake. As shown in Fig. 1, 30 to 150 μmol/L hydrogen peroxide suppressed the glucose uptake stimulated by 100 nmol/L insulin in a dose-dependent manner, with all values taken in the presence of hydrogen peroxide significantly lower than that in insulin alone. However, basal glucose uptake without insulin stimulation in the presence of 150 μmol/L hydrogen peroxide was significantly reduced when compared to the value in the corresponding controls, suggesting damage of cellular function. Since consistent suppression of insulin-stimulated glucose uptake was observed without reduction of basal glucose uptake in the case of 100 μmol/L hydrogen peroxide, we chose this concentration for inducing insulin resistance in the remainder of this study.

3.2. Protective effect of gliclazide against oxidative stress in 3T3L1 adipocytes

Under basal conditions without hydrogen peroxide, addition of 0.1 to 10 μmol/L gliclazide did not stimulate glucose uptake into 3T3L1 adipocytes (1857 ± 220 [dpm per dish], 1753 ± 115, 1922 ± 198, 1741 ± 128, and 1825 ± 167 in 0, 0.1, 1, 5, and 10 μmol/L gliclazide, respectively). To determine whether gliclazide can protect against oxidative stress–induced insulin resistance, we examined the effect of gliclazide on insulin-stimulated glucose uptake under hydrogen peroxide treatment. As shown in Fig. 2A, 5 μmol/L gliclazide again did not affect basal glucose uptake in the presence of hydrogen peroxide without insulin. However, in the presence of insulin, 100 μmol/L hydrogen peroxide alone significantly suppressed glucose uptake to 65.9% ± 7.8% of control values, but addition of 5 μmol/L gliclazide significantly restored the glucose uptake to 93.3% ± 6.6%, a figure not statistically different from the control values. A range of 1 to 10 μmol/L gliclazide also significantly, and dose-dependently, restored insulin-induced glucose uptake under hydrogen peroxide treatment (Fig. 2B).

For comparison, these same experiments were reproduced using the known anti-oxidant NAC in place of gliclazide. Although glucose uptake induced by insulin under hydrogen peroxide was reduced to 62.0% ± 8.0% of control values, adding 10 mmol/L NAC significantly restored glucose uptake to 91.2% ± 8.5%, which was not statistically different from that in the presence of insulin alone, as indicated in Fig. 3A. Like gliclazide, treatment with NAC also restored the insulin-stimulated glucose uptake under 100 μmol/L hydrogen peroxide in concentrations of 0.1 to 10 mmol/L dose dependently (Fig. 3B). In contrast to the effects of 5 μmol/L gliclazide or NAC, 5 μmol/L glibenclamide failed to restore glucose uptake in the presence of 100 μmol/L hydrogen peroxide and insulin (Fig. 4A). A range of 0.1 to 10 μmol/L glibenclamide likewise showed no significant improvement on insulin-stimulated glucose uptake under hydrogen peroxide treatment (Fig. 4B). Compositely, these data indicate that sulfonylurea gliclazide protects against insulin resistance induced by hydrogen peroxide in 3T3L1 adipocytes, correlating gliclazide activity with that of the known anti-oxidant NAC, whereas sulfonylurea glibenclamide displays no such activity.

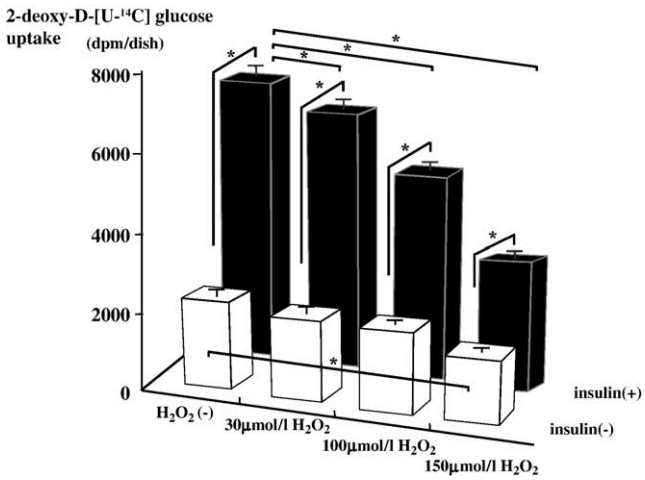

Fig. 1. Inhibition of insulin-induced glucose uptake by various concentrations of hydrogen peroxide in differentiated 3T3L1 adipocytes. White and black bars represent conditions without and with 100 nmol/L insulin, respectively, under hydrogen peroxide. Values are means ± SEM (n = 6). *$P < .01$.

