Reduced sensitivity of dihydroxyacetone on ATP-sensitive K+ channels of pancreatic beta cells in GK rats

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Summary In the GK (Goto-Kakizaki) rat, a genetic model of non-insulin-dependent diabetes mellitus, glucose-induced insulin secretion is selectively impaired. In addition, it has been suggested by previous studies that impaired glucose metabolism in beta cells of the GK rat results in insufficient closure of ATP-sensitive K+ channels (K_{ATP} channels) and a consequent decrease in depolarization, leading to a decreased insulin release. We have recently reported that the site of disturbed glucose metabolism is probably located in the early stages of glycolysis or in the glycerol phosphate shuttle. In the present study, in order to identify the impaired metabolic step in diabetic beta cells, we have investigated insulin secretory capacity by stimulation with dihydroxyacetone (DHA), which is known to be directly converted to DHA-phosphate and to preferentially enter the glycerol phosphate shuttle. In addition, using the patch-clamp technique, we also have studied the sensitivity of DHA on the K_{ATP} channels of beta cells in GK rats. The insulin secretion in response to 5 mmol/l DHA with 2.8 mmol/l glucose was impaired, and DHA sensitivity of the K_{ATP} channels was reduced in beta cells of GK rats. From these results, we suggest that the intracellular site responsible for impaired glucose metabolism in pancreatic beta cells of GK rats is located in the glycerol phosphate shuttle. [Diabetologia (1994) 37: 1082–1087]

Key words Dihydroxyacetone, ATP-sensitive K+ channels, GK rat, glycerol phosphate shuttle, pancreatic beta cell.

It is well known that glucose-induced insulin secretion from pancreatic beta cells is selectively impaired in patients with non-insulin-dependent diabetes mellitus (NIDDM) [1] and in animal models of NIDDM such as the GK (Goto-Kakizaki) rat [2]. We have recently reported that in GK rats one of the mechanisms of this abnormality is insufficient closure of ATP-sensitive K+ channels (K_{ATP} channels), which is

attributable to impaired intracellular glucose metabolism. Moreover, evidence has suggested that the disturbance is located in the steps of glycolysis between glucose 6-phosphate and glyceraldehyde 3-phosphate or in the glycerol phosphate shuttle [3]. This shuttle is thought to be a direct link in the metabolic pathway between glycolysis and mitochondrial oxidation, and has been reported to be most active in pancreatic beta cells among mammalian tissues [4, 5], and the augmentation of intracellular glucose metabolism is modulated mainly by its activity [6]. Accordingly, the decreased glucose metabolism in beta cells in NIDDM is probably caused by functional defects in this shuttle. Dihydroxyacetone (DHA)-phosphate, an intermediate substrate in the glycolytic pathway, is known to preferentially enter the glycerol phosphate shuttle [7]. Thus, in order to ascertain the possibility of impaired function of the shuttle in the beta cells in NIDDM, we investigated the

effect of DHA on insulin secretion and K$_{ATP}$-channel activity in GK rats.

Materials and methods

Male GK rats and control Wistar rats at 7 weeks of age were supplied by Pharmaceutical Research Laboratories-II (Takeda Chemical Industry, Co. Ltd., Osaka, Japan). The animals were fed on standard laboratory chow ad libitum until the study. Both GK rats (serum glucose: 18.1 ± 0.5 mmol/l, $n = 22$) and controls (serum glucose: 8.7 ± 0.4 mmol/l, $n = 19$) were between 8 and 12 weeks of age when they were used.

Preparation of islet cells. Pancreatic islets were isolated by collagenase digestion. The islets were dispersed by a previously described method [8] and cultured on small glass coverslips overnight using RPMI-1640 medium containing 11.1 mmol/l glucose supplemented with fetal calf serum (10%), penicillin (100 μ/ml), and streptomycin (0.1 mg/ml). In the patch-clamp studies, each coverslip was transferred to the test chamber placed on an inverted microscope.

