J. Biochem. 127, 289–295 (2000)

Difference in Mechanism between Glyceraldehyde- and Glucose-Induced Insulin Secretion from Isolated Rat Pancreatic Islets[1]

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Received November 5, 1999; accepted November 17, 1999

The effects of D-glyceraldehyde and glucose on islet function were compared in order to investigate the difference between them in the mechanism by which they induce insulin secretion. The stimulation of insulin secretion from isolated rat islets by 10 mM glyceraldehyde was not completely inhibited by either 150 μM diazoxide (an opener of ATP-sensitive K^+ channels) or 5 μM nitrendipine (an L-type Ca^{2+}-channel blocker), whereas the stimulation of insulin secretion by 20 mM glucose was completely inhibited by either drug. The insulin secretion induced by glyceraldehyde was less augmented by 100 μM carbachol (a cholinergic agonist) than that induced by glucose. The stimulation of myo-inositol phosphate production by 100 μM carbachol was more marked in islets incubated with the hexose than with the triose. The content of glyceraldehyde 3-phosphate, a glycolytic intermediate, in islets incubated with glyceraldehyde was far higher than that in islets incubated with glucose, whereas the ATP content in islets incubated with the triose was significantly lower than that in islets incubated with the hexose. These results suggest that glyceraldehyde not only mimics the effect of glucose on insulin secretion but also has the ability to cause the secretion of insulin without the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. The reason for the lower potency of the triose than the hexose in stimulating insulin secretion is also discussed.

Key words: glucose, glyceraldehyde, glycolytic intermediates, insulin secretion, metabolism, pancreatic islet.

D-Glyceraldehyde is a potent insulin secretagogue that is believed to stimulate insulin secretion *via* its metabolism in pancreatic islets (*1–8*). This triose has been suggested to enter the glycolytic pathway *via* its phosphorylation to glyceraldehyde 3-phosphate (GAP) and subsequently to follow the same fate as glucose, thus mimicking the effect of glucose on islet β-cells (*4*, *8*, *9*). The regulation of insulin secretion by glucose, though not yet precisely clear, is believed to involve two major mechanisms. The best characterized pathway serves to increase the cytoplasmic concentration of Ca^{2+} in β-cells through the following sequence of events (*10*, *11*): metabolism of glucose leads to an increase in the ATP/ADP ratio and closure of ATP-sensitive K^+ (K^+_{ATP}) channels, resulting in depolarization of the plasma membrane and influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. The other mechanism involves a K^+_{ATP} channel–independent pathway (*12*, *13*): glucose enhances the effect of increased intracellular Ca^{2+} concentrations on insulin exocytosis at a distal site in stimulus-secretion coupling.

MacDonald (*14*) reported that, besides stimulating insulin release in islets *via* entering metabolism through its phosphorylation by triokinase, glyceraldehyde may also be phosphorylated directly by the GAPDH reaction to form glycerate 1-phosphate, which is probably unmetabolizable in islets. He suggested that an increased NADH/NAD ratio induced by the second reaction would have an important implication in the insulinotropic action of glyceraldehyde.

More recently, Elliott *et al.* (*15*, *16*) proposed that the stimulation of insulin secretion from HIT-T15 hamster insulinoma cells by glyceraldehyde did not require metabolism of the triose within the cell and that the electrogenic transport of the triose across the plasma membrane, possibly *via* H^+ cotransport, might lead to depolarization and hence to Ca^{2+} entry into the cell.

The mechanism of glyceraldehyde-induced insulin secretion is thus disputable, and the difference in mechanism between glyceraldehyde- and glucose-induced insulin secretion is not yet clear. In this study, we investigated the effect of diazoxide (an opener of K^+_{ATP} channels; *12*, *13*), carbachol (an agonist of the muscarinic receptor; *17*, *18*), and nitrendipine (an L-type Ca^{2+}-channel blocker; *19*, *20*) on glyceraldehyde- and glucose-induced insulin secretion from rat islets. In addition, we measured the contents of glycolytic intermediates, inositol phosphates, and ATP and the pro-

[1] This work was supported in part by a Grant-in-Aid (to I.M.) from the High-Tech Research Center Project of the Ministry of Education, Science, Sports, and Culture of Japan.

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Abbreviations: BSA, bovine serum albumin; DHAP, dihydroxyacetone phosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; GAP, glyceraldehyde 3-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6P, glucose 6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; K^+_{ATP} channel, ATP-sensitive K^+ channel; KRB buffer, Krebs-Ringer bicarbonate buffer.

duction of lactate and pyruvate in islets incubated with glyceraldehyde or glucose.

MATERIALS AND METHODS

Materials—Diazoxide, carbachol, D-glyceraldehyde, DL-glyceraldehyde 3-phosphate, and auxiliary enzymes used for the measurement of glycolytic intermediates, lactate and pyruvate production, and triose-phosphate isomerase activity were purchased from Sigma (St. Louis, MO, USA). RPMI 1640 and fetal bovine serum were obtained from Gibco (Grand Island, NY, USA). An ATP assay kit, nitrendipine, and collagenase were purchased from Wako (Osaka).

Measurement of Glycolytic Intermediates—Pancreatic islets from female Wistar rats weighing 270–320 g (Clea Japan, Tokyo) were isolated by a collagenase digestion method (*21*). Batches of 30–50 islets were preincubated for 30 min at 37°C in an atmosphere of 5% CO_2/95% O_2 in 5 ml of preincubation medium, *i.e.*, RPMI 1640 containing 10% fetal bovine serum and 32 μM glutathione together with 2.8 mM glucose, then incubated for 10 or 30 min in 5 ml of incubation medium (RPMI 1640 supplemented with 10% fetal bovine serum and 32 μM glutathione together with 10 mM glyceraldehyde or 20 mM glucose). After removal of the medium, the islets were quickly washed with ice-cold Krebs-Ringer bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 24.2 mM $NaHCO_3$; pH 7.4; KRB buffer), then sonicated in 70 μl of 0.4 M $HClO_4$.