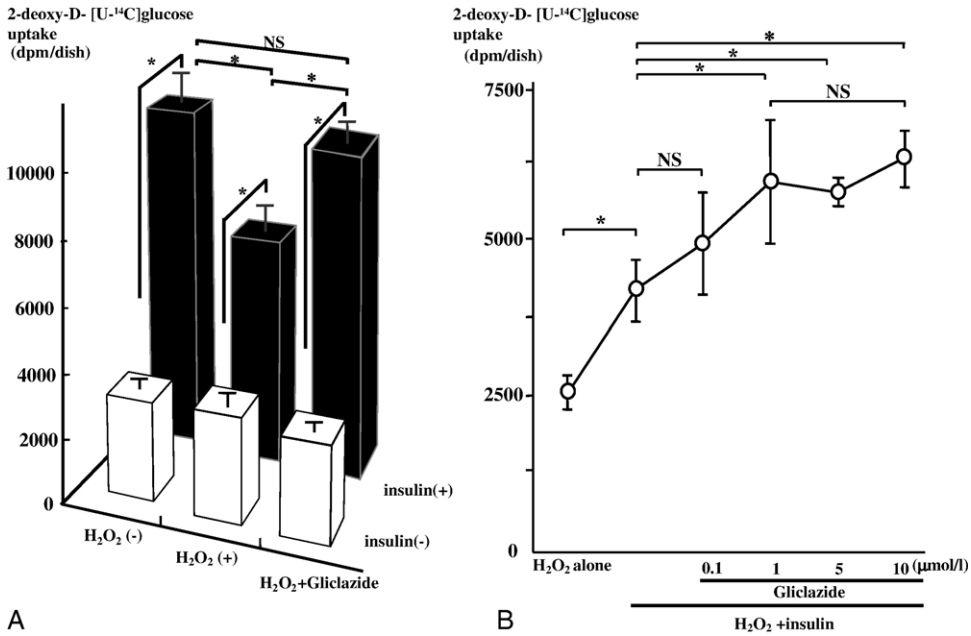

Fig. 2. A, Protective effect of gliclazide against the inhibition of insulin-induced glucose uptake under 100 μmol/L hydrogen peroxide. White and black bars indicate conditions without and with 100 nmol/L insulin, respectively, under hydrogen peroxide alone or plus gliclazide. B, Gliclazide dose-dependently restores insulin-induced glucose uptake in the presence of hydrogen peroxide. Values are means ± SEM (n = 6). *P < .01. NS indicates not significant.

3.3. Gliclazide effect on insulin-stimulated GLUT4 translocation under oxidative stress

Live, insulin-stimulated 3T3L1 adipocytes expressing GLUT4-eGFP were imaged to evaluate GLUT4 translocation over time in the absence of drug (Fig. 5). As illustrated in Fig. 6, for each drug then tested, changes in insulin-stimulated GLUT4-eGFP translocation were monitored under hydrogen peroxide before and 20 minutes after the stimulation. The addition of 100 μmol/L hydrogen peroxide alone did not affect intracellular localization of GLUT4-eGFP (Fig. 6A, upper panels), and insulin alone failed to induce significant GLUT4 translocation to the plasma membrane under hydrogen peroxide (Fig. 6A, lower panels). These controls confirm functional disturbances of insulin signaling in the presence of hydrogen peroxide. In an