Measurement of insulin secretory capacity. Insulin secretory capacity was assessed by the batch incubation method as previously reported [3] with freshly isolated islets from GK and control rats. The islets were pre-incubated with Krebs-Ringer-bicarbonate buffer (KRBB) medium containing 5.5 mmol/l glucose and 0.2% bovine serum albumin (BSA) at 37°C for 30 min because it has previously been reported [2] that response to glucose in islets of GK rats was recovered after 50 min under conditions with no glucose. Batches of about 10 islets were then incubated with KRBB medium supplemented with test materials and 0.2% BSA at 37°C for 30 min. The test materials (DHA, glyceraldehyde, and ketoisocaproate; were all purchased from Nacalai Tesque, Kyoto, Japan) were dissolved immediately before incubation. Glibenclamide (Hoechst, Frankfurt, Germany) was prepared as 1 mmol/l stock solutions in dimethylsulphoxide (DMSO), and was dissolved just before the experiments. An aliquot was taken from the solution of each batch for later measurement of insulin by radioimmunoassay.

Electrophysiological measurements. Single-channel recording in cell-attached configuration was made as described previously [8]. The bathing solution was composed of (in mmol/l) 135 NaCl, 5 KCl, 2 CaCl$_2$, 2 MgSO$_4$, and 5 HEPES (pH 7.4 with NaOH), with a pipette solution containing 140 KCl, 2 CaCl$_2$, and 5 HEPES. Dispersed cells of more than 10-μm diameter were used in the experiments because non-beta cells are reported to be of smaller size in rats [9]. The experiments were done using at least three different preparations to ascertain reproducibility.
    Data currents were recorded through a patch-clamp amplifier (EPC-7, List Electronic, Darmstadt, Germany) and stored on videotape via a pulse code modulation converting system (PCM501, Sony, Tokyo, Japan) for later analysis by computer (PC-98XL, NEC, Tokyo, Japan). Data on the effect of DHA were recorded continuously for 180 s at a sampling frequency of 0.5 kHz through a Bessel type filter (1 kHz). The analysis in the assessment of concentration-response relationship was performed using the records between 120 s and 180 s. The time course for inhibition of K$_{ATP}$-channel activity by 5 mmol/l DHA was assessed from the data recorded for 180·s by means of a previously reported method [3]. The channel activity [I] was expressed as the mean patch current (N × P$_o$ × i),

where N, P$_o$, and i represent the number of channels in the patch membrane, the open probability, and the unit amplitude of single channel current, respectively. The mean patch current was calculated as the average difference between baseline current (no open current) and open channel currents. The channel activity in the control solution was expressed as I$_c$, and I/I$_c$ was determined as the relative channel activity. The experiments were performed at room temperature (22–25°C).

Statistical analysis

Results were expressed as mean ± SEM (number of observations). Statistical significance was evaluated by unpaired Student's t-test or by chi-squared test and p less than 0.05 was considered significant.

Results

Comparison of the effect of DHA and glyceraldehyde on K$_{ATP}$ channels and insulin secretion. As shown in Figure 1 A, 1 mmol/l DHA had no inhibitory effect on K$_{ATP}$-channel activity of pancreatic beta cells, but 2 mmol/l DHA had a small and 5 mmol/l had a considerable inhibitory effect, in a reversible manner. Figure 1 B shows the dose-dependency of the inhibitory effects of DHA, but even 10 mmol/l DHA did not abolish K$_{ATP}$-channel activity completely. Compared with the effects of glyceraldehyde which we have previously reported [3], the effects of DHA are significantly smaller at the concentrations of 1, 2, and 5 mmol/l ($p < 0.05$). On the other hand, 5 or 10 mmol/l DHA in the absence of glucose did not enhance insulin secretion, but glyceraldehyde alone initiated insulin secretion at the concentration of 5 mmol/l ($p < 0.01$), with almost complete suppression of K$_{ATP}$-channel activity, and 10 mmol/l glyceraldehyde further potentiated insulin secretion ($p < 0.01$, Fig. 1 C).