The sonicate was centrifuged, and the supernatant was neutralized with 200 mM Hepes-NaOH buffer (pH 7.0) and 1 M K_2CO_3. After centrifugation of the neutralized solution, the supernatant obtained was analyzed for glycolytic intermediates. The intermediates were assayed using an enzymatic cycling system for NADH amplification (*22*). The assay of glucose 6-phosphate (G6P), as an example, was performed as follows: The first-step reaction was conducted in 50 mM Hepes-NaOH buffer (pH 8.0) containing 0.01 mM NAD, 0.2 mM EDTA, 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin (BSA), 1 unit/ml glucose-6-phosphate dehydrogenase (G6PDH), and islet extract (10 μl) in a total volume of 27 μl. After incubation for 25 min at 30°C, 3 μl of 1 M NaOH was added to stop the reaction, and the mixture was incubated for 20 min at 60°C to destroy any remaining NAD. In the second step (amplification step for NADH), the reaction mixture containing 30 μl of sample solution from the first step, 100 mM Hepes-NaOH buffer (pH 7.5), 1 mM dithiothreitol, 0.2 mg/ml BSA, 280 mM ethanol, 2 mM oxaloacetate, 16 units/ml alcohol dehydrogenase, and 4 units/ml malate dehydrogenase in a total volume of 100 μl was incubated for 30 min at 30°C. The reaction was stopped by heating the mixture at 100°C for 3.5 min. In the third step, malate formed in the second step was assayed by incubating 100 μl of sample solution in 50 mM 2-amino-2-methyl-1-propanol-HCl buffer (pH 9.9) containing 0.18 mM NAD, 0.1 mg/ml BSA, 9 mM glutamate, 0.8 unit/ml malate dehydrogenase, and 1 unit/ml aspartate transaminase in a total volume of 600 μl for 25 min at 30°C. The NADH formed was then measured by fluorometry at 340 nm excitation and 450 nm emission. The islet G6P content was obtained from a calibration curve made with standard G6P (0.5–4 pmol). G6PDH in the first-step reaction was omitted for the measurement of a blank value.

For the assay of fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (FBP), dihydroxyacetone phosphate (DHAP), and GAP, the following enzymes in addition to the appropriate substrates were used in the respective first-step reactions: 1 unit/ml G6PDH and 1 unit/ml glucose-6-phosphate isomerase for F6P; 0.1 unit/ml GAPDH and 0.5 unit/ml aldolase for FBP; 0.1 unit/ml GAPDH and 1 unit/ml triose-phosphate isomerase for DHAP; and 0.1 unit/ml GAPDH for GAP.

Measurement of Insulin Secretion—Batches of four islets were preincubated for 30 min in 1 ml of preincubation medium (described above), then incubated for 30 min in 1 ml of incubation medium (described above). In some experiments, 150 μM diazoxide was present during both preincubation and incubation. Carbachol (100 μM) or nitrendipine (5 μM) was present during incubation in some experiments. Nitrendipine was dissolved in dimethylsulfoxide and diluted to a final solvent concentration of 0.01% (v/v). Insulin in media obtained after the incubation was measured by an enzyme immunoassay with a commercial kit.

Measurement of Inositol Phosphates—The inositol phosphate contents in islets were measured as already described (*23*). Briefly, batches of 120–150 islets were preincubated for 150 min in an atmosphere of 5% CO_2/95% O_2 at 37°C in silicone-treated borosilicate tubes (12 × 75 mm) in 0.8 ml of KRB buffer supplemented with 2 mg/ml BSA, 2.8 mM glucose, and 16 μCi myo-[2-^{3}H]inositol. After preincubation, the medium was removed, and the labeled islets were washed five times with 1 ml of KRB buffer containing 2.8 mM glucose, 2 mg/ml BSA, 10 mM LiCl, and 1 mM unlabeled myo-inositol. The prelabeled islets were incubated for 30 min in 0.5 ml of incubation medium (described above) containing 10 mM LiCl and 1 mM unlabeled myo-inositol. In some experiments, 100 μM carbachol was present during incubation. Incubations were terminated by the addition of 1.5 ml of chloroform–methanol–HCl (200:100:1, v/v/v). The mixtures were shaken, then centrifuged to separate the two phases. Free myo-inositiol and inositol phosphates in the upper, aqueous phase were eluted in a stepwise manner by anion-exchange chromatography, and the ^{3}H radioactivity in fractions was counted after addition of 10 ml of organic counting scintillant.

Measurement of Lactate and Pyruvate Production—Batches of 10–20 islets were preincubated for 30 min in 1 ml of KRB buffer containing 2.8 mM glucose and 2 mg/ml BSA, then incubated for 10 or 30 min in 1 ml of KRB buffer supplemented with 10 mM glyceraldehyde or 2.8 or 20 mM glucose together with 2 mg/ml BSA. Lactate in media was measured as described previously (*24*). RPMI 1640 supplemented with 10% fetal bovine serum was not used as a medium in this experiment, since the fetal bovine serum itself contains lactate and pyruvate.

For the assay of pyruvate in media, the reaction mixture (400 μl) containing 100 mM Tris-HCl buffer (pH 8.0), 1 mM $MgSO_4$, 0.5 mM EDTA, 1 mM dithiothreitol, 0.5 mM NAD, 0.15 mM CoA, 1 mM thiamine pyrophosphate, 0.1 U/ml pyruvate dehydrogenase, and sample solution (250 μl) was incubated for 20 min at 30°C. Fluorescence of NADH was then measured at excitation and emission wavelengths of 340 and 450 nm, respectively. Islet-free media instead of sample solutions were used to obtain blank values.

