Inhibition of Glucose-Induced Insulin Secretion Through Inactivation of Glucokinase by Glyceraldehyde

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D-Glyceraldehyde irreversibly inhibited rat liver glucokinase in a concentration-dependent manner. The inactivation of glucokinase by glyceraldehyde was blocked by the presence of its substrates such as glucose and mannose. Glucokinase was highly sensitive to glyceraldehyde compared with some other glycolytic enzymes (from animal tissues) including hexokinase, glucose-6-phosphate isomerase, 6-phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase. The amino acid analysis of untreated and glyceraldehyde-treated glucokinase suggested that glyceraldehyde-induced inactivation of glucokinase is caused by glycation of Lys residues of the enzyme by the triose. Treatment of pancreatic islets with 6 mM glyceraldehyde for 1 h at 37°C caused both inactivation of glucokinase and inhibition of glucose-induced insulin secretion. Another glucose-phosphorylating enzyme (hexokinase) in pancreatic islets, however, was little affected by glyceraldehyde. In addition, glyceraldehyde did not affect the insulin secretory responses of islets to nonglucose secretagogues such as glyceraldehyde and Leu. When pancreatic islets were cultured with a lower concentration (1 mM) of glyceraldehyde for a longer time (17 h) in the presence of 10 mM glucose to mimic the in vivo conditions, both glucokinase activity and glucose-induced insulin secretion were again decreased. This study demonstrates that glucose-induced insulin secretion is impaired by glyceraldehyde through the inactivation of glucokinase. The implication of this finding in the pathophysiology of type II diabetes is discussed. *Diabetes* 42:1003–09, 1993

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Type II diabetes, non-insulin-dependent diabetes mellitus; DETAPAC, diethylenetriaminepentaacetic acid; G-6-P, glucose-6-phosphate; BSA, bovine serum albumin; FBS, fetal bovine serum; DTT, dithiothreitol; $[S]_{0.5}$, half-maximal saturation; n_H, Hill coefficient.

Glucokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) has been suggested as a key enzyme in the recognition of glucose as an insulin secretagogue in pancreatic islets (1–6). We recently reported that the inhibitory action of 2-cyclohexen-1-one on glucose-induced insulin secretion is exerted mainly, but not solely, by inactivation of glucokinase (7). During the course of that study, we found that the inactivation of glucokinase by 2-cyclohexen-1-one was augmented by the presence of D-glyceraldehyde, and that this augmentation was attributable to inactivation of glucokinase by glyceraldehyde. Because it is highly probable that glyceraldehyde is produced in pancreatic islets, we thought it would be meaningful to study the inactivation of the enzyme by the biological substance glyceraldehyde. In this study, we dealt with the properties, mechanism, and meaning of glucokinase inactivation effected by glyceraldehyde. Because liver glucokinase is quite similar to islet glucokinase in various properties (8) and is easy to obtain in large amounts, we used glucokinase purified from rat liver instead of rat islet glucokinase in this study.

RESEARCH DESIGN AND METHODS

D-Glyceraldehyde was purchased from Aldrich (Milwaukee, WI); catalase (EC 1.11.1.6) from Boehringer-Mannheim (Mannheim, Germany); DETAPAC from Tokyo Kasei (Tokyo, Japan). Dihydroxyacetone, D-threose, D-erythrose, monosaccharide phosphates, 6-phosphofructokinase (EC 2.7.1.11, from rabbit liver), methylglyoxalase (EC 4.4.1.5), and superoxide dismutase (EC 1.15.1.1, from bovine erythrocytes) were obtained from Sigma (St. Louis, MO). RPMI-1640 medium and FBS were obtained from Gibco (Grand Island, NY); glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12, from rabbit muscle) and pyruvate kinase (EC 2.7.1.40, from pig heart) from Oriental Yeast (Osaka, Japan).