Insulin secretory capacity by glyceraldehyde and DHA in GK rats and controls. Figure 2 A shows the insulin secretory capacity of glyceraldehyde in the absence of glucose. Glyceraldehyde (5 mmol/l) initiated insulin secretion to the same extent in control rats ($170 \pm 14\%$, $n = 13$) and in GK rats ($173 \pm 25\%$, $n = 11$). Insulin secretion in response to 10 mmol/l glyceraldehyde was further potentiated to $497 \pm 17\%$ in control [$n = 5$] and to $440 \pm 56\%$ in GK rats [$n = 5$], without significant difference. The addition of 1 μmol/l glibenclamide to 5 mmol/l glyceraldehyde elicited marked insulin release in both groups ($549 \pm 38\%$ in control [$n = 5$] and $515 \pm 31\%$ in GK rats [$n = 5$]).
    In control rats, 2 mmol/l DHA did not initiate insulin secretion in the presence of 2.8 mmol/l glucose (data not shown), but 5 mmol/l DHA under the same conditions dramatically enhanced insulin secre-
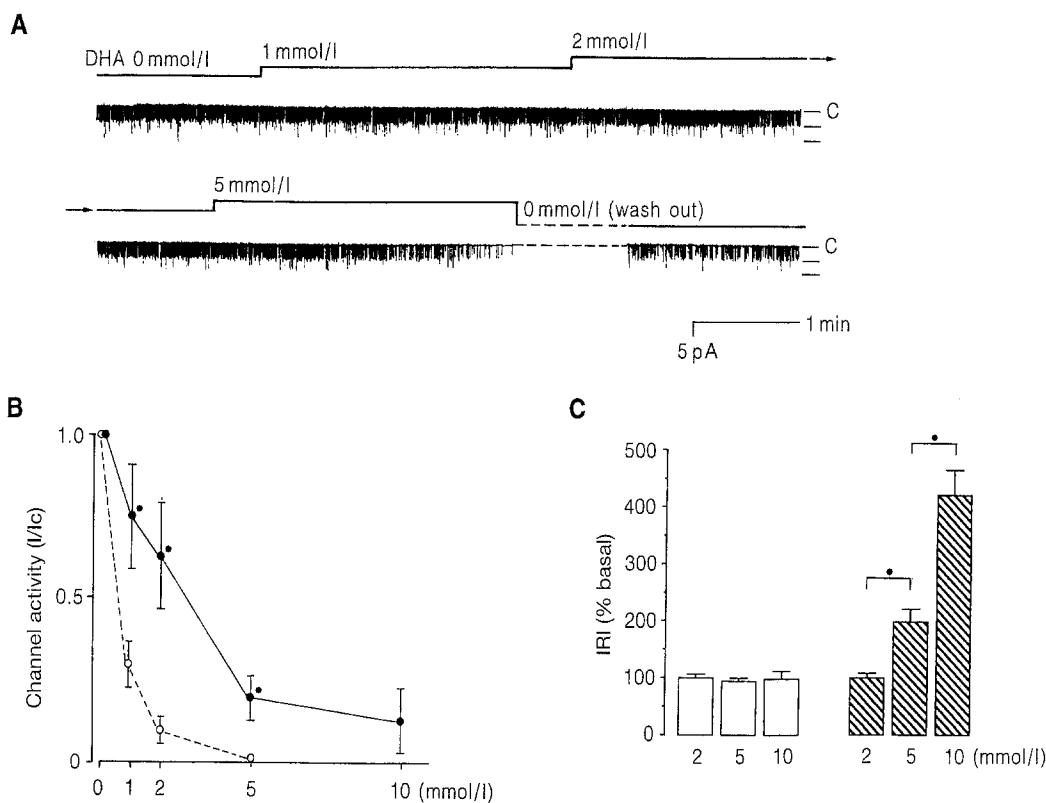
Fig. 1. (A–C) Effect of DHA on sensitivity of K_{ATP}-channel activity in normal single beta cells and insulin secretion in normal islets. **A:** Representative trace shows the inhibitory effect of K_{ATP} channels when increasing concentrations of DHA were added. Data recorded at 0 mV of pipette potential in cell-attached configuration. **B:** Dose-dependency of inhibitory effect of DHA on K_{ATP}-channel activity (-●-). Number of observation is 5–6 for each point. Effect of glyceraldehyde on K_{ATP} channels described in our previous report (---O---) [3]. *$p < 0.05$. **C:** Insulin secretory capacity expressed as the percentage of basal secretion in response to DHA □ or glyceraldehyde ▨. The control insulin secretion by 2 mmol/l DHA and 2 mmol/l glyceraldehyde was 546 ± 34 pg · islet⁻¹ · 30 min⁻¹ ($n = 10$) and 476 ± 41 pg · islet⁻¹ · 30 min⁻¹ ($n = 12$), respectively. *$p < 0.001$