Measurement of ATP—Three islets were preincubated for

30 min at 37°C in 200 µl of preincubation medium (described above), then incubated in 200 µl of incubation medium (described above) for 5, 10, or 30 min. After removal of the medium, islets were sonicated in 800 µl of 80 mM $HClO_4$. The sonicate was neutralized with 40 µl of 200 mM Hepes-NaOH buffer (pH 7.0) and 40 µl of 1 M K_2CO_3, then centrifuged at 11,000 ×*g* for 10 min at 4°C. The ATP content in the supernatant was determined by measurement of luminescence with a luminometer (Lumat LB 9507, EG&G Berthold, Germany) after addition of luciferin–luciferase preparation to the islet homogenates. The recovery of ATP added to sonicates at 6 pmol/100 µl was 97 ± 3% (mean ± SE of 6 determinations), indicating that the assay method is satisfactorily valid.

Measurement of Triose-Phosphate Isomerase Activity—Ten freshly isolated islets were sonicated in 50 mM Hepes-NaOH buffer (pH 7.6) containing 1 mM EDTA, 1 mM dithiothreitol, and 1 mg/ml BSA, and centrifuged for 10 min at 10,000 ×*g* at 4°C. Triose-phosphate isomerase activity in the extract was measured by a similar method to that described by Gracy (*25*). Brieffy, the assay was performed at 30°C in 250 mM Hepes-NaOH buffer (pH 7.6) containing 0.15 mM NADH, 1 unit/ml glycerol-3-phosphate dehydrogenase, 0.05–1 mM DL-GAP, and sample solution (20 µl) in a total volume of 400 µl. The rate of the decrease in absorbance at 340 nm was followed: The K_m and V_{max} values were determined by the Lineweaver-Burk plot method.

Assay of DNA—DNA in islet sonicates was assayed by the method of Labarca and Paigen (*26*).

Statistical Analysis—The statistical analyses were performed by the method of Dunnett with the level of significance at $p < 0.05$.

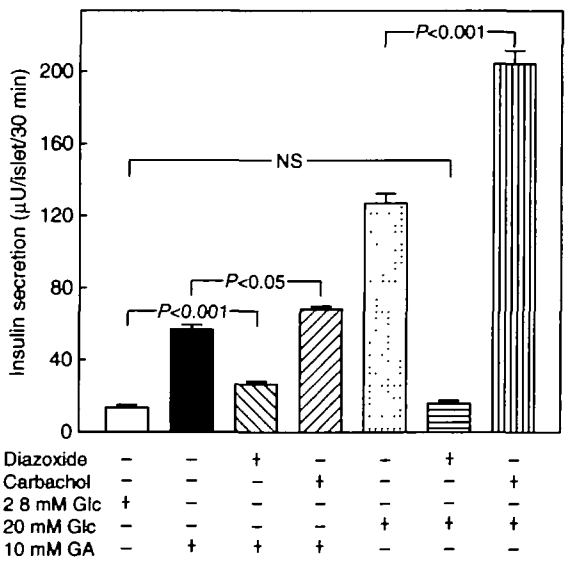

Fig. 1. **Effect of diazoxide and carbachol on insulin secretion induced by either 20 mM glucose (Glc) or 10 mM glyceraldehyde (GA).** After preincubation for 30 min, pancreatic islets were incubated with glucose (2.8 or 20 mM) or glyceraldehyde (10 mM) for 30 min. In some experiments, 150 µM diazoxide was present during both preincubation and incubation. Carbachol (100 µM) was present during incubation in some experiments. Insulin in media was assayed. Values are means±SE of six to eight determinations. NS, not significant.

RESULTS

Insulin Secretion—The effects of diazoxide and carbachol on glucose- or glyceraldehyde-induced insulin secretion are shown in Fig. 1. The insulin secretion induced by 10 mM glyceraldehyde was markedly higher than the basal insulin secretion observed in the presence of 2.8 mM glucose, but was lower than half of that induced by 20 mM glucose. The increase in insulin secretion in response to 20 mM glucose was completely suppressed by 150 µM diazoxide. The glyceraldehyde-induced insulin secretion was also inhibited by diazoxide, but the level of secretion was still higher than the basal level. The stimulation of insulin secretion by 20 mM glucose was augmented by 67% by 100 µM carbachol; in contrast, that by 10 mM glyceraldehyde was increased to a lesser degree (by 24%) by the drug.

The insulin secretion observed in the presence of both 20 mM glucose and 5 µM nitrendipine was almost the same as the basal insulin secretion (Fig. 2). On the other hand, glyceraldehyde-induced insulin secretion in the presence of the Ca^{2+}-channel blocker was 70% higher than the basal insulin secretion.

Islet Glycolytic Intermediate Contents—The contents of all glycolytic intermediates analyzed (G6P, F6P, FBP, DHAP, and GAP) were higher in islets incubated with 20 mM glucose than in islets incubated with 2.8 mM glucose (Fig. 3). In contrast, only GAP was significantly increased in islets incubated with 10 mM glyceraldehyde relative to that in islets incubated with 2.8 mM glucose. Also, the GAP contents in islets incubated with 10 mM glyceraldehyde for 10 or 30 min were respectively 8.9 or 5.1 times those in islets incubated with 20 mM glucose for the same periods. Surprisingly, the amount of DHAP in islets incubated with 10 mM glyceraldehyde was not higher than that in islets incubated with 2.8 mM glucose and was far lower than that