Purification of enzymes. Liver glucokinase was purified as described previously (9). G-6-P isomerase (EC 5.3.1.9) was partially purified from rat skeletal muscle (Y.T., I.M., and J.O., unpublished observations). Hexokinase type I (EC 2.7.1.1) was partially purified from rat erythrocytes by ion-exchange chromatography on a column of DE-52 (Whatman, Maidstone, England) according to the method of Serafini et al. (10). Hexokinase type II (EC 2.7.1.1) from rat skeletal muscle was also prepared by use of a column of DE-52 by the method of Grossbard and Schimke (11).

Treatment of enzymes. Enzyme solutions (about 2 U/ml) were prepared with buffer A (pH 7.0) consisting of 20 mM KH_2PO_4, 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 5% (vol/vol) glycerol, and 1 mg/ml BSA. Aliquots (5 μl) of the enzyme solutions were mixed with 85 μl of buffer A supplemented, if necessary, with hexose, glutathione, superoxide dismutase, catalase, $CuCl_2$, $FeCl_3$, or DETAPAC. The mixture was preincubated for 1 min at 37°C, and incubation was started by addition of 10 μl of a solution containing glyceraldehyde (10–200 mM) or some other sugar (including monosaccharide phosphate; 100 mM). After incubation for the indicated period, 70 μl of the reaction mixture was taken out and immediately used for the assay of enzyme activity. Control experiments were conducted in parallel with test experiments by replacement of the solution of glyceraldehyde or some other sugar with buffer A.

Preparation of glyceraldehyde-treated glucokinase. Pure rat liver glucokinase (1 U, 3.4 μg) was incubated for 1 h at 37°C in 0.5 ml of buffer A containing 20 mM glyceraldehyde. The reaction mixture was applied to an Econo-Pac 10-DG column (Bio-Rad, Richmond, CA) previously equilibrated with buffer A. The glyceraldehyde-treated glucokinase was eluted at a rate of 0.5 ml/min, fractions of 0.5 ml were collected, and all of the fractions containing glucokinase activity were pooled.

Assays of enzymes. All spectrophotometric assays were performed in a total volume of 1 ml. The activity of liver glucokinase was determined as described previously (12). Because the K_m of glucokinase with respect to glucose was not altered by treatment of the enzyme with glyceraldehyde as described in RESULTS, glucokinase was generally assayed at a single glucose concentration (50 mM) whether treated or untreated with glyceraldehyde. The V_{max} of glucose phosphorylation by glucokinase and the K_m for glucose were determined by the Hanes-Woolf plot with five levels of glucose concentration over a range of 15–80 mM. The $[S]_{0.5}$ and n_H values were evaluated by the Hill plot with five levels of glucose concentration over a range of 5–20 mM. Hexokinase type I and hexokinase type II activities were measured by the glucokinase assay method, except that the glucose concentration was changed to 1 mM. G-6-P isomerase activity was determined with fructose-6-phosphate used as the substrate as described previously (13). The methods for the assay of 6-phosphofructokinase (14), glyceraldehyde-3-phosphate dehydrogenase (15), and pyruvate kinase (16) were described previously. Activities of glucokinase and hexokinase in pancreatic islets were assayed by fluorometry according to the method of Trus et al. (17).

Short-term incubation of islets. Pancreatic islets were isolated from fed female Wistar rats (weighing 300–350 g, Clea Japan, Tokyo, Japan) according to the method described previously (7). Batches of five islets were incubated for 1 h at 37°C in 1 ml of buffer B (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.2 mM $NaHCO_3$, 0.2% BSA, and 2.8 mM glucose [pH 7.4]) supplemented with 6 mM glyceraldehyde, washed with buffer B at room temperature, and then further incubated for 30 min at 37°C in 1 ml of buffer B in an attempt to eliminate the possible direct influence of glyceraldehyde on subsequent glucose-induced insulin secretion. The next and final step, incubation of islets for insulin secretion, was performed for 1 h at 37°C in 1 ml of buffer B supplemented with 20 mM glucose, 10 mM glyceraldehyde, or 15 mM Leu. Before this last incubation, some batches were used for the assay of glucokinase and hexokinase activities. All the incubations were performed in an atmosphere of 95% O_2/5% CO_2. In control experiments, the first incubation of islets was performed in the absence of glyceraldehyde. Insulin was assayed by an enzyme immunoassay with a kit from Mitsui (Tokyo, Japan), with human insulin as the standard.