tion (197 ± 21 % vs under 2.8 mmol/l glucose alone, $p < 0.001$; $n = 13$). On the other hand, 10 mmol/l DHA failed to enhance it further (180 ± 17 % [$n = 13$], Fig. 2 B). In GK rats, 5 or 10 mmol/l DHA elicited no significant insulin secretion even in the presence of 2.8 mmol/l glucose (106 ± 9 % at 5 mmol/l [$n = 14$], 105 ± 6 % at 10 mmol/l [$n = 14$]), but 1 μmol glibenclamide in the presence of 5 mmol/l DHA and 2.8 mmol/l glucose enhanced it to the same extent as in controls (215 ± 24 % in GK [$n = 13$] and 261 ± 27 % in control [$n = 13$] vs in the presence of 2.8 mmol/l glucose alone; Fig. 2 B). Insulin secretion elicited by 5 mmol/l ketoisocaproate at 2 mmol/l glyceraldehyde in both groups was not significantly different (286 ± 32 % in control [$n = 9$] and 317 ± 32 % in GK rats [$n = 10$]).

Effect of DHA on K_{ATP} channels in GK and control rats. Figure 3 A shows examples of the time course of inhibition of K_{ATP}-channel activity by 5 mmol/l DHA without glucose in both groups. In the control groups, inhibition of the channel activity became predominant after 60 s and only a few channel events were observed after 120 s. On the other hand, the clear inhibitory effect was not recognized until 180 s in GK rats. As shown in Figure 3 B, the inhibitory effect of 5 mmol/l DHA on K_{ATP}-channel activity was smaller throughout the courses in the GK groups. In particular, the difference was significant after 80 s ($p < 0.05$) and I/I_c values after 180 s remained 0.56 ± 0.14 [$n = 19$] in GK rats against 0.20 ± 0.07

[$n = 17$] in controls ($p < 0.05$). Tested cells of both groups showed a temporary activation of channel activity to the same extent at an early phase (35 % [6 of 17] in control and 53 % [10 of 19] in GK rats, not significant by chi-squared test).

Discussion

GK rats have been recognized as a hereditary NIDDM rat model with characteristics of selective impaired glucose-induced insulin secretion [2, 3, 10]. Giroix and collaborators [11] have recently reported that insulin release in response to secretagogues other than glucose was also impaired in 17-week-old GK rats. However, the discrepancy might arise from the difference in the age of the rats, since islets of GK rats at 16 weeks of age are reported to suffer from histological damage [12], and it is possible that GK rats of an advanced age have highly impaired beta-cell function with a poor insulin response to any secretagogue. On the other hand, several reports, including our study, have indicated that islets of younger GK rats keep a selectivity for glucose stimulation in the impairment of insulin secretion [2, 3].

We demonstrate here that insulin secretion by stimulation of DHA alone is within the range of basal secretion. Although several previous studies [13–15] have demonstrated that DHA as well as glyceraldehyde is an initiator for insulin secretion in rat islets, the ability of DHA to initiate insulin secretion was