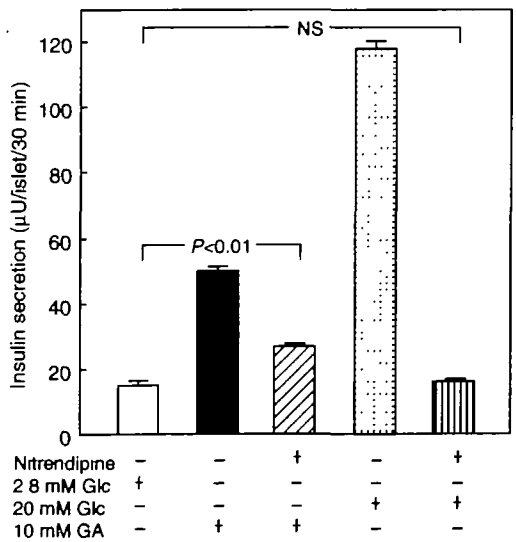

Fig. 2. **Effect of nitrendipine on insulin secretion induced by either 20 mM glucose (Glc) or 10 mM glyceraldehyde (GA).** After preincubation for 30 min, pancreatic islets were incubated with glucose (2.8 or 20 mM) or glyceraldehyde (10 mM) for 30 min. Nitrendipine (5 µM) was present during incubation in some experiments. Insulin in media was assayed. NS, not significant.

in islets incubated with 20 mM glucose.

Islet Inositol Phosphate Contents—myo-Inositol (1)-phosphate, myo-inositol (1,4)-bisphosphate, and myo-inositol (1,4,5)-trisphosphate levels in islets incubated with 10 mM glyceraldehyde or 20 mM glucose were clearly higher than those in islets incubated with 2.8 mM glucose (Table I). The contents of the monophosphate, bisphosphate, trisphosphate, and total myo-inositol phosphates in islets incubated with 10 mM glyceraldehyde were significantly higher than those in islets incubated with 20 mM glucose. Each of the three myo-inositol phosphates and their total in islets incubated with 20 mM glucose were 31–56% higher in the presence of 100 μM carbachol than in the absence of the drug. Carbachol increased the levels of total myo-inositol phosphates and myo-inositol monophosphate in islets incubated with 10 mM glyceraldehyde by 18 and 20%, respectively.

Lactate and Pyruvate Production in Islets—The levels of lactate and pyruvate production by islets incubated for 10 or 30 min with 10 mM glyceraldehyde were higher than the corresponding values for islets incubated with 20 mM glucose (Fig. 4). After incubation for 30 min, the amounts of lactate and pyruvate produced from 10 mM glyceraldehyde were respectively about 2 and 4 times higher than those from 20 mM glucose.

Islet ATP Contents—The ATP content in islets incubated for 5 or 10 min with 20 mM glucose was significantly higher than that in islets incubated with 2.8 mM glucose, but at 30 min the difference was no longer significant (Fig.

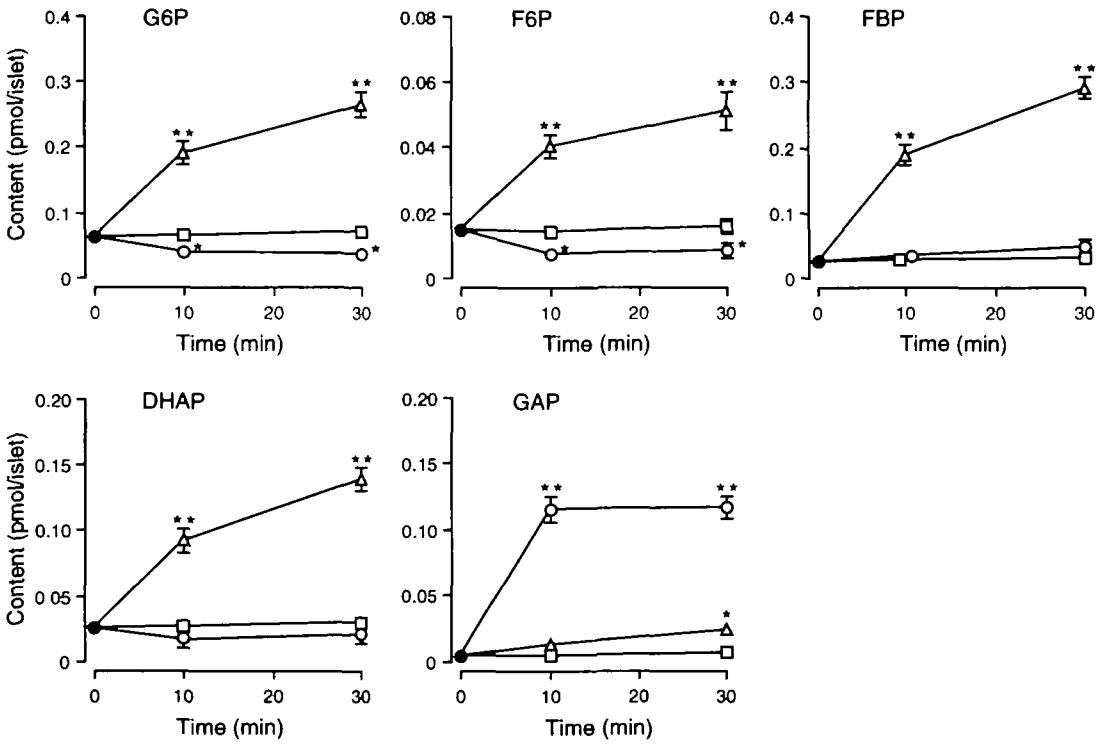

Fig. 3. **Glycolytic intermediate contents in pancreatic islets incubated under different conditions.** Islets were preincubated with 2.8 mM glucose for 30 min, incubated with 2.8 mM glucose (□), 20 mM glucose (△), or 10 mM glyceraldehyde (○) for 10 or 30 min, then analyzed for glycolytic intermediate contents. Values are means ± SE of five determinations. G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate. $^{*}p < 0.01$, $^{**}p < 0.001$ *vs.* 2.8 mM glucose.