Long-term incubation of islets. Groups of ~100 islets were cultured in 5 ml of RPMI-1640 medium containing 10 mM glucose, 2 mM Gln, 32 μM glutathione, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 10% FBS, and 1 mM glyceraldehyde. The culture was maintained for 17 h at 37°C in an atmosphere of 5% CO_2/95% O_2, after which the islets were incubated for 30 min at 37°C in buffer B and then assayed for glucose-phosphorylating activity and glucose-induced insulin secretion.

Amino acid analysis. Purified rat liver glucokinase (4 μg) was incubated for 2 h at 37°C in 1 ml of buffer A containing 5 or 20 mM glyceraldehyde. Glyceraldehyde-treated and untreated glucokinase was dialyzed exhaustively against distilled water and freeze-dried. Hydrolysis was performed in 6 M HCl for 24 h at 110°C in vacuo. Amino acid analysis of the hydrolyzate was conducted on an automatic amino acid analyzer (model 835, Hitachi, Tokyo, Japan). The analysis was performed in duplicate, and the data were expressed as the mean of two determinations. The number of each of the amino acids was calculated by assuming that Phe residues would not be altered by treatment with glyceraldehyde, and that the number of Phes per mole of glucokinase would be 18 (18).

Protein assay. Protein was assayed by the Bradford method with a kit from Bio-Rad and BSA as a standard.

Statistical analysis. The unpaired Student's t test was used to evaluate the statistical significance of the data. $P < 0.05$ was considered significant.

RESULTS

Glyceraldehyde-induced decrease of glucokinase activity was time dependent and also concentration dependent (Fig. 1). The curves in Fig. 1 show that the decrease

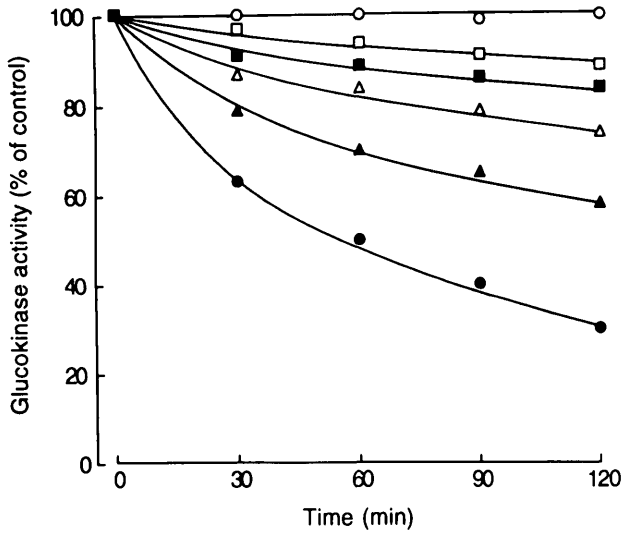

FIG. 1. Time courses of inactivation of glucokinase by glyceraldehyde. Liver glucokinase was incubated for various periods at 37°C without (○) or with glyceraldehyde (□, 1 mM; ■, 2.5 mM; △, 5 mM; ▲, 10 mM; ●, 20 mM) and then assayed for enzyme activity. Data are means of three experiments.

in activity was enhanced with time at any glyceraldehyde concentration, even when the incubation was continued over 2 h.

Glucokinase activity measured just after incubation with glyceraldehyde (20 mM) for 1 h at 37°C was not different from that measured after gel filtration (data not shown). And incubation of glyceraldehyde-treated glucokinase with 5 mM glutathione for 1 h at 37°C did not result in recovery of the activity (data not shown).