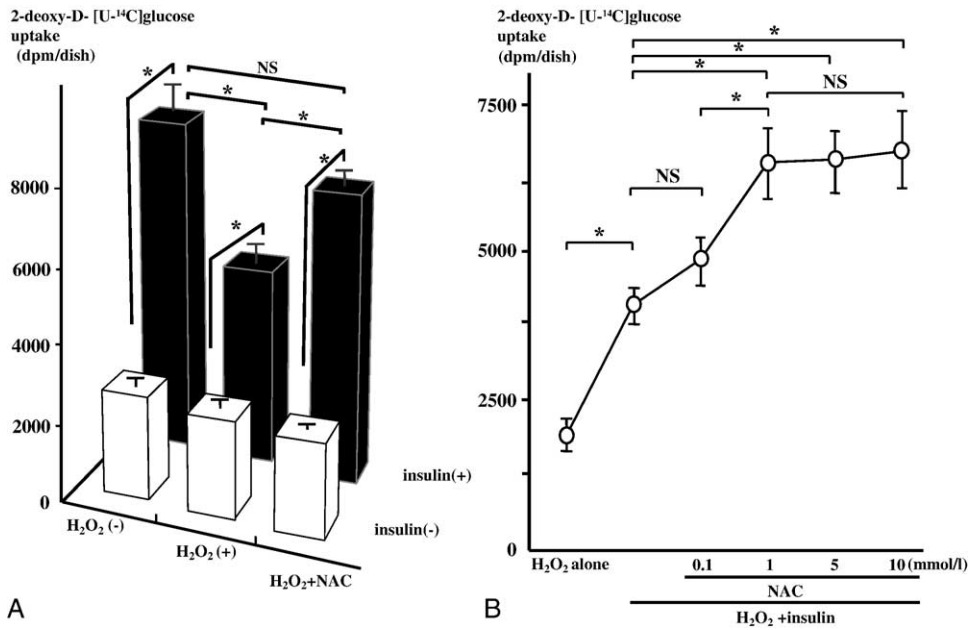

Fig. 3. A, Effect of NAC on the inhibition of insulin-induced glucose uptake by 100 μmol/L hydrogen peroxide. White and black bars indicate the conditions without and with 100 nmol/L insulin, respectively, under hydrogen peroxide alone or plus NAC. B, Dose-dependent recovery by NAC of the reduced insulin-induced glucose uptake under hydrogen peroxide. Values are means ± SEM (n = 6). *P < .01.

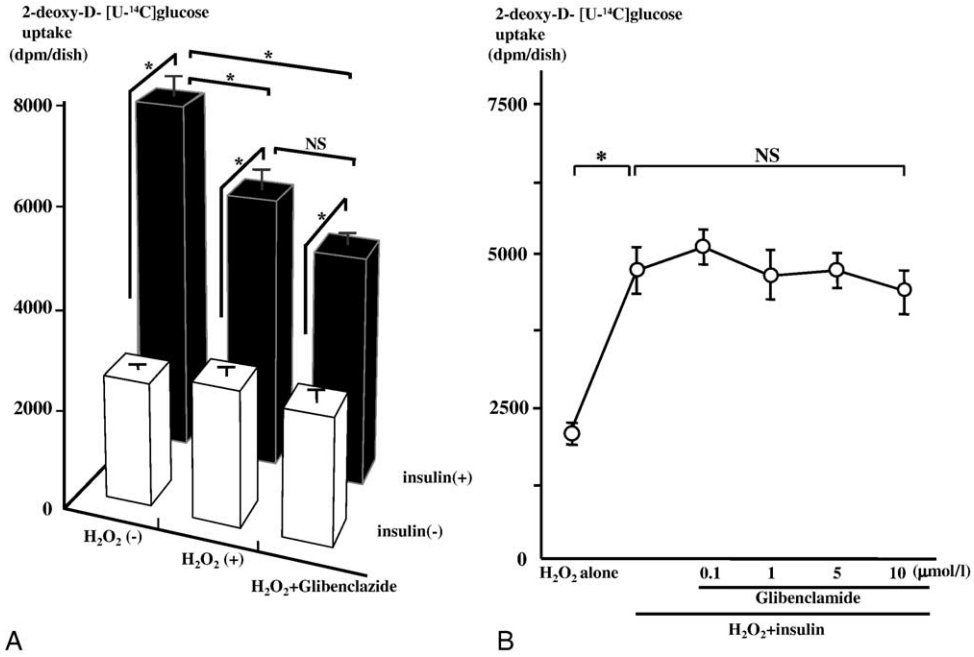

Fig. 4. A, Failure of glibenclamide to affect the inhibition of insulin-stimulated glucose uptake by 100 μmol/L hydrogen peroxide. White and black bars represent the conditions without and with 100 nmol/L insulin, respectively, under hydrogen peroxide alone or plus glibenclamide. B, Glibenclamide does not restore glucose uptake induced by insulin under hydrogen peroxide. Values are means ± SEM (n = 6). *P < .01.