TABLE I. **Effect of incubation conditions on [^{3}H]inositol phosphate production by prelabeled pancreatic islets.** Batches of 150 islets were preincubated for 150 min with myo-[2-^{3}H]inositol plus 2.8 mM glucose to label their inositol-containing lipids. After a washing, the islets were incubated for 30 min at 37°C with 1 mM unlabeled myo-inositol plus the additives indicated. Under all incubation conditions, LiCl (10 mM) was also included to prevent degradation of inositol phosphates. Data are the means ± SE of four separate determinations.

Incubation conditions	[^{3}H]Inositol phosphate production (cpm/islet/30 min)			
	$InsP_1$	$InsP_2$	$InsP_3$	Total InsP
2.8 mM Glc	219 ± 5	33 ± 2	20 ± 2	272 ± 7
20 mM Glc	670 ± 13	82 ± 2	43 ± 2	795 ± 14
20 mM Glc+100μM Cch	1,045 ± 31$^{\#\#}$	108 ± 2$^{\#\#}$	59 ± 2$^{\#}$	1,212 ± 30$^{\#\#}$
10 mM GA	748 ± 14**	102 ± 4**	58 ± 2**	908 ± 19**
10 mM GA+100μM Cch	899 ± 23$^{\#}$	111 ± 2	57 ± 3	1,067 ± 20$^{\#}$

Glc, glucose; Cch, carbachol; GA, glyceraldehyde; $InsP_1$, myo-inositol (1)-phosphate; $InsP_2$, myo-inositol (1, 4)-bisphosphate; $InsP_3$, myo-inositol (1,4,5)-trisphosphate; InsP, myo-inosistol phosphataes. $^{*}p < 0.01$, $^{**}p < 0.005$ 10 mM GA *vs.* 20 mM Glc. $^{\#}p < 0.005$, $^{\#\#}p < 0.001$ *vs.* values in the absence of 100 μM Cch.

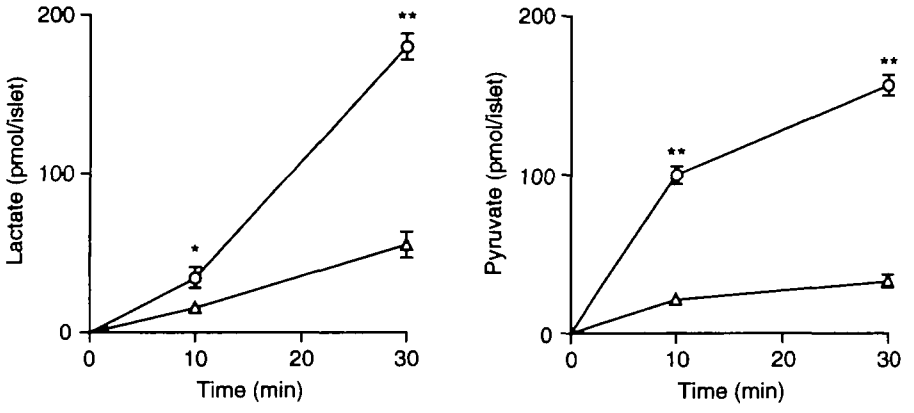

Fig. 4. **Lactate and pyruvate production in pancreatic islets incubated with either 20 mM glucose (△) or 10 mM glyceraldehyde (○).** After preincubation with 2.8 mM glucose for 30 min, islets were incubated with 2.8 mM glucose, 20 mM glucose, or 10 mM glyceraldehyde for 10 or 30 min. Lactate and pyruvate in the medium were assayed enzymatically. The values shown were obtained by subtraction of the basal values (29 ± 1 and 127 ± 10 pmol lactate/islet or 32 ± 2 and 45 ± 3 pmol pyruvate/islet at incubation times of 10 and 30 min, respectively) observed in the incubation with 2.8 mM glucose. Values are means ± SE of five to seven determinations. $^{*}p < 0.01$, $^{**}p < 0.001$ *vs.* 20 mM glucose.

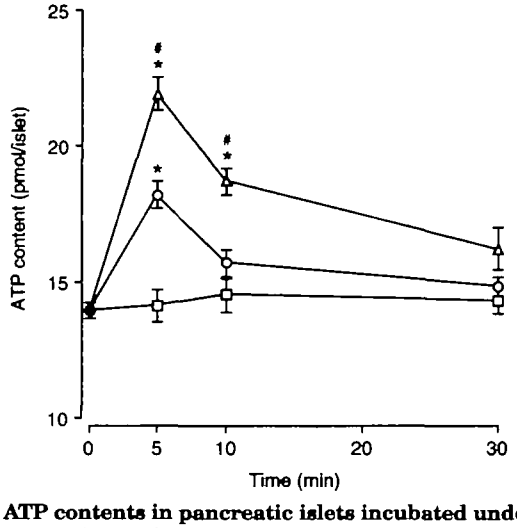

Fig. 5. **ATP contents in pancreatic islets incubated under different conditions.** Islets were preincubated with 2.8 mM glucose for 30 min, incubated with 2.8 mM glucose (□), 20 mM glucose (△), or 10 mM glyceraldehyde (○) for the indicated periods, then analyzed for ATP content. Values are means ± SE of six determinations. $^{*}p < 0.005$ *vs.* 2.8 mM glucose; $\#p < 0.005$ *vs.* 10 mM glyceraldehyde.

5). In islets incubated with 10 mM glyceraldehyde, the ATP content was higher than that in islets incubated with 2.8 mM glucose only after incubation for 5 min. However, the islet ATP content after 5 min of incubation with the triose was significantly lower than after the same period of incubation with 20 mM glucose.

Triose-Phosphate Isomerase Activity—The K_m and V_{max} values for GAP of islet triose-phosphate isomerase, the enzyme catalyzing the isomerization between GAP and DHAP, were 0.71 mM and 9,780 mol/kg DNA/h, respectively.