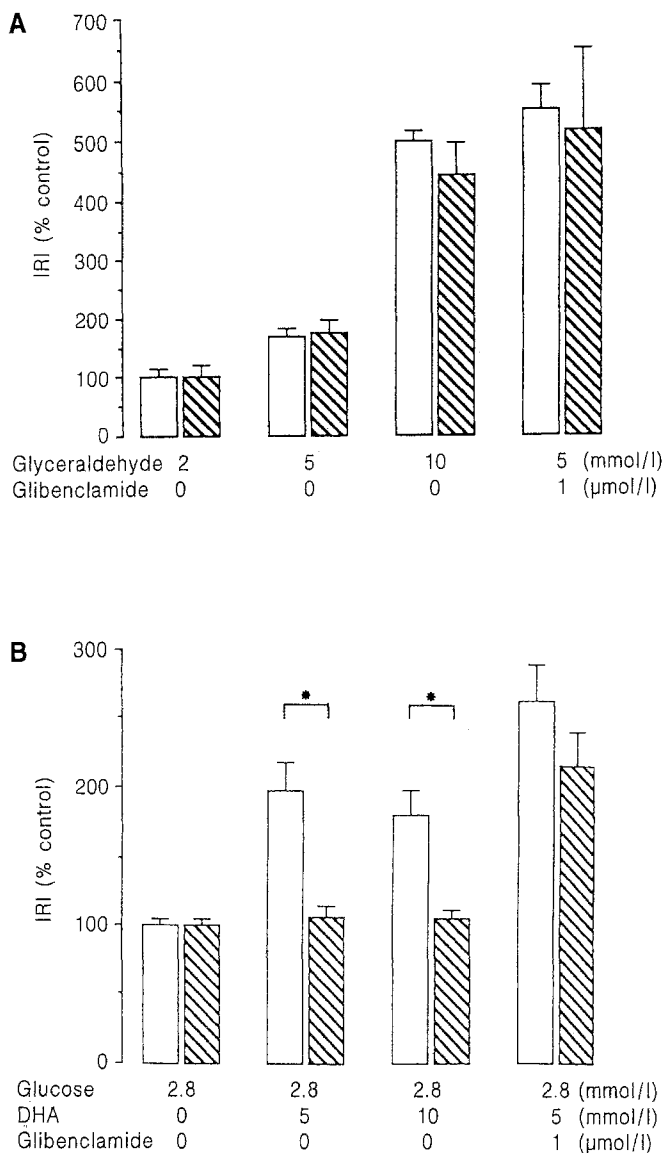**Fig. 2. (A, B)** Insulin secretion by stimulation with glyceraldehyde or DHA in control (☐) and GK (▨) groups. The results are expressed as the percentage of those elicited by 2 mmol/l glyceraldehyde (**A**) or 2.8 mmol/l glucose (**B**). Glibenclamide was used in order to examine insulin secretory capacity from islets of both groups in state of complete closure of K_{ATP} channels. **A:** The dose-dependent effect of glyceraldehyde on insulin release in both groups. The control values stimulated with 2 mmol/l glyceraldehyde were 476 ± 41 pg · islet^{-1} · 30 min^{-1} in control [$n = 11$] and 437 ± 26 pg · islet^{-1} · 30 min^{-1} in GK rats [$n = 12$], were not significantly different. **B:** Dose-dependent effect of DHA on insulin secretion in both groups. The control insulin secretion in response to 2.8 mmol/l glucose was not significantly different between the two groups, whose values were 878 ± 51 pg · islet^{-1} · 30 min^{-1} ($n = 13$) and 846 ± 77 pg · islet^{-1} · 30 min^{-1} ($n = 14$) in control and GK rats, respectively. $p < 0.001$

considerably less than that of 1–4 mmol/l glyceraldehyde or 5.5 mmol/l glucose. Accordingly, our results are not necessarily inconsistent with previous findings.

The inhibition of the K_{ATP}-channel has been widely accepted as a key step in the initiation of in-

sulin secretion induced by fuel substrates such as glucose, glyceraldehyde and ketoisocaproate [3, 16], but the almost complete suppression of its activity is thought to be necessary for the initiation. In the present study, DHA was found to elicit insulin secretion in the presence of 2.8 mmol/l glucose from pancreatic islets of control rats. However, DHA alone failed to initiate insulin release, and its inhibitory effect on the channel activity was apparently less than that of glyceraldehyde, a known initiator of insulin secretion. Even at 10 mmol/l, K_{ATP}-channel activity remained at about 10 % of the control state. In our preliminary studies, pyruvate also incompletely inhibited the channel activity even at 40 mmol/l with an I/I$_c$ value of about 0.1 (unpublished observation by Y. Tsuura and H. Ishida), a concentration which has been reported to have no effect on insulin secretion [17]. These findings seem to be consistent with the "spare channel hypothesis" of Cook et al. [18].