The kinetic characteristics of glyceraldehyde-treated glucokinase were compared with those of untreated enzymes (Table 1). Glucokinase was incubated with 20 mM glyceraldehyde for 1 h at 37°C and then assayed with various glucose concentrations (5–80 mM). The V_{max} of treated glucokinase was reduced to 50% of that of untreated enzyme, whereas other characteristics (glucose K_m, $[S]_{0.5}$, and n_H) were not significantly different between treated and untreated glucokinase.

The abilities of various hexoses to prevent the glyceraldehyde-induced inactivation of glucokinase were examined by incubation of the enzyme with 5 mM glyceraldehyde in the presence of 20 and 100 mM hexose (Table 2). Of the hexoses used, glucose and mannose are substrates of glucokinase, whereas 3-*O*-methylglucose and galactose are neither substrates nor inhibitors. The inactivation of glucokinase by glyceraldehyde was significantly attenuated by glucose and mannose but not by 3-*O*-methylglucose and galactose. A high concentration of glucose (100 mM; 14 times the K_m of glucokinase) completely protected the enzyme from inactivation by glyceraldehyde. The protection by glucose against glucokinase inactivation by glyceraldehyde was concentration dependent up to at least 20 mM (Fig. 2). Note that the detrimental action of glyceraldehyde was protected by only 50–55%, even in the presence of 20 mM glucose.

Unlike glucokinase, such glycolytic enzymes as glyceraldehyde-3-phosphate dehydrogenase and pyruvate

TABLE 1
Kinetic characteristics of glyceraldehyde-treated and untreated glucokinase

	Glucokinase	
	Untreated	Glyceraldehyde-treated
V_{max} (mol · g enzyme^{-1} · h^{-1})	19.5 ± 0.2	9.8 ± 0.3*
K_m (mM)	5.3 ± 0.4	5.8 ± 0.3
$[S]_{0.5}$ (mM)	7.7 ± 0.3	7.9 ± 0.2
n_H	1.50 ± 0.02	1.45 ± 0.01

Data are means ± SD of 5 experiments. Liver glucokinase was treated with glyceraldehyde (20 mM) for 1 h at 37°C and then assayed for the enzyme activity with various concentrations (5–80 mM) of glucose.
*$P < 0.001$ vs. untreated glucokinase.

TABLE 2
Effect of hexoses of glyceraldehyde-induced inactivation of glucokinase

Treatment	Glucokinase activity (% of control)
Control	100 ± 2
Glyceraldehyde	68 ± 3
Plus 20 mM glucose	88 ± 1*
Plus 100 mM glucose	101 ± 3*
Plus 20 mM mannose	80 ± 3*
Plus 100 mM mannose	90 ± 2*
Plus 20 mM 3-*O*-methylglucose	68 ± 2
Plus 100 mM 3-*O*-methylglucose	68 ± 1
Plus 20 mM galactose	67 ± 1
Plus 100 mM galactose	69 ± 2

Data are means ± SD of 5 experiments. Liver glucokinase was incubated with glyceraldehyde (5 mM) for 3 h at 37°C in the presence or absence of hexose (20 and 100 mM) and then assayed for the enzyme activity.
*$P < 0.001$ vs. glyceraldehyde alone.

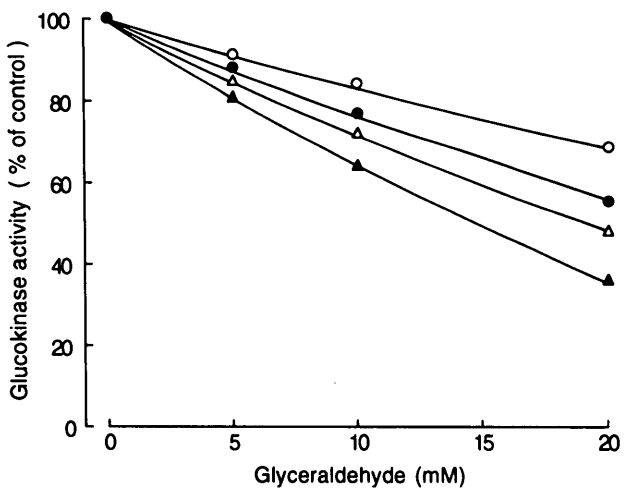

FIG. 2. Effect of glucose concentration on glyceraldehyde-induced inactivation of glucokinase. Liver glucokinase was incubated for 1 h at 37°C with indicated concentrations of glyceraldehyde in the absence (▲) or presence of glucose (△, 5 mM; ●, 10 mM; ○, 20 mM) and then assayed for enzyme activity. Data are means of three experiments.