additional control, no GLUT4 translocation occurred with gliclazide in the absence of insulin stimulation (Fig. 6B, upper panels). However, addition of 5 μmol/L gliclazide to cells treated with hydrogen peroxide and insulin clearly showed GLUT4 intracellular translocation (Fig. 6B, lower panels). Addition of 10 mmol/L NAC in the presence of hydrogen peroxide resulted in insulin-stimulated GLUT4 translocation similar to that seen with gliclazide (Fig. 6C). However, as shown in Fig. 6D, hydrogen peroxide treatment followed by 5 μmol/L glibenclamide did not cause any significant changes in GLUT4 distribution upon insulin stimulation.

The magnitude of GLUT4-eGFP translocation over time was calculated as the ratio of the peripheral/cellular fluorescence at the specified timepoints. As demonstrated in Fig. 7, no significant changes in GLUT4 translocation were seen in the presence of vehicle alone or 100 μmol/L hydrogen peroxide alone. Hydrogen peroxide followed by addition of 5 μmol/L gliclazide did not induce GLUT4 translocation in the absence of insulin. But a significant increase in insulin-induced translocation was observed in hydrogen peroxide- and gliclazide-treated cells by 4 minute after insulin stimulation. GLUT4 translocation was still significantly enhanced at 20 minutes, and values in the insulin-stimulated state were similar to those induced by insulin alone. Cells treated with hydrogen peroxide and 10 mmol/L NAC showed a similarly enhanced GLUT4 translocation at and beyond 4 minutes after insulin stimulation. However, no significant increase in insulin-stimulated GLUT4 translocation was visible in hydrogen peroxide–treated and 5 μmol/L glibenclamide-treated cells.

4. Discussion

Accelerated ROS formation has been recognized as a direct consequence of chronic hyperglycemia. Increased oxidative stress is thought to impair insulin secretion from

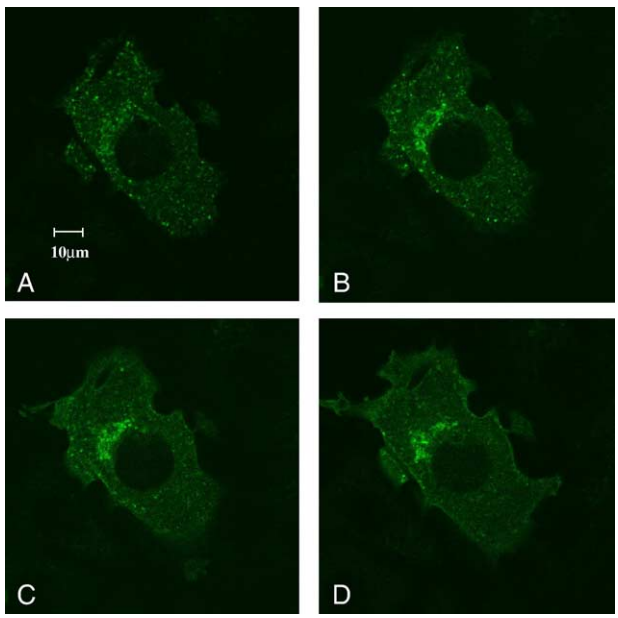

Fig. 5. Time course of GLUT4-eGFP translocation in 3T3L1 adipocytes under 100 nmol/L insulin stimulation. A, 0 minutes; B, 4 minutes; C, 10 minutes; D, 20 minutes. The apparent translocation was observed at the timepoint of 4 minutes and thereafter.