The possible explanation for the difference in inhibitory potency on K_{ATP}-channels between DHA and glyceraldehyde may be based on their distinct phosphorylation pathways. DHA has been reported to be phosphorylated exclusively by triokinase with a very low K_m value, while glyceraldehyde has been known to also be phosphorylated by glyceraldehyde 3-phosphate dehydrogenase with high K_m value (4.8 mmol/l) [19]. Accordingly, 5 mmol/l glyceraldehyde is thought to suppress K_{ATP}-channel activity through ATP production by preferentially entering the distal portion of glycolysis via glyceraldehyde 3-phosphate dehydrogenase, and also the mitochondrial oxidation system. On the other hand, ATP from DHA could be produced through the glycerol phosphate shuttle as well as glycolysis.

The inhibitory effect of DHA on K_{ATP}-channel activity was found to be lower in beta cells of GK rats compared to controls. Since the properties of the K_{ATP}-channel in GK rats have been shown not to be altered [3], the reduced sensitivity of DHA on the K_{ATP}-channel activity could be due to impaired utilization of DHA for intracellular ATP production. The conversion of DHA to DHA-phosphate by triokinase might thus be reduced in GK rats, but this hypothesis does not seem probable because triokinase activity has been reported to be abundantly present in islets [19], and this step could not become rate-limiting. In addition, the K_{ATP}-channel activity was found to be temporarily activated in the early phase after DHA loading in beta cells of both groups to the same extent. This transient activation is perhaps attributable to ATP consumption through the phosphorylation steps by triokinase, similar to the case of glucose which is known to convert to fructose-1,6-bisphosphate through two phosphorylation steps [20, 21]. The defective phosphorylation of DHA is, therefore, unlikely to be responsible for the im-
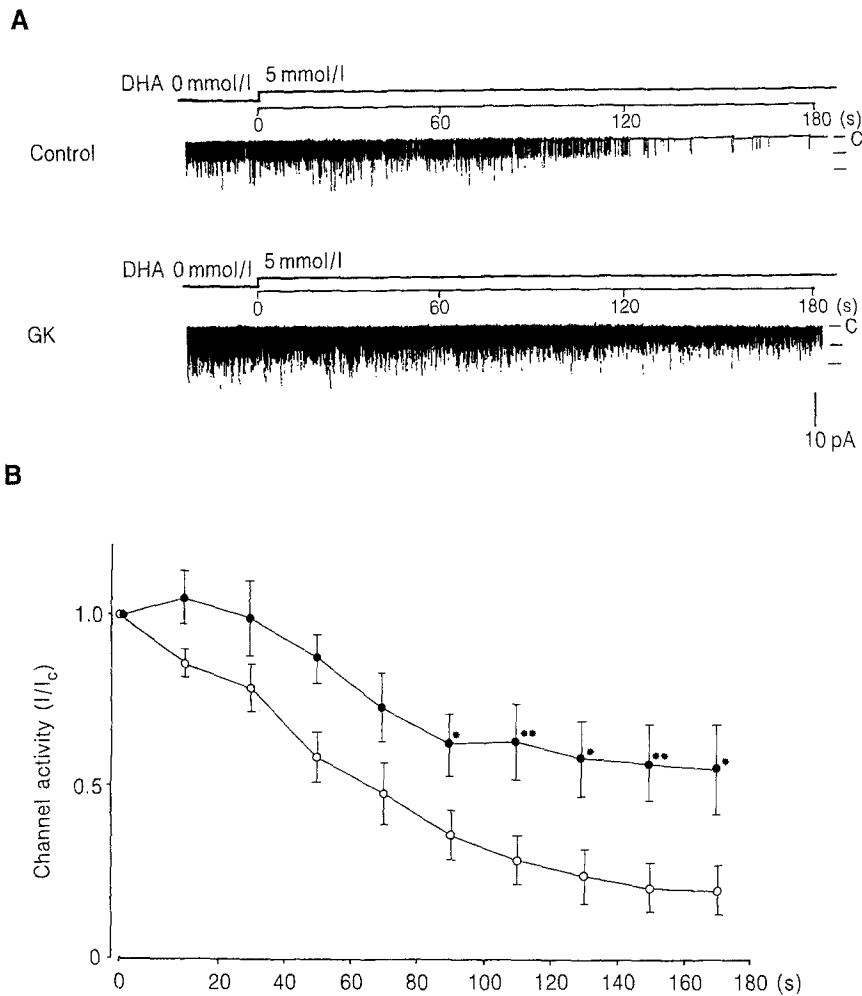
A