DISCUSSION

The view proposed by Elliott *et al.* (*15*), that the electrogenic H^+-coupled transport of glyceraldehyde across the plasma membrane in HIT-T15 insulinoma cells leads to depolarization without the closure of K^+_{ATP} channels and to Ca^{2+} entry into the cell, is unlikely to be applicable to the insulinotropic action of the triose in native β-cells, since diazoxide, an opener of K^+_{ATP} channels, evidently inhibited the insulin secretion by the triose (Fig. 1). The incomplete elimination of the glyceraldehyde stimulation of insulin secretion by nitrendipine in contrast to the complete elimination of the glucose stimulation (Fig. 2) is compatible with our view that at least part of the insulin secretion induced by the triose is independent of Ca^{2+} influx through L-type Ca^{2+} channels.

The finding of much higher GAP contents in islets incubated with 10 mM glyceraldehyde than in islets incubated with 20 mM glucose (Fig. 3) calls into question the view of McDonald (*14*) that the phosphorylation of glyceraldehyde by triokinase, because of the very low activity of the enzyme, is not rapid enough to explain the stimulation of insulin release by metabolism of the triose *via* the glycolytic pathway.

It is known that glucose-induced insulin secretion is completely inhibited by diazoxide (*12*, *13*). Diazoxide causes the opening of K^+_{ATP} channels in the plasma membrane of β-cells, resulting in the closure of voltage-dependent Ca^{2+} channels. We reproduced this effect of diazoxide, as shown in Fig. 1. The insulin secretion induced by glyceraldehyde, however, was not completely inhibited by diazoxide, indicating that the mechanism of stimulation of insulin secretion by the triose is not necessarily identical with that by the hexose: while the greater part of the insulin secretion induced by the triose is sensitive to diazoxide, *i.e.*, dependent on K^+_{ATP} channels, a part is resistant to diazoxide, *i.e.*, independent of those channels.

Potentiation of glucose-induced insulin secretion by carbachol was reported previously (*17*, *18*). Our data on the effect of carbachol on glucose-induced insulin secretion (Fig. 1) agree well with these reports. The mechanism by which carbachol potentiates glucose-induced insulin secretion is not yet clear, but it has been suggested that this cholinergic agonist enhances the activity of phospholipase C in β-cells, leading to an increase in the hydrolysis of phosphoinositides to diacylglycerol and inositol phosphates, and subsequently to the activation of protein kinase C by diacylglycerol (*17*, *18*). Less marked potentiation by carbachol of insulin secretion induced by glyceraldehyde in contrast to that by glucose (Fig. 1) again indicates that the triose does not simply mimic the effect of glucose on insulin secretion.

The amount of GAP (about 0.12 pmol/islet) in islets incu-

bated with 10 mM glyceraldehyde for 10 or 30 min (Fig. 3) corresponds to an intracellular concentration of 60 μM, if the mean volume of rat islets is taken to be 2 nl (*27*). MacDonald *et al.* (*28*) suggested that GAP stimulates inositol phosphate formation in rat islets by activating phospholipase C, which activation is half maximal at 25 μM. These considerations raise the possibility that the phospholipase C activation by GAP, resulting in the stimulation of formation of diacylglycerol and myo-inositol (1,4,5)-trisphosphate [a second messenger that releases Ca^{2+} from intracellular stores (*29*)], is involved in the insulin secretion induced by glyceraldehyde in the presence of diazoxide. Higher inositol phosphate production in islets incubated with 10 mM glyceraldehyde relative to that obtained with 20 mM glucose (Table I) is compatible with this view. Activation of phospholipase C by GAP may explain the less marked stimulation by carbachol of both insulin secretion and inositol phosphate production induced by glyceraldehyde relative to glucose (Fig. 1 and Table I): the effect of phospholipase C activation by carbachol on both parameters may be less marked in islets incubated with the triose than in islets incubated with the hexose. Our view should be carefully verified, however, because some investigators have expressed incompatible views (*30, 31*). Persaud *et al.* (*30*) reported that the activation of protein kinase C, *i.e.*, the formation of diacylglycerol, is not required for glyceraldehyde stimulation of insulin secretion from rat islets. Gasa *et al.* (*31*) demonstrated that overexpression of some isoform of phospholipase C in rat islets elicited a large increase in inositol phosphate accumulation, but had no effect on insulin secretion.

The magnitude of the maximum secretory response induced by glyceraldehyde (10–15 mM) is known to be approximately half of or a little lower than that triggered by glucose (20 mM) (*2, 4, 8*). Our observation shown in Fig. 1 agrees well with this notion. Alcázar *et al.* (*8*) suggested that the maximum capacity to metabolize glyceraldehyde through islet glycolysis, probably set by the activity of triokinase, is less than half of that to metabolize glucose, and that this determines the lower secretory response to the triose relative to the hexose. A markedly higher content of GAP in islets incubated with 10 mM glyceraldehyde than in those incubated with 20 mM glucose, however, raises a question about their suggestion.