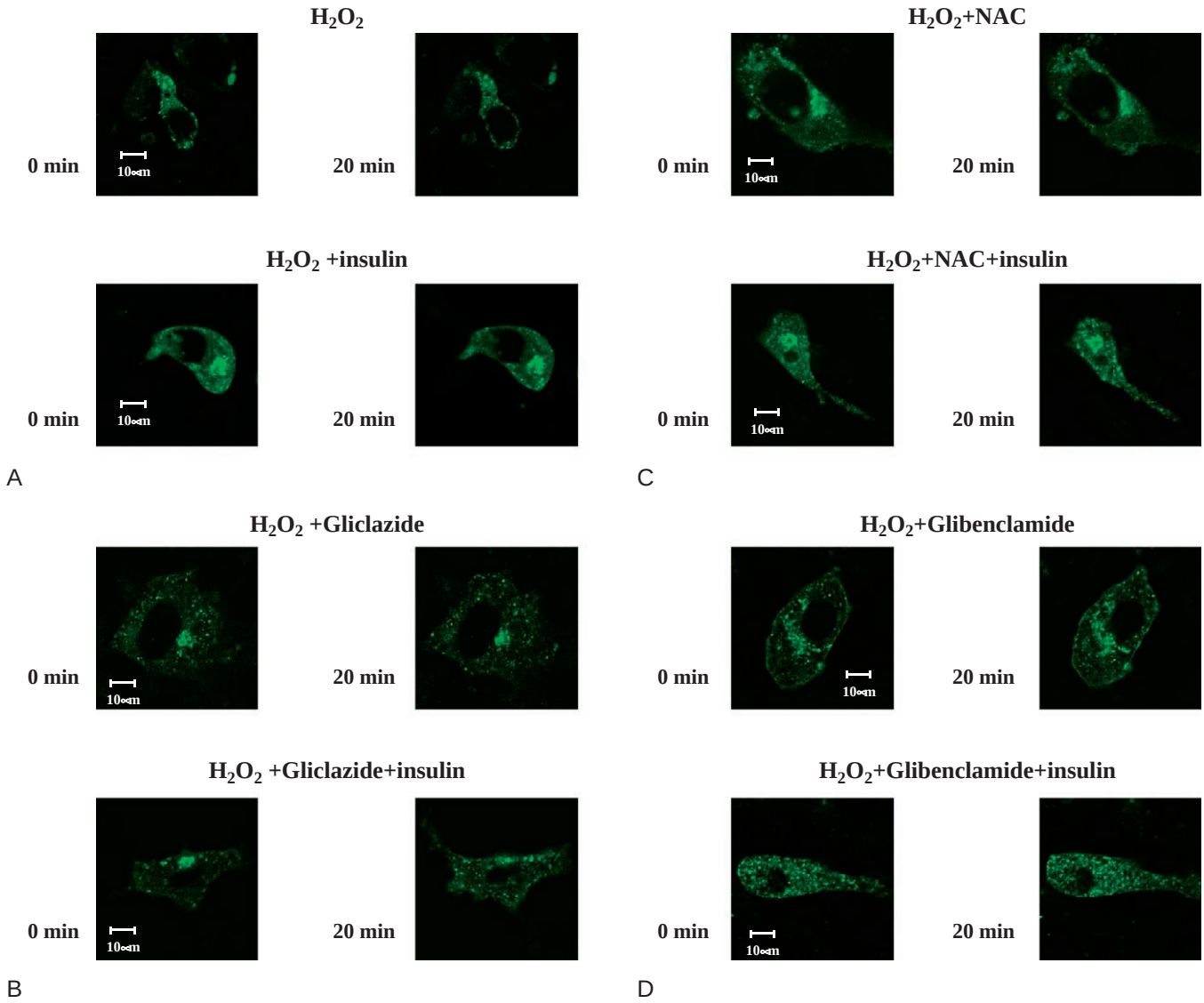

Fig. 6. Illustration of changes in insulin-stimulated GLUT4-eGFP localization with the designated test agents under hydrogen peroxide. A, Failure of 100 nmol/L insulin stimulation to induce significant GLUT4 translocation under hydrogen peroxide. B, GLUT4 translocation observed after insulin stimulation in the presence of 5 μmol/L gliclazide under hydrogen peroxide. C, Insulin-stimulated GLUT4 translocation shown with 10 mmol/L NAC even under hydrogen peroxide. D, No significant GLUT4 translocation occurred under 5 μmol/L glibenclamide plus hydrogen peroxide and insulin.

beta cells, worsen insulin resistance in target tissues, and possibly promote diabetic complications [4,6,19-21].