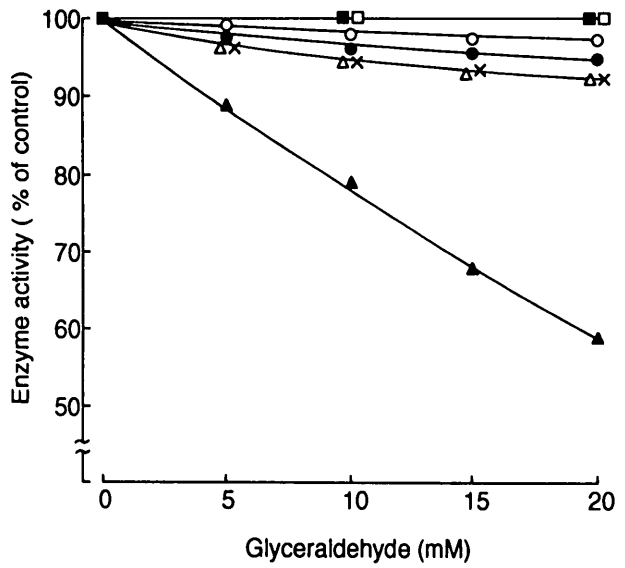

FIG. 3. Effect of glyceraldehyde concentration on activities of glycolytic enzymes. Each enzyme was incubated with indicated concentrations of glyceraldehyde for 30 min at 37°C, and the residual enzyme activity was measured. Data are means of three experiments. (■), Pyruvate kinase; (□), glyceraldehyde-3-phosphate dehydrogenase; (○), hexokinase type I; (●), hexokinase type II; (△), G-6-P isomerase; (X), 6-phosphofructokinase; (▲), liver glucokinase.

kinase were not inactivated at all by glyceraldehyde; and hexokinase type I, hexokinase type II, G-6-P isomerase, and 6-phosphofructokinase were only slightly altered by it (Fig. 3).

Scavengers and inducers of activated oxygen species did not affect the inactivation of glucokinase by glyceraldehyde (data not shown). The compounds tested included superoxide dismutase (1000 U/ml), catalase (100 U/ml), DETAPAC (1 mM), glutathione (5 mM), DTT (5 mM), $CuCl_2$ (100 μM), and $FeCl_3$ (100 μM).

We examined whether liver glucokinase would be inactivated when incubated with monosaccharides and monosaccharide phosphates. Of 14 compounds (10 mM) tested, D-threose, D-erythrose, and D-glyceraldehyde-3-phosphate decreased the enzyme activity by 27, 18, and 25%, respectively, when incubated with the enzyme for 2 h at 37°C (Table 3). By comparison, D-glyceraldehyde decreased glucokinase activity by 43% under the same conditions (Table 3). With regard to glucokinase inactivation by glyceraldehyde-3-phosphate, methylglyoxal, which is the degradation product of glyceraldehyde-3-phosphate (19), might also participate in the inactivation of glucokinase. In fact, when glucokinase was incubated with glyceraldehyde-3-phosphate in the presence of 10 mM glutathione and 20 U/ml methylglyoxalase to convert methylglyoxal to S-lactoylglutathione, the inhibition value was reduced to 12%.