The islet β-cell has two hydrogen shuttles, the glycerol phosphate shuttle and the malate-aspartate shuttle, to oxidize cytosolic NADH (*32*), most of which is probably formed metabolically at the step in glycolysis catalyzed by GAPDH. Recent papers reported that neither ATP produced directly by glycolysis nor ATP generated during operation of the citric acid cycle participates significantly in closure of K^+_{ATP} channels that regulate fuel-mediated insulin secretion, and that the ATP produced by oxidation of NADH, formed in the glycolytic pathway, *via* hydrogen shuttle systems is critically required for the regulation of insulin secretion (*33, 34*). Incidentally, one (*34*) of these papers reported that islets from mice homozygous for the disrupted gene of mitochondrial glycerol-3-phosphate dehydrogenase, a rate-limiting enzyme for the glycerol phosphate shuttle, had similar ability to wild-type mouse islets to secrete insulin in response to glucose. This does not, however, preclude a role for the glycerol phosphate shuttle in rat islets, since, as in mouse islets, it is not known to what degree the glycerol phosphate shuttle is involved in the NADH shuttle system in rat islets. It is also conceivable that the disruption of the glycerol phosphate shuttle by the gene manipulation was compensated for by an increase in the malate-aspartate shuttle activity. The remarkably lower content of DHAP, which acts as the acceptor of the hydrogen of NADH in the glycerol phosphate shuttle, in islets incubated with 10 mM glyceraldehyde than in those incubated with 20 mM glucose (Fig. 3) suggests that the function of the glycerol phosphate shuttle is inefficiently elicited in islets incubated with glyceraldehyde, resulting in the lower insulin secretion upon stimulation by the triose than by the hexose. The lower content of ATP in islets incubated with 10 mM glyceraldehyde than with 20 mM glucose (Fig. 5) supports this view. The higher GAP content (Fig. 3) and higher rate of lactate and pyruvate production (Fig. 4) in glyceraldehyde-stimulated islets than in glucose-stimulated islets indicate that not only faster production of pyruvate by glycolysis but also more transformation of pyruvate to lactate took place in the former islets. The lower ability of glyceraldehyde than glucose to stimulate insulin secretion in spite of the higher ability of the triose to produce GAP implies a disparity between the two sugars in the mechanism of stimulation of insulin secretion.

The DHAP/GAP ratios in tissues such as human erythrocytes (*35*), rat liver (*36*), and rat muscle (*36*) were reported to be 2.8–12.2. The ratios for islets incubated with 10 mM glyceraldehyde for 10 and 30 min were 0.13 and 0.18, respectively; whereas those for islets incubated with 20 mM glucose for the same time periods were 7.0 and 6.0, respectively (Fig. 3). The reason for the very low ratio in glyceraldehyde-stimulated islets is unknown. One possible reason may be that the activity of islet triose-phosphate isomerase, in spite of its high V_{max} value (9,780 mol/kg DNA/h) compared with GAPDH and fructose-bisphosphate aldolase (144 and 39 mol/kg DNA/h, respectively) in rat islets (unpublished data), is not fully elicited in islet β-cells, and its level of activity is not sufficient to cause equilibration between GAP and DHAP. It seems unlikely that DHAP is consumed by the retrograde reaction, starting from the condensation between DHAP and GAP, of the glycolytic pathway, since the contents of FBP, F6P, and G6P in glyceraldehyde-stimulated islets are not higher than those is islets incubated with 2.8 mM glucose (Fig. 3).

In conclusion, our results suggest that there are at least two pathways of stimulus–secretion coupling that mediate the effects of glyceraldehyde on insulin secretion. In one of them, the triose is metabolized *via* the glycolytic pathway after its phosphorylation and thereby stimulates insulin secretion through the closure of K^+_{ATP} channels in a manner analogous to glucose. In the other one, the activation of phospholipase C by GAP, produced at a high level by phosphorylation of glyceraldehyde, causes the hydrolysis of phosphoinositides, resulting in the stimulation of insulin secretion without the closure of K^+_{ATP} channels. In addition, our data also suggest that the lower insulin secretion induced by glyceraldehyde than by glucose may be due to the deficient functioning of the glycerol phosphate shuttle on account of low levels of DHAP in islets incubated with the triose.