It has been reported that plasma free-radical concentrations correlate positively with fasting levels of plasma insulin and negatively with whole-body glucose utilization under diabetic condition [22]. In in vivo studies and animal models of type 2 diabetes mellitus, anti-oxidants have been shown to improve insulin sensitivity [23,24], as specially seen for α-lipoic acid (LA), an analog of octanoic acid. These findings are in accord with clinical trials which showed that treatment with LA, vitamin E, vitamin C, or glutathione improved insulin-mediated glucose disposal in insulin-resistant individuals and/or type 2 diabetic patients [25,26]. Overall, these studies support anti-oxidants as beneficial in type 2 diabetes mellitus therapy.

In several former studies [27-29], hydrogen peroxide was shown to acutely enhance glucose transport into adipocytes via putative mechanisms not involving the insulin receptor. Yet chronic exposure to micromolar hydrogen peroxide was recently observed to result in disruption of the insulin-stimulated IRS-1 and PI-3-kinase cellular redistribution in 3T3L1 adipocytes [10]. It has been shown in vitro that ROS and oxidative stress lead to the activation of multiple serine kinase cascades [30]. Multiple potential targets of serine phosphorylation exist in the insulin receptor and the IRS family of proteins. Increased serine phosphorylation of IRS-1 and IRS-2 has been proposed as a molecular basis for insulin resistance [31,32]. Serine phosphorylation of IRS proteins would inhibit their binding to the juxtamembrane region of the insulin receptor and also impair the ability of

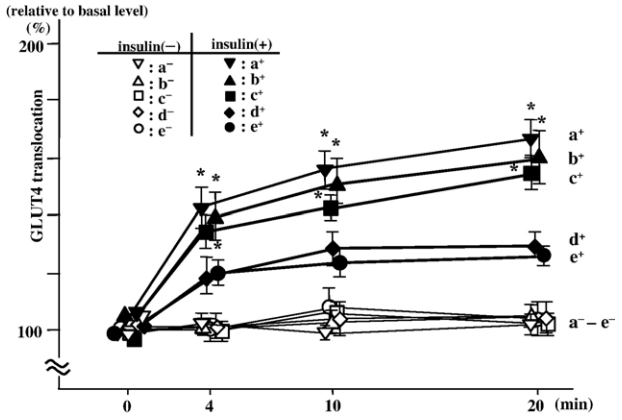

Fig. 7. Time course of GLUT4-eGFP translocation under insulin stimulation. White and negative superscripts (a^{-}-e^{-}) indicate conditions without insulin. Black and positive superscripts (a^{+}-e^{+}) indicate conditions with 100 nmol/L insulin. a, Without hydrogen peroxide or any agent; b, hydrogen peroxide plus gliclazide; c, hydrogen peroxide plus NAC; d, hydrogen peroxide plus glibenclamide; e, hydrogen peroxide alone. Values are means ± SEM (n = 4). *P < .01 vs e^{+}.

IRS proteins to undergo insulin-induced tyrosine phosphorylation. The involvement of c-Jun NH_2-terminal kinase/stress-activated protein kinases, p38 mitogen–activated protein kinases, inhibitory protein κB kinase β, and hexosamine has been suggested as an intervening mechanism of serine phosphorylation [30,33-35]. In L6 muscle cells, for example, hydrogen peroxide–mediated inhibition of insulin-stimulated glucose transport was accompanied by activation of p38 mitogen–activated protein kinase [34]. In addition, salicylate, an inhibitor of inhibitory protein κB kinase β, has been observed to restore insulin sensitivity in insulin target cells, possibly through decreasing serine phosphorylation of IRS proteins [36].