The amino acid analysis revealed that only the Lys content was lower in glyceraldehyde-treated glucokinase than in untreated enzyme, and that a more marked decrease in the Lys content was observed when glucokinase was treated with a higher concentration (5 vs. 20 mM) of glyceraldehyde (Table 4). The contents of some amino acids (e.g., Thr, Ser, Val, Met, and Ile) observed

TABLE 3
Effect of monosaccharides and monosaccharide phosphates on glucokinase activity

Treatment	Glucokinase activity (% of control)
Control	100
D-Glyceraldehyde	57
Dihydroxyacetone	100
D-Threose	73
D-Erythrose	82
D-Ribose	100
D-Xylose	100
D-Glucose	100
D-Mannose	100
D-Fructose	100
D-Glyceraldehyde-3-phosphate	75
Dihydroxyacetone phosphate	100
D-Ribose-5-phosphate	100
D-G-6-P	100
D-Fructose-6-phosphate	100
D-Fructose-1,6-bisphosphate	100

Data are means of 3 experiments. Liver glucokinase was incubated with monosaccharide or monosaccharide phosphate (10 mM) for 2 h at 37°C and then assayed for the enzyme activity.

for untreated glucokinase were lower than those deduced from cloned cDNA for rat liver glucokinase (18) for various reasons related to the acid hydrolysis: decomposition (Thr and Ser), insufficient hydrolysis (Val and Ile), and oxidation (Met).

Treatment of pancreatic islets with 6 mM glyceraldehyde for 1 h at 37°C in the presence of 2.8 mM glucose attenuated both glucokinase activity and glucose-induced insulin secretion (Table 5). Another glucose-phosphorylating enzyme (hexokinase), however, was

TABLE 4
The amino acid composition of glyceraldehyde-treated and untreated glucokinase

Amino acid	Untreated glucokinase	Glyceraldehyde-treated glucokinase		Reported value*
		5 mM	20 mM	
Asp + Asn	40.0	39.8	39.6	41
Thr	22.1	22.0	21.9	24
Ser	26.6	26.3	26.2	32
Glu + Gln	57.2	58.5	57.3	61
Pro	8.8	9.0	8.7	9
Gly	40.0	39.8	39.2	40
Ala	28.1	27.8	27.7	29
Val	34.4	33.9	33.6	37
Met	20.2	19.5	20.2	24
Ile	15.9	15.9	15.8	18
Leu	41.1	41.0	40.7	43
Tyr	9.7	9.6	9.4	10
Phe	18.0	18.0	18.0	18
Lys	23.9	22.0	17.9	23
His	10.4	10.3	11.0	10
Arg	29.1	28.3	28.8	30

Liver glucokinase was treated for 2 h at 37°C without or with glyceraldehyde (5 or 20 mM) and then analyzed for its amino acid composition.
*Reported previously (18).

TABLE 5
Short-term effect of a high glyceraldehyde concentration on glucose-phosphorylating activity and insulin secretion in pancreatic islets

Treatment	Glucose-phosphorylating activity (pmol · 90 min^{-1} · islet^{-1})		Insulin secretion (pmol · h^{-1} · islet^{-1})		
	Glucokinase activity	Hexokinase activity	Secretion by glucose	Secretion by glyceraldehyde	Secretion by leucine
Control	68 ± 4	63 ± 4	1.36 ± 0.21	0.49 ± 0.10	0.52 ± 0.12
Glyceraldehyde	57 ± 3*	65 ± 5	1.05 ± 0.22*	0.49 ± 0.15	0.49 ± 0.08

Data are means ± SD of 5–7 and 10–12 experiments for glucose-phosphorylating activity and insulin secretion, respectively. Pancreatic islets were incubated with 6 mM glyceraldehyde for 1 h at 37°C in the presence of 2.8 mM glucose. Then, after the islets had been incubated for 30 min at 37°C in the absence of glyceraldehyde, they were analyzed for glucose-phosphorylating activity and insulin secretion induced by 20 mM glucose, 10 mM glyceraldehyde, or 15 mM Leu. Insulin secretion is expressed by subtraction of the basal insulin secretion (0.19 and 0.27 pmol · h^{-1} · islet^{-1} in control and glyceraldehyde-treated islets, respectively) from the observed insulin secretion.
*$P < 0.001$ vs. control.

little affected by glyceraldehyde. Neither glyceraldehyde- nor Leu-induced insulin secretion in pancreatic islets was affected by the glyceraldehyde treatment (Table 5). When pancreatic islets were incubated with a lower concentration (1 mM) of glyceraldehyde for a longer time (17 h) in the presence of 10 mM glucose to mimic the in vivo conditions, both glucokinase activity and glucose-induced insulin secretion were again decreased (Table 6).