REFERENCES

1. Ashcroft, S.J.H., Weerasinghe, L.C.C., and Randle, P.J. (1973) Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. *Biochem. J.* **132**, 223–231
2. Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., and Täljedal, I.-B. (1974) The pancreatic β-cell recognition of insulin secretagogues. *Arch. Biochem. Biophys.* **162**, 448–457
3. Hahn, H.-J., Ziegler, M., and Mohr, E. (1974) Inhibition of glucagon secretion by glucose and glyceraldehyde on isolated islets of Wistar rats. *FEBS Lett.* **49**, 100–102
4. Malaisse, W.J., Herchuelz, A., Levy, J., Sener, A., Pipeleers, D.G., Devis, G., Somers, G., and Van Obberghen, E. (1976) The stimulus-secretion coupling of glucose-induced insulin release. XIX. The insulinotropic effect of glyceraldehyde. *Mol. Cell. Endocrinol.* **4**, 1–12
5. Zawalich, W.S., Dye, E.S., Rognstad, R., and Matschinsky, F.M. (1978) On the biochemical nature of triose- and hexose-stimulated insulin secretion. *Endocrinology* **103**, 2027–2034
6. Zawalich, W.S. (1979) Intermediary metabolism and insulin secretion from isolated rat islets of Langerhans. *Diabetes* **28**, 252–260
7. MacDonald, M.J. (1981) High content of mitochondrial glycerol-3-phosphate dehydrogenase in pancreatic islets and its inhibition by diazoxide. *J. Biol. Chem.* **256**, 8287–8290
8. Alcázar, O., Giné, E., Qiu-Yue, Z., and Tamarit-Rodriquez, J. (1995) The stimulation of insulin secretion by D-glyceraldehyde correlates with its rate of oxidation in islet cells. *Biochem. J.* **310**, 215–220
9. Wollheim, C.B., Dunne, M.J., Peter-Riesch, B., Bruzzone, R., Pozzan, T., and Petersen, O.H. (1988) Activators of protein kinase C depolarize insulin-secreting cells by closing K^+ channels. *EMBO J.* **7**, 2443–2449
10. Newgard, C.B. and McGarry, J.D. (1995) Metabolic coupling factors in pancreatic β-cell signal transduction. *Annu. Rev. Biochem.* **64**, 689–719
11. Prentki, M. (1996) New insights into pancreatic β-cell metabolic signaling in insulin secretion. *Eur. J. Endocrinol.* **134**, 272–286
12. Gembal, M., Gilon, P., and Henquin, J.C. (1992) Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. *J. Clin. Invest.* **89**, 1288–1295
13. Aizawa, T., Sato, Y., Ishihara, F., Taguchi, N., Komatsu, M., Suzuki, N., Hashizume, K., and Yamada, T. (1994) ATP-sensitive K^+ channel-independent glucose action in rat pancreatic β-cell. *Am. J. Physiol.* **266**, C622–C627
14. MacDonald, M.J. (1989) Does glyceraldehyde enter pancreatic islet metabolism *via* both the triokinase and the glyceraldehyde phosphate dehydrogenase reactions? A study of these enzymes in islets. *Arch. Biochem. Biophys.* **270**, 15–22
15. Elliott, A.C., Trebilcock, R., Yates, A.P., and Best, L. (1993) Stimulation of HIT-T15 insulinoma cells by glyceraldehyde does not require its metabolism. *Eur. J. Biochem.* **213**, 359–365
16. Davies, J., Tomlinson, S., Elliott, A.C., and Best, L. (1994) A possible role for glyceraldehyde transport in the stimulation of HIT-T15 insulinoma cells. *Biochem. J.* **304**, 295-299
17. Persaud, S.J., Jones, P.M., Sugden, D., and Howell, S.L. (1989) The role of protein kinase C in cholinergic stimulation of insulin secretion from rat islets of Langerhans. *Biochem. J.* **264**, 753–758
18. Kelley, G.G., Zawalich, K.C., and Zawalich, W.S. (1995) Synergistic interaction of glucose and neurohumoral agonists to stimulate islet phosphoinositide hydrolysis. *Am. J. Physiol.* **269**, E575–E582
19. Komatsu, M., Schermerhorn, T., Aizawa, T., and Sharp, G.W.G. (1995) Glucose stimulation of insulin release in the absence of extracellular Ca^{2+} and in the absence of any increase in intracellular Ca^{2+} in rat pancreatic islets. *Proc. Natl. Acad. Sci. USA* **92**, 10728–10732
20. Tsuura, Y., Fujimoto, S., Kajikawa, M., Ishida, H., and Seino, Y. (1999) Regulation of intracellular ATP concentration under conditions of reduced ATP consumption in pancreatic islets. *Biochem. Biophys. Res. Commun.* **261**, 439–444
21. Miwa, I., Murata, T., Mitsuyama, S., and Okuda, J. (1990) Participation of glucokinase inactivation in inhibition of glucose-induced insulin secretion by 2-cyclohexen-1-one. *Diabetes* **39**, 1170–1176
22. Kato, T., Berger, S.J., Carter, J.A., and Lowry, O.H. (1973) An enzymatic cycling method for nicotinamide-adenine dinucleotide with malic and alcohol dehydrogenases. *Anal. Biochem.* **53**, 86–97
23. Morin, L., Giroix, M.-H., Gangnerau, M.-N., Bailbé, D., and Porta, B. (1997) Impaired phosphoinositide metabolism in glucose-incompetent islets of neonatally streptozotocin-diabetic rats. *Am. J. Physiol.* **272**, E737–E745
24. Miwa, I., Fukatsu, H., Toyoda, Y., and Okuda, J. (1990) Anomeric preference of glucose utilization in human erythrocytes loaded with glucokinase. *Biochem. Biophys. Res. Commun.* **173**, 201–207
25. Gracy, R.W. (1975) Triosephosphate isomerase from human erythrocytes in *Methods in Enzymology* (Wood, W.A., ed.) Vol. **41**, pp. 442–447, Academic Press, New York
26. Labarca, C. and Paigen, K. (1980) A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* **102**, 344–352
27. Erecinska, M., Bryla, J., Michalik, M., Meglasson, M.D., and Nelson, D. (1992) Energy metabolism in islets of Langerhans. *Biochim. Biophys. Acta* **1101**, 273–295
28. MacDonald, M.J., Mertz, R.J., and Rana, R.S. (1989) Glyceraldehyde phosphate: An insulin secretagogue with possible effects on inositol phosphate formation in pancreatic islets. *Arch. Biochem. Biophys.* **269**, 194–200
29. Berridge, M.J. and Irvine, R.F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312**, 315–321
30. Persaud, S.J., Jones, P.M., and Howell, S.L. (1991) Activation of protein kinase C is not required for glyceraldehyde-stimulated insulin secretion from rat islets. *Biochim. Biophys. Acta* **1095**, 183–185
31. Gasa, R., Trinh, K.Y., Yu, K., Wilkie, T.M., and Newgard, C.B. (1999) Overexpression of G11α and isoforms of phospholipase C in islet β-cells reveals a lack of correlation between inositol phosphate accumulation and insulin secretion. *Diabetes* **48**, 1035–1044
32. MacDonald, M.J. (1990) Elusive proximal signals of β-cells for insulin secretion. *Diabetes* **39**, 1461–1466
33. Dukes, I.D., McIntyre, M.S., Mertz, R.J., Philipson, L.H., Roe, M.W., Spencer, B., and Worley, J.F.III (1994) Dependence on NADH produced during glycolysis for β-cell glucose signaling. *J. Biol. Chem.* **269**, 10979–10982
34. Eto, K., Tsubamoto, Y., Terauchi, Y., Sugiyama, T., Kishimoto, T., Takahashi, N., Yamauchi, N., Kubota, N., Murayama, S., Aizawa, T., Akanuma Y., Aizawa, S., Kasai, H., Yazaki, Y., and Kadowaki, T. (1999) Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. *Science* **283**, 981–985
35. Minakami, S. and Yoshikawa, H. (1966) Studies on erythrocyte glycolysis. II. Free energy changes and rate limiting steps in erythrocyte glycolysis. *J. Biochem.* **59**, 139–144
36. Veech, R.L., Raijman, L., Dalziel, K., and Krebs, H.A. (1969) Disequilibrium in the triose phosphate isomerase system in rat liver. *Biochem. J.* **115**, 837–842