Gliclazide was previously shown to act as a general free-radical scavenger. An azabicyclo-octyl ring in this sulfonylurea drug, as pictured in Fig. 8, has been proposed to be responsible for its anti-oxidant property [14]. Hydrogen peroxide was used here as a trigger of oxidative stress because hydrogen peroxide is known to act physiologically during most oxidative processes. And we observed that gliclazide significantly, and dose-dependently, improves glucose uptake in 3T3L1 adipocytes in vitro; this improvement in glucose uptake resembled the effect seen for known anti-oxidant NAC. Notably, glibenclamide, which has a sulfonylurea moiety like gliclazide but does not contain the azabicyclo-octyl ring, failed to improve insulin resistance. This suggests a mechanism for gliclazide's reduction in insulin resistance that is also dependent upon anti-oxidant activity, as ascribable to its azabicyclo-octyl ring. We found that the reduction of GLUT4 translocation under oxidative stress was also improved by gliclazide. Therefore the improvement in glucose uptake into 3T3L1 adipocytes may be due, at least in part, to the increase in translocated GLUT4 in the plasma membrane. We speculate that gliclazide may restore GLUT4 translocation by reducing serine phosphorylation on target proteins such as PI-3-kinase and IRS-1, which would restore the insulin signaling. A precedent for this speculation regarding oxidative stress–sensitive molecules is found in the report that another anti-oxidant, LA, could protect against hydrogen peroxide–induced insulin resistance in 3T3L1 adipocytes, but did not prevent IRS-1 degradation nor serine phosphorylation [37]. We therefore hypothesize that the site of action of gliclazide for anti-oxidant activities is located at the putative site(s) distal to that for IRS-1/PI-3-kinase activation. Future studies analyzing the phosphorylation state of insulin-signaling proteins in response to oxidative stress, with or without gliclazide treatment, would help to address this speculation.

The concentrations of gliclazide we used here (1-10 μmol/L) are considered consistent with blood concentrations for this drug in clinical use [38]. Using our specified concentrations, we found that gliclazide alone did not stimulate glucose uptake in 3T3L1 adipocytes under basal conditions without added insulin. In contrast to our findings, Rodriguez et al [39] recently demonstrated that very high concentrations of gliclazide (300 μg/mL ≒ 930 μmol/L) elicited a direct stimulatory effect on glucose uptake by rat hindquarter skeletal muscle in vitro. Rodriguez et al further found that gliclazide's effect correlated with enhanced IRS-1/PI-3-kinase–associated activity. The precise reason for the discrepancy between findings in skeletal muscle and adipocytes is not clear. However, a chief consideration for these differences is the nearly 100-fold difference in gliclazide concentrations applied. This suggests cautious consideration of cell type and drug concentration in pursuing molecular target(s) involved in gliclazide's activity for any future pharmacological purposes.

In the present study, we demonstrated for the first time that sulfonylurea gliclazide can overcome insulin resistance induced by oxidative stress in cultured adipocytes. This beneficial effect appears independent of the insulinotropic action through gliclazide binding to high-affinity sulfonyl-

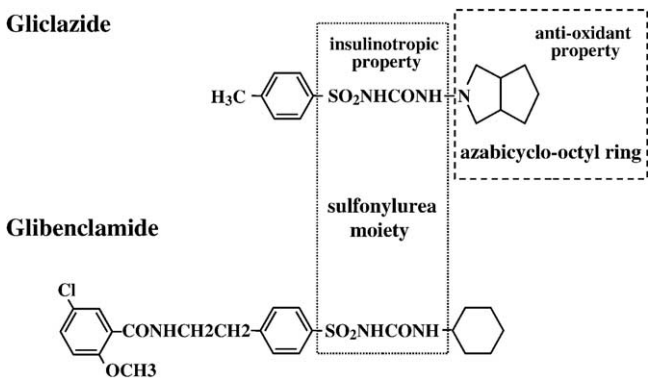

Fig. 8. Comparison of the chemical structure between gliclazide and glibenclamide. Both drugs possess the sulfonylurea moiety which provides an insulinotropic property. However, gliclazide additionally contains an azabicyclo-octyl ring, which has been suggested to be responsible for the anti-oxidant properties of gliclazide.

urea receptor of beta cells; sulfonylurea glibenclamide, which also exerts an insulinotropic effect, did not demonstrate anti-oxidant capacity. As improved glycemic control in vivo has been speculated to beneficially reduce oxidative stress in diabetic patients, the anti-oxidant activities of gliclazide derived from its azabicycro-octyl ring could add a pharmacological advantage to this drug. Gliclazide's ability to increase insulin sensitivity and GLUT4 translocation could further augment the hypoglycemic effect of released insulin from pancreatic beta cells and could, in turn, preserve better glycemic control in the treatment of type 2 diabetic patients by protecting beta cell function.

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