B

Fig. 3. (A, B) Inhibitory effect of 5 mmol/l DHA on K_{ATP}-channel activity in control and GK rats. **A:** Representative traces show the time course of inhibition by DHA in control (upper panel) and GK rats (lower panel). The traces were recorded at 0 mV of pipette potential in cell-attached configuration. **B:** Time course of inhibition of K_{ATP}-channel activity by 5 mmol/l DHA in control (O, $n = 17$) and GK rats (●, $n = 19$). The I_c values were 3.77 ± 0.79 pA in control ($n = 17$) and 3.59 ± 0.65 pA in GK rats ($n = 19$). $*p < 0.05$; $**p < 0.02$

paired inhibition of K_{ATP}-channel activity by DHA in diabetic beta cells.

Since the insulin secretion induced by glyceraldehyde was not impaired in GK rats and its inhibitory effect on K_{ATP}-channel activity has been found to be identical in the two groups in our recent study [3], the distal steps after glyceraldehyde 3-phosphate in glycolysis including glyceraldehyde 3-phosphate dehydrogenase activity should be functionally intact. Therefore, the most likely candidate responsible for decreased glucose metabolism is thought to be a malfunction of the glycerol phosphate shuttle where DHA-phosphate enters. FAD-linked glycerophosphate dehydrogenase is known to be the key enzyme of this shuttle [7], and its activity has been reported to be reduced in islets in NIDDM rat models including GK rats [22–24], consistent with our observations. However, our results could not completely rule out the possibility that triose phosphate isomerase is also disturbed in beta cells of GK rats. Further investigations are needed to clarify this issue.

In conclusion, DHA can inhibit K_{ATP}-channel activity and enhance insulin secretion in the presence of glucose. In GK rats, however, the sensitivity of DHA on the K_{ATP}-channels was reduced, presum-

ably due to the insufficient utilization of DHA for intracellular ATP production. These results are consistent with the hypothesis that the glycerol phosphate shuttle is functionally impaired in NIDDM beta cells.

References

1. Ward WK, Beard JC, Halter JB, Pfeiffer MA, Porte D (1984) Pathophysiology of insulin secretion in non-insulin dependent diabetes mellitus. Diabetes Care 7: 491–502
2. Portha B, Serradas P, Bailbe D, Suzuki K, Goto Y, Giroix MH (1991) β-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Diabetes 40: 486–491
3. Tsuura Y, Ishida H, Okamoto Y et al. (1993) Glucose sensitivity of ATP-sensitive K⁺ channels is impaired in β cells of

the GK rat, a new genetic model of NIDDM. Diabetes 42: 1446–1453

4. MacDonald MJ (1981) High content of mitochondrial glycerol-3-phosphate dehydrogenase in pancreatic islets and its inhibition by diazoxide. J Biol Chem 256: 8287–8290

5. Rasschaert J, Ling Z, Malaisse WJ (1993) Effect of streptozotocin and nicotinamide upon FAD-glycerophosphate dehydrogenase activity and insulin release in purified pancreatic B-cells. Mol Cell Biochem 120: 135–140

6. Sener A, Malaisse WJ (1992) Hexose metabolism in pancreatic islets: Ca^{2+}-dependent activation of the glycerol phosphate shuttle by nutrient secretagogues. J Biol Chem 267: 13251–13256