DISCUSSION

In the first set of experiments (Fig. 1), we confirmed our preliminary observation (7) that glucokinase activity is decreased by glyceraldehyde. The failure of gel filtration to relieve glucokinase inhibition by glyceraldehyde indicates that once the inhibition is caused, it is not released in the absence of glyceraldehyde, i.e., the inhibition is irreversible. The data on protection of glucokinase by various hexoses from the action of glyceraldehyde suggest that the key interaction of glucokinase with glyceraldehyde occurs at or near the active site of the enzyme. The action of glyceraldehyde on glucokinase was found to be appreciably selective, although not specific, because glyceraldehyde little or slightly inactivated other glycolytic enzymes such as hexokinase type I, hexokinase type II, G-6-P isomerase, 6-phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase.

Monosaccharides including glyceraldehyde can be autoxidized, and the autoxidation is a transition metal-catalyzed process that generates α-ketoaldehydes and activated oxygen species (20,21). The α-ketoaldehydes and the activated oxygen species formed by autoxidation have been proposed to contribute to the covalent attachment of monosaccharide to protein (20,22) and to cause protein cleavage (22), respectively. It seemed possible, therefore, that glyceraldehyde might exert its inhibitory action on glucokinase through autoxidation. In fact, the autoxidation of glyceraldehyde was reported to result in the inactivation of erythrocyte ATPase activities (23). This view, however, is not consistent with our findings that scavengers of oxygen radicals (superoxide dismutase, catalase, glutathione, and DTT), autoxidation-accelating agents ($CuCl_2$ and $FeCl_3$), and a metal-chelating agent (DETAPAC) did not affect the inactivation of glucokinase by glyceraldehyde. Moreover, our observation of no inactivation of glucokinase by dihydroxyacetone, which has a higher susceptibility to autoxidation than glyceraldehyde (21), does not suggest any relevance of autoxidation to glucokinase inactivation. In addition, the fact that glucokinase inactivation by glyceraldehyde was not released by incubation with glutathione may exclude the

TABLE 6
Long-term effect of a low glyceraldehyde concentration on glucose-phosphorylating activity and insulin secretion in pancreatic islets

Treatment	Glucose-phosphorylating activity (pmol · 90 min^{-1} · islet^{-1})		Glucose-induced insulin secretion (pmol · h^{-1} · islet^{-1})
	Glucokinase activity	Hexokinase activity	
Control	56 ± 3	69 ± 4	1.20 ± 0.20
Glyceraldehyde	40 ± 6*	72 ± 5	0.82 ± 0.19*

Data are means ± SD of 7 and 18 experiments for glucose-phosphorylating activity and insulin secretion, respectively. Pancreatic islets were cultured with 1 mM glyceraldehyde for 17 h at 37°C in the presence of 10 mM glucose. After the islets had been subsequently incubated for 30 min at 37°C in the absence of glyceraldehyde, they were analyzed for glucose-phosphorylating activity and insulin secretion induced by 20 mM glucose. Insulin secretion is expressed by subtraction of the basal insulin secretion (0.18 and 0.22 pmol · h^{-1} · islet^{-1} in control and glyceraldehyde-treated islets, respectively) from the observed secretion.
*$P < 0.001$ vs. control.