7. Leahy JL, Bonner-Weir S, Weir GC (1992) β-cell dysfunction induced by chronic hyperglycemia. Diabetes Care 15: 442–455

8. Tsuura Y, Ishida H, Okamoto Y et al. (1992) Impaired glucose sensitivity of ATP-sensitive K$^+$ channels in streptozotocin-induced NIDDM rats. Diabetes 41: 861–865

9. Pipeleers DG, Pipeleers-Marichal MA (1981) A method for the purification of single A, B and D cells and for the isolation of coupled cells from isolated rat islets. Diabetologia 20: 654–663

10. Kimura K, Toyota T, Kakizaki M, Kudo M, Takebe K, Goto Y (1982) Impaired insulin secretion in the spontaneous diabetes rats. Tohoku J Ext Med 137: 453–459

11. Giroix MH, Vesco L, Portha B (1993) Functional and metabolic perturbations in isolated pancreatic islets from the GK rat, a genetic model of noninsulin-dependent diabetes. Endocrinology 132: 815–822

12. Goto Y, Kakizaki M (1981) The spontaneous-diabetes rat: a model of noninsulin dependent diabetes mellitus. Proc Jpn Acad 57: 381–384

13. Zawalich WS, Dye ES, Rognstad R, Matschinsky FM (1978) On the biochemical nature of triose- and hexose-stimulated insulin secretion. Endocrinology 103: 2027–2034

14. MacDonald MJ, Fahien LA (1988) Glyceraldehyde phosphate and methyl esters of succinic acid: two new potent insulin secretagogues. Diabetes 37: 997–999

15. MacDonald MJ, Mertz R, Rana RS (1989) Glyceraldehyde phosphate: an insulin secretagogue with possible effects on inositol phosphate formation in pancreatic islets. Arch Biochem Biophys 269: 194–200

16. Misler S, Falke LC, Gillis K, McDaniel ML (1986) A metabolite-regulated potassium channel in rat pancreatic B cells. Proc Natl Acad Sci USA 83: 7119–7123

17. Sener A, Kawazu S, Hutton JC et al. (1978) The stimulus-secretion coupling of glucose-induced insulin release. Biochem J 176: 217–232

18. Cook DL, Satin LS, Ashford MLJ, Hales CN (1988) ATP-sensitive K$^+$ channels in pancreatic β-cells: spare-channel hypothesis. Diabetes 37: 495–498

19. MacDonald MJ (1989) Does glyceraldehyde enter pancreatic islet metabolism via both the triokinase and the glyceraldehyde phosphate dehydrogenase reaction? Arch Biochem Biophys 270: 15–22

20. Hayashi S, Horie M, Tsuura Y, Ishida H, Okada Y, Seino Y, Sasayama S (1993) Disopyramide blocks pancreatic ATP-sensitive K$^+$ channels and enhances insulin release. Am J Physiol 265: C337–C342

21. Arkhammar P, Nilsson T, Rorsman P, Berggren PO (1987) Inhibition of ATP-regulated K$^+$ channels precedes depolarization-induced increase in cytoplasmic free Ca^{2+} concentration in pancreatic β-cells. J Biol Chem 262: 5448–5454

22. Östenson CG, Abdel-Halim SM, Rasschaert J, Malaisse-Lagae F, Meuris S, Sener A, Efendic S (1993) Deficient activity of FAD-linked glycerophosphate dehydrogenase in islets of GK rats. Diabetologia 36: 722–726

23. Giroix MH, Rasschaert J, Sener A, Leclercq-Meyer V, Bailbe D, Portha B, Malaisse WJ (1992) Study of hexose transport, glycerol phosphate shuttle and Krebs cycle in islets injected with streptozotocin during the neonatal period. Mol Cell Endocrinol 83: 95–104

24. Giroix MH, Rasschaert J, Bailbe D, Leclercq-Meyer V, Sener A, Portha B, Malaisse WJ (1991) Impairment of glycerol phosphate shuttle in islets from rats with diabetes induced by neonatal streptozocin. Diabetes 40: 227–232