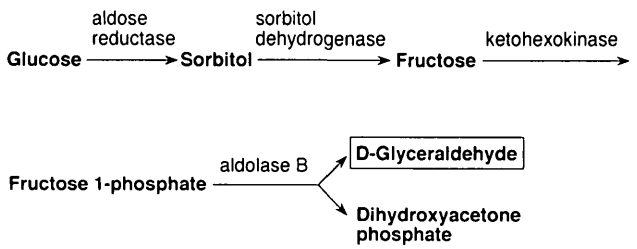

FIG. 4. Possible pathway of D-glyceraldehyde formation in pancreatic islets.

participation of disulfide-bond formation in the glucokinase inactivation. On the other hand, the data in Table 4 strongly suggest that glucokinase is inactivated by glyceraldehyde through glycation of Lys residues of the enzyme.

Glucokinase activity and glucose-induced insulin secretion in pancreatic islets were significantly decreased when islets were incubated with 6 mM glyceraldehyde for 1 h at 37°C (Table 5). Many works suggest that glucokinase is a regulatory step for glucose-induced insulin secretion (1–7). It is conceivable, therefore, that the impairment of glucose-induced insulin secretion by glyceraldehyde may be attributable to the inactivation of glucokinase. MacDonald et al. (24) recently reported that glyceraldehyde exerts a cytotoxic effect on islet β-cells by causing a decrease in the NAD/NADH ratio, the depletion of ATP, and the accumulation of acidic products, i.e., glyceric acid and glycerate-1-phosphate. However, involvement of such toxicity in glyceraldehyde inhibition of insulinotropism is questionable, because glyceraldehyde attenuated only glucose-induced insulin secretion without affecting glyceraldehyde- and Leu-induced insulin secretion (Table 5); that is, if the glyceraldehyde inhibition of insulinotropism would be attributable to cytotoxicity, the insulin secretory response of islet β-cells would be impaired to some degree regardless of the secretagogue.

No paper has hitherto reported on the content of D-glyceraldehyde in pancreatic islets. Only one publication (25) has reported the tissue content of D-glyceraldehyde as far as we know: the amount of D-glyceraldehyde in cow and rabbit lenses was reported to be 4–15 nmol/g wet weight. It is possible, however, that glyceraldehyde is produced from glucose in islet β-cells by the pathway shown in Fig. 4, because all of the enzymes in the pathway have been reported to exist in pancreatic islets. The polyol pathway, which consists of aldose reductase (EC 1.1.1.21) and sorbitol dehydrogenase (EC 1.1.1.14), can catalyze the conversion of glucose to fructose via sorbitol (26). Fructose is phosphorylated by ketohexokinase (EC 2.7.1.3) to produce fructose-1-phosphate (27). And, the phosphohexose has been suggested to be split by aldolase B (EC 4.1.2.13) to D-glyceraldehyde and dihydroxyacetone phosphate (27). We are now developing a microassay method for tissue D-glyceraldehyde.

It is widely accepted that fructose formation through the polyol pathway is increased in many tissues in the diabetic state compared with the normal state (28). Thus, the islet glyceraldehyde content would be increased in the diabetic state, if glyceraldehyde would be produced by the above-mentioned pathway. The pancreatic β-cell desensitization to glucose has been reported to be induced by chronic hyperglycemia (29–32). These considerations suggest the possibility that the inactivation of glucokinase by glyceraldehyde may contribute to the β-cell dysfunction in situations of chronic hyperglycemia. With regard to this matter, note that both glucokinase activity and glucose-induced insulin secretion were significantly decreased when islets were incubated with a relatively low concentration (1 mM) of glyceraldehyde for a long time (17 h) in the presence of glucose (10 mM), a compound protective against glyceraldehyde action. Some investigators recently reported that culturing of isolated pancreatic islets in high glucose for prolonged periods caused the induction of both glucokinase activity and glucose-stimulated insulin release (33,34). However, their results do not exclude the possibility that not only the induction of glucokinase activity, but also the inactivation of glucokinase by glyceraldehyde, took place during the culture period. It remains to be solved whether glyceraldehyde formation participates in the detrimental effect of chronic hyperglycemia on glucose-induced insulin secretion